Venous thromboembolism in patients with cancer undertaking chemotherapy

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Abstract

Background
Venous thromboembolism (VTE) is an umbrella term for the pathological development of blood clots in the venous system. These include pulmonary embolism (PE), deep and superficial venous thromboses. A cancer patient’s risk of developing VTE is at least 4-fold that of the general population and can increase markedly when undertaking chemotherapy. With advances in radiological imaging, increasing numbers of clinically unsuspected VTEs are diagnosed on routine cancer staging scans. This may be because the patient is asymptomatic, the clinician may be unaware of the increased risk or the non-specific presenting symptoms and signs may be attributed to the underlying cancer. VTE is ranked as the second biggest killer of cancer inpatients and a leading killer of outpatients worldwide.

Study aims
1. Establish the prevalence/cumulative incidence of venous thromboembolism (VTE) in cancer patients undertaking chemotherapy at the Canterbury Regional Cancer and Haematology Service (CRCHS).

2. Identify potential clinicopathological biomarkers predictive or diagnostic for VTE development in cancer patients undertaking chemotherapy.

3. Identify differences in these potential biomarkers between cancer patients and volunteers without cancer at baseline assessment.

4. Assess the performance of the Khorana score, Khorana/Ay score (The Vienna model) and PROTECHT score as risk assessment models (RAMs) for predicting VTE development in cancer patients undertaking chemotherapy.

5. Investigate the utility of a calibrated, automated fluorogenic thrombin generation assay in predicting/diagnosing VTE development in cancer patients, at baseline and undertaking chemotherapy.
6. Investigate changes in thrombomodulin and antithrombin levels during chemotherapy and their association with VTE in cancer patients undertaking chemotherapy.

7. Investigate ratios of thrombin generation variables, thrombomodulin and or antithrombin in cancer patients undertaking chemotherapy and their association with VTE.

8. Investigate changes in angiopoietin-1, angiopoietin-2 and/or soluble Tie-2 receptor levels in cancer patients undertaking chemotherapy and their association with VTE.

9. Investigate ratios of angiopoietin-1, angiopoietin-2 and soluble Tie-2 receptor levels with each other in cancer patients undertaking chemotherapy and their association with VTE.

**Methods**

A prospective, observational study was performed, through the Canterbury Regional Cancer and Haematology Service, to determine the baseline prevalence and cumulative incidence of clinically suspected and unsuspected VTE during chemotherapy treatment. Clinicopathological findings and published risk assessment models (RAMs) were analysed to assess their utility in VTE diagnosis or risk prediction. Adult patients with solid tumour malignancies, lymphoma or myeloma were consented to undergo serial clinical assessments, blood tests and radiological imaging. Exclusion criteria included patients who had received chemotherapy or radiation treatments in the preceding three months or were on anticoagulation except for aspirin or clopidogrel. Clinicopathological findings were compared with age- and gender-matched volunteers without cancer, including plasma levels of variables of a calibrated, automated thrombin generation assay (TGA) and the angiopoietin (Ang)-Tie2 angiogenesis pathway.

**Results**

Between July 2011 and August 2013, 203 cancer patients and 50 volunteers without cancer were recruited. Fifty one (25.1%) cancer patients were diagnosed with VTE, including 17 (8.4% prevalence) patients at baseline
assessment, prior to commencing chemotherapy. All baseline VTEs were clinically unsuspected including 12 PEs with the most proximal site of thrombus being the lobar pulmonary arteries in 5 patients. Thirty one patients undertaking chemotherapy were subsequently diagnosed with VTE during the follow up period. Overall, 60% of the VTE events were clinically unsuspected. RAMs for VTE development showed high specificity and negative predictive value but were not sensitive with a low positive predictive value and were unable to predict clinically unsuspected VTE development. Adaptations of the RAMs, using local population-derived cut-offs, did not significantly improve the precision of the models. The Christchurch percentile adaptation of the Khorana/Ay RAM, however, was the only model in which none of the patients classified as low risk, subsequently developed VTE on chemotherapy.

The D-dimer was a strong marker of VTE risk and development with higher baseline plasma levels seen in patients with cancer compared with volunteers without cancer. Plasma D-dimer concentrations ≥270ng/mL may predict for the development of VTE in cancer patients on chemotherapy within 100 days and, in combination with an Eastern Collaborative Oncology Group performance status >1, may aid in diagnosing VTE prior to commencing chemotherapy. Any increase in D-dimer concentration, after serial testing, was associated with VTE development on chemotherapy with a one-off concentration of ≥355ng/mL associated with VTE diagnosis (sensitivity 100%, specificity 75%). Plasma levels of TGA and the Ang-Tie2 pathway variables were different in cancer patients compared with volunteers without cancer and changed during chemotherapy treatment, but only a decrease in the angiopoietin-2:soluble Tie2 (sTie-2) ratio, after serial testing, was independently associated with VTE development on chemotherapy.

Conclusions
VTE risk is high in cancer patients with many patients presenting with significant clot burden that is clinically unsuspected. Although low risk patients can be reliably identified using RAMs, it is challenging to identify high risk patients in order to tailor thromboprophylaxis. Assessment of the ECOG performance status and plasma concentrations of the D-dimer and Ang-2:sTie2
ratio in cancer patients may be associated with VTE development and warrant further investigation.
“A thesis of one strong marriage, two beautiful children, four houses and approximately 4,000 earthquakes.”
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### List of abbreviations commonly used

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<th>Description</th>
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<tbody>
<tr>
<td>ACCP</td>
<td>American College of Chest Physicians</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>Ang-1</td>
<td>Angiopoietin-1</td>
</tr>
<tr>
<td>Ang-2</td>
<td>Angiopoietin-2</td>
</tr>
<tr>
<td>APC</td>
<td>Activated protein C</td>
</tr>
<tr>
<td>APL</td>
<td>Acute promyeloctic leukaemia</td>
</tr>
<tr>
<td>APTT</td>
<td>Acquired partial thromboplastin time</td>
</tr>
<tr>
<td>AT</td>
<td>Antithrombin</td>
</tr>
<tr>
<td>ASCO</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>BCS</td>
<td>Budd-Chiari syndrome</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CATS</td>
<td>Cancer and thrombosis study</td>
</tr>
<tr>
<td>CCF</td>
<td>Congestive cardiac failure</td>
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<tr>
<td>CHCH PC</td>
<td>Christchurch percentile</td>
</tr>
<tr>
<td>CHL</td>
<td>Canterbury Health Laboratories</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclo-oxygenase</td>
</tr>
<tr>
<td>CRP/ hsCRP</td>
<td>C-reactive protein/ high sensitivity C-reactive protein</td>
</tr>
<tr>
<td>CTEPH</td>
<td>Chronic thromboembolic pulmonary hypertension</td>
</tr>
<tr>
<td>CTPA</td>
<td>Computed tomography pulmonary angiogram</td>
</tr>
<tr>
<td>CVAD</td>
<td>Central venous access device</td>
</tr>
<tr>
<td>CVC</td>
<td>Central venous catheter</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>DVT</td>
<td>Deep venous or vein thrombosis</td>
</tr>
<tr>
<td>EC</td>
<td>Endothelial cell</td>
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<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>EPCR</td>
<td>Endothelial protein C receptor</td>
</tr>
<tr>
<td>ESMO</td>
<td>European Society of Medical Oncology</td>
</tr>
<tr>
<td>ETP</td>
<td>Endogenous thrombin potential</td>
</tr>
<tr>
<td>FDP</td>
<td>Fibrin degradation product</td>
</tr>
<tr>
<td>GBM</td>
<td>Glioblastoma multiforme</td>
</tr>
<tr>
<td>GCSF</td>
<td>Granulocyte Colony Stimulating Factor</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HMG CoA</td>
<td>3-hydroxy-3-methylglutaryl-coenzyme A</td>
</tr>
<tr>
<td>HMVEC</td>
<td>Human microvascular endothelial cells</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Human umbilical vein endothelial cells</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin 1 beta</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalised Ratio</td>
</tr>
<tr>
<td>IVC</td>
<td>Inferior vena cava</td>
</tr>
<tr>
<td>JAK2</td>
<td>Janus Kinase 2</td>
</tr>
<tr>
<td>LMWH</td>
<td>Low molecular weight heparin</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MDCT</td>
<td>Multi-detector computed tomography</td>
</tr>
<tr>
<td>MP</td>
<td>Microparticle</td>
</tr>
<tr>
<td>MPN</td>
<td>Myeloproliferative neoplasm</td>
</tr>
<tr>
<td>MOH NZ</td>
<td>Ministry of Health New Zealand</td>
</tr>
<tr>
<td>MVT</td>
<td>Mesenteric venous or vein thrombosis</td>
</tr>
<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear Factor Kappa B</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
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Venous thromboembolism in cancer patients undertaking chemotherapy
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>TM</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor alpha</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>TT</td>
<td>Thrombin time</td>
</tr>
<tr>
<td>UFH</td>
<td>Unfractionated heparin</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper limit of normal</td>
</tr>
<tr>
<td>uPA</td>
<td>Urokinase like plasminogen activator</td>
</tr>
<tr>
<td>USS</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Vascular endothelial growth factor receptor</td>
</tr>
<tr>
<td>V/Q</td>
<td>Ventilation/ perfusion</td>
</tr>
<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
</tr>
<tr>
<td>vWF</td>
<td>Von Willebrand factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WPB</td>
<td>Weibel-Palade body</td>
</tr>
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**Chemotherapy abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABVD</td>
<td>Doxorubicin, bleomycin, vinblastine, dacarbazine.</td>
</tr>
<tr>
<td>AC</td>
<td>Doxorubicin and cyclophosphamide.</td>
</tr>
<tr>
<td>CAPOX</td>
<td>Capecitabine and oxaliplatin.</td>
</tr>
<tr>
<td>CHOP</td>
<td>Cyclophosphamide, doxorubicin, vincristine, prednisone.</td>
</tr>
<tr>
<td>CMF</td>
<td>Cyclophosphamide, methotrexate, 5-fluorouracil.</td>
</tr>
<tr>
<td>CMFVP</td>
<td>Cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, prednisone.</td>
</tr>
<tr>
<td>EC</td>
<td>Epirubicin and cyclophosphamide.</td>
</tr>
<tr>
<td>ECF</td>
<td>Epirubicin, cisplatin, 5-fluorouracil.</td>
</tr>
<tr>
<td>EOF</td>
<td>Epirubicin, oxaliplatin, 5-fluorouracil.</td>
</tr>
<tr>
<td>EOX</td>
<td>Epirubicin, oxaliplatin, capecitabine.</td>
</tr>
<tr>
<td>FEC</td>
<td>5-fluorouracil, epirubicin, cyclophosphamide.</td>
</tr>
<tr>
<td>FOLFIRINOX</td>
<td>5-fluorouracil, leucovorin, irinotecan, oxaliplatin.</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>5-fluorouracil, leucovorin, oxaliplatin.</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-fluorouracil.</td>
</tr>
<tr>
<td>GDP</td>
<td>Gemcitabine, dexamethasone, cisplatin.</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.</td>
</tr>
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Introduction

Venous thromboembolism (VTE) is an umbrella term describing the pathological development of a thrombus (blood clot) or thrombi (blood clots) within the venous system of the body. They have been demonstrated anywhere within the superficial or deep venous systems termed superficial venous thrombosis and deep venous thrombosis (DVT), respectively. The term ‘VTE’ also encompasses clot within the pulmonary (lung) vasculature where the term pulmonary embolism (PE) is used. Many people associate VTE development with periods of immobility such as the post-operative period and long haul aircraft flights.

Extensive published research has investigated VTE risk, pathophysiology, diagnosis, management and outcome in medical and surgical patients. Subgroups with a higher predisposition for developing VTE than the general population have been identified. Research focusing on these patients have shown that certain factors, including medical conditions or diseases, independently impact on the risk of VTE development, and that VTE prevention and treatment should ideally be tailored to the individual patient. Cancer patients have been identified as one subgroup with an increased risk for VTE development compared with the general population.

The interactions between the human body’s coagulation (clotting) mechanisms and cancer have been documented since the 19th century initially by Jean-Baptiste Bouillaud (1823) and then by Armand Trousseau (1865), who described his eponymous syndrome of migrating superficial thrombophlebitis and underlying gastric malignancy. Tragically Trousseau diagnosed his own gastric cancer, having developed a lower extremity DVT, and died 6 months later (1-5). Christian Albert Theodor Bilroth, a pioneer of surgical techniques, including the first gastrectomy, laryngectomy and oesophagectomy procedures, was also the first to postulate the involvement of the body’s coagulation mechanisms in the development of cancer metastases when he noted the presence of tumour cells within a thrombus (2, 6). Rudolf Virchow, the father of cellular pathology, coined the term pulmonary embolism following his conclusions that thrombi in the lungs are the result of fragments of blood clot.
(emboli) that have broken off from a thrombus that has developed elsewhere in the body and travelled (embolised) to the pulmonary vasculature(7). Pulmonary embolism (pl. emboli) can develop de novo within the pulmonary arterial tree as well as embolise from other areas of the venous system.

Over the last 15 years increasing preclinical and clinical research have improved our knowledge of the relationship between VTE and cancer. However, the underlying pathophysiological mechanisms for VTE development in cancer patients remain poorly understood, limiting clinical guidance on VTE prevention, diagnosis and management. The results of the Fundamental Research in Oncology and Thrombosis (FRONTLINE) survey on thrombosis and cancer also suggest that not all clinicians are aware of the risks of, and outcomes from, VTE occurrence and anticoagulation treatment in cancer patients(8).

VTE is ranked as the second biggest killer of hospitalised cancer patients(9-11). Compared to the general non-cancer population there is at least a 4-fold increase in the risk of developing VTE in cancer patients, which increases 6-7 fold for a patient receiving chemotherapeutic agents. This risk is modified by many clinical and biological parameters as well as the primary site of the cancer and the type of chemotherapeutic agents used(12, 13). Virchow’s triad, as first described in the 1950s, long after Rudolf Virchow had died, links thrombosis risk to the constituents of the blood, the flow of that blood and/or the integrity of the vessel in which the blood flows(14). These criteria have been further researched to give us more insight into the complex interactions of various stimuli leading not only to thrombosis but also tumour associated angiogenesis, tumour growth and metastasis formation(15).

To date, VTE prevalence data in cancer patients have been obtained predominantly from retrospective studies, but the true prevalence has been underestimated as DVT and PE may be clinically unsuspected(16). Unsuspected VTE are being diagnosed more often on routine cancer staging scans due to improvements in computed tomography (CT) imaging resolution(17). In addition, autopsy studies have suggested PE prevalence to be as high as 70% in medical patients, but these studies have involved small numbers of patients and
included subjects without cancer. Discovery of unsuspected VTE poses management dilemmas with regards to clinical significance, uncertainty whether they propagate to become clinically distinct thromboses impacting on patients’ quality of life and function and whether they will become life-threatening or life-ending if left untreated. It is speculated whether these thrombi embolised or developed in situ. Clinicians weigh up treating these patients with anticoagulants because of the significant risk of morbidity and mortality as a result of bleeding complications and the duration of anticoagulation in cancer patients is yet to be established. Some of these issues are unlikely to be resolved in a prospectively designed clinical trial due to ethical concerns about withholding anticoagulation but they are all often considered in clinical practice.

Studies investigating medical inpatients without cancer have shown that rates of asymptomatic VTE are far higher than symptomatic VTE, with a strong association between the presence of asymptomatic DVT and the subsequent development of symptomatic VTE. These medical patients, however, do not have a statistically significant higher mortality rate(18, 19). No prospective data, to date, have been published describing the natural history and/or outcome of asymptomatic VTE in cancer patients and it remains controversial in some circumstances as to whether or not to treat them, although the American College of Chest Physicians’ (ACCP) 2012 guidelines recommend anticoagulation for all VTE events(20).

As well as being potentially life threatening, VTE can reduce an individual’s quality of life, performance status and potential fitness for any planned surgery, chemotherapy or radiation treatment. It is, however, an area of debate as to whether the increased mortality rate in cancer patients with VTE is directly related to the effect of the VTE, or whether VTE and high mortality rate are both sequelae of a more aggressive cancer.

The diagnosis of VTE in cancer patients currently relies on the patients’ non-specific presenting symptoms and/or signs and the treating physician’s index of suspicion for the presence of VTE. Diagnosis of VTE is made even more complex when the patient has pulmonary metastases or comorbidities such as chronic obstructive pulmonary disease or cardiac failure.
Clinical decision tools used in medical patients, such as the Wells Criteria in conjunction with the D-dimer blood test, are commonly employed in clinical practice for those that present with symptoms suggestive of VTE(21). Those who present with a D-dimer result within the laboratory-stipulated normal range and are classified as low probability on the Wells Criteria score do not require further investigation as they have a very low probability of having VTE. Those patients that are classified as intermediate or high risk and/or have a D-dimer result above the normal range fulfil the criteria for further investigation with radiological imaging. Clinical decision tools such as these are of limited utility in a cohort of cancer patients of whom more than 80% may have a D-dimer above the normal range due to the impact of their cancer and/or treatments(22).

Primary prevention of VTE or thromboprophylaxis, therefore, appears to be a sensible approach but with evidence suggesting that the risk of significant bleeding on anticoagulants is increased in cancer patients it would be prudent to tailor prophylactic anticoagulation to those cancer patients at high risk for VTE and not treat low risk patients(23). The Khorana score is a published tool for predicting VTE risk in cancer patients commencing chemotherapy and is currently being validated in clinical trials as to its utility(24).

The demand for simple, practical and reproducible investigational tools is high in all areas of medical practice. While no model can be perfect in its predictive abilities it can be used as an initial screen to guide the use of more expensive and potentially harmful tests or interventions. This may, in turn, ease the burden on the health system and potentially reduce the morbidity and inconvenience experienced by patients.

This prospectively designed research study investigated the prevalence of both suspected and unsuspected VTE in cancer patients undertaking chemotherapy treatment at the tertiary referral Canterbury Regional Cancer and Haematology Service (CRCHS) in the South Island of New Zealand. It also continued investigations into clinical variables and biomarkers in the peripheral blood that may improve the diagnosis of VTE in cancer patients or identify those at high risk for developing VTE.
Chapter 1
Literature Review
Chapter 1 Literature Review

The vascular system and coagulation

1.1 The vascular system

The vascular system is a pipeline of interconnecting, tubular vessels which transport blood throughout the body. At a basic level it is split into two parts. The venous system (the veins and venules) returns deoxygenated blood from all regions of the body to the right side of the heart. From there the pulmonary arteries transfer this blood to the lungs where carbon dioxide, a waste product of metabolism, can be removed and the blood oxygenated (by gas exchange). Oxygenated blood then returns to the left side of the heart through the pulmonary veins, and is pumped to the peripheral tissues via the arterial system (the arteries and arterioles) to maintain the body’s metabolism (see figure 1).

Figure 1 A basic overview of the vascular system
The flow of blood through the vasculature not only relies on the pumping action of the heart but also on contractions of muscles within the walls of the blood vessels. Arteries contain more muscle within their walls than veins and facilitate unidirectional flow at a high pressure. Veins contain thin layers of muscle within their walls and carry blood at a significantly lower pressure than arteries. They are easy to physically compress and/or occlude extrinsically so are reliant on (see figure 2):

- valves to prevent reverse flow of blood from the peripheries
- the squeezing action, induced by contractions of the large muscles of the limbs on adjacent veins
- changes in intrathoracic and intra-abdominal pressure with respiration to maintain the blood return to the heart and counteract the pooling effects of gravity.

**Figure 2 Venous valves and the lower limb muscle pump**

The venous system contains superficial and deep veins. Superficial veins return blood from the most peripheral areas of the body to the larger deep veins, via perforator vessels, which feed into a network of vessels of enlarging calibre as they move closer to the heart, with the largest and most proximal being the superior (returning venous blood from above the heart) and inferior (returning venous blood from below the heart) vena cavae.
1.2 The pulmonary vasculature

The main pulmonary artery exits the heart posteriorly and superiorly from the pulmonary valve of the right ventricle and splits into the right and left pulmonary arteries, at approximately the level of the 5th thoracic vertebra, to supply each lung with blood. Each pulmonary artery splits into lobar arteries supplying the lobes of each lung which then further split into segmental and subsegmental arteries as the tree of blood vessels branches out, roughly following the airways that pervade the lung parenchyma towards the alveoli (see figure 3). The alveoli are tiny air sacs (average diameter 200 micrometres) where gas exchange occurs, via alveolar capillaries, to remove carbon dioxide and obtain oxygen to maintain metabolism. The oxygenated blood is then carried back from the lungs via the pulmonary venous network, through the interlobular septae of each lung. Each lung lobe has a pulmonary vein that courses towards the heart. On the right side the upper and middle lobe veins usually join together so that two pulmonary veins from each side generally pass through the pericardium and separately feed into the heart at the posteroinferior aspect of the left atrium.

Figure 3 Pulmonary artery anatomy
1.3 Coagulation

Coagulation (thrombogenesis or haemostasis) is the process by which a blood clot or thrombus is produced. When a thrombus (pl. thrombi) develops within the body’s blood vessels it is termed thrombosis and, depending on where it develops within the vascular system, it is termed an arterial or venous thrombosis. Coagulation is a finely controlled set of physiological processes which minimise blood loss following injury. Without effective coagulation humans would bleed to death from minor injuries sustained from everyday living.

1.4 Physiological coagulation

Historically the process of blood coagulation has been conceptualised into three parts, based primarily on our ability to measure these components in the laboratory (see figure 4):

- The intrinsic pathway.
- The extrinsic pathway.
- The (final) common pathway.

It was observed that blood would clot inside the body but required stimuli from substances outside of (extrinsic to) the blood. Contrasting this was the fact that when blood was dropped onto an artificial surface, such as glass, the coagulation process still took place suggesting that the blood (intrinsically) contained all that was required to facilitate coagulation. This prompted early research to split coagulation into two separate pathways which were stimulated in different ways but resulted in a common goal (common pathway) of thrombin generation. Extensive research has further identified the complex sequence of events during the coagulation process, which is termed the coagulation or clotting cascade. The 3-pathway hypothesis is thought to be somewhat outdated and inaccurate, as the pathways interact with each other regardless of the stimulus. However it provides a good foundation for learning how the coagulation process occurs.
Venous thromboembolism in cancer patients undertaking chemotherapy

Figure 4 Overview of coagulation
1.4.1 The intrinsic (contact) pathway
Several proteins are involved in the initiation of the intrinsic or contact activation pathway. Factor XII, prekallikrein and high molecular weight kininogen (HMWK) are activated by contact with a negatively charged surface which we now understand may actually be inside (e.g. phospholipid) or outside the body and, therefore, not necessarily artificial.

A complex of activated Factor XII (XIIa) and HMWK in turn activates factor IX (IXa) which, in complex with activated factor VIII (VIIIa), activates factor X (Xa). Initially small amounts of VIIIa are present but feedback from Xa, and thrombin produced by the action of Xa, stimulate increased activation of factor VIII. The rest of the intrinsic pathway uses the same pathway as the extrinsic pathway (the common pathway) which integrates factors V, prothrombin and fibrinogen(25, 26).

The physiological function of contact pathway activation is, as yet, unknown and differs in humans from other animal species. Factor XII deficiency does not cause obvious abnormalities in humans, yet in mice and baboons manifests an antithrombotic phenotype(27). Whales and dolphins, in fact, lack factor XII altogether. Human contact pathway activation, in vivo, always appears to be pathologically stimulated by events including contact with artificial surfaces (e.g. central venous access device tips), blood and platelet activation (e.g. during cardiopulmonary bypass procedures) and administration of intravenous treatments (e.g. albumin, immunoglobulin) containing kallikrein or factor XIIa(28).

1.4.2 The extrinsic pathway
After injury damaged tissues will require repair. Some of that injury will damage local blood vessels which leads to bleeding as the endothelial lining, on the abluminal side of the blood vessel wall, has been breached. Tissue Factor (TF) is normally sequestered under the intact endothelial lining of blood vessels, likely in an encrypted conformation. Prothrombotic phospholipid (rich in phosphatidylserine (PS) is normally enriched on the cytoplasmic side of the platelet membrane phospholipid bilayer. Upon vessel injury, TF and PS are exposed to circulating blood which contains serine protease enzymes also known as coagulation factors. This rapidly commences a chain of events to aid
repair of the damage to the blood vessel by plugging the injury with a thrombus, maintain blood vessel integrity and prevent life threatening blood loss(29). 

Inactive coagulation Factor VII (FVII) zymogen possesses a defective active site which only becomes fully active when it combines with TF and transforms to its active state (FVIIa:TF complex)(30). This interaction is the major initiator of the extrinsic coagulation pathway by activating factor X to Xa which, in turn, cleaves prothrombin (factor II) to thrombin (factor IIa)(31) (see figure 5).

**Figure 5 Coagulation initiation**

This initial process is relatively inefficient and yields only a small concentration of thrombin, but positive feedback from the resulting thrombin prompts an explosive propagation of its own production through factor XI (and the intrinsic pathway). Thrombin also activates factors V (Va) and VIII (VIIIa) as well as platelets and monocytes. Factor Va interacts with Factor Xa (the prothrombinase complex) and Factor VIIIa interacts with factor IXa (the tenase complex), both in the presence of calcium and phospholipids, to increase thrombin production by upregulating Xa activity, and via the intrinsic pathway, respectively (see figure 6).
**1.4.3 Thrombin**

The serine protease thrombin (activated Factor II (FIIa)), discovered by Dr. Andrew Buchanan in the nineteenth century, is the central enzyme of the coagulation process(32). It is also involved in many other physiological processes of the body including anticoagulation, fibrinolysis, tissue repair, wound healing, platelet and endothelial activation and inflammation(33).

Thrombin is a 37kDa molecule produced when prothrombin is cleaved by the prothrombinase complex (factor Va, factor Xa, calcium and phospholipid). Phospholipid provides a negatively charged surface on which the process takes place. Prothrombin is predominantly formed in the liver but small amounts are also produced in the gastrointestinal tract, brain, kidney and spleen. Like Factors VII, IX, X and protein C, thrombin and prothrombin are vitamin K-dependent for their function. Vitamin K cannot be produced endogenously in humans and can only be obtained from the diet(34, 35).

Thrombin is well known for its central function within the coagulation cascade. Its main procoagulant effect is to convert fibrinogen to fibrin monomers which spontaneously multimerise. It also activates FXIII (fibrin stabilising factor or the Hageman Factor) which promotes cross linking between fibrin strands. Thrombin also:
- inhibits fibrinolysis by activation of thrombin-activatable fibrinolysis inhibitor (TAFI).

- induces the adhesion molecule P-selectin to be expressed on endothelial cells and platelets. In the blood, tissue factor microparticles bearing the P-selectin glycoprotein ligand-1 (PSGL-1) bind to P-selectin at the site of thrombus and support its propagation.

Fibrinogen monomers are soluble molecules in the blood composed of two large globular D domains split by a smaller globular E domain. During the coagulation cascade, thrombin converts fibrinogen to the gel-like substance fibrin by catalysing the removal of fibrinopeptides A and B from the E domain. This leads to polymerisation of the molecules through linking between the E domain of one molecule and the D-domain of another to produce double-stranded protofibrils. These protofibrils then laterally associate and continue to grow and branch to form a 3-dimensional web which is stabilised by covalent linkages between adjacent D domains through the action of FXIIIa(36) (see figure 7).

**Figure 7 Fibrin production**

![Fibrin production diagram](image-url)
Thrombin is composed of two polypeptide chains linked by a disulfide bond. The A (light) chain has no proven functional role, but the B (heavy) chain contains 4 identified binding sites (35) (see figure 8). The sodium binding site plays an important role in determining if thrombin will play a pro- or anticoagulant role (see figure 9). Under physiological conditions, approximately 60% of thrombin molecules are bound to sodium. If a sodium ion is bound to this site it influences changes in thrombin structure which promote the binding of procoagulant molecules such as fibrinogen and Factors V, VIII and XI. In the absence of bound sodium, thrombin promotes anticoagulation through its binding with thrombomodulin to activate protein C, a potent natural anticoagulant (37).

**Figure 8 The basic structure of thrombin**
Adjacent to the active site are two anionic binding exosites termed I and II. Exosite I interacts predominantly with hydrophobic contacts and exosite II interactions are largely ionic in nature(38). The importance of these sites, along with the active site, is in their ability to recognise and bind various substrates which influence the function of thrombin.

**1.4.3.1 Procoagulant interactions of thrombin**

Exosite I, in concert with the active site, binds fibrinogen and also hirudin, a potent natural inhibitor of thrombin, found in the saliva of the leech, *Hirudo medicinalis*. Both exosites I and II are involved in the recognition of Factors V and VIII and, in their active forms, act as cofactors as part of the prothrombinase and tenase complexes respectively, to increase thrombin production by positive feedback mechanisms(37). Fibrinogen and Factors V, VIII and, in part XI, are directly changed by the interactions with thrombin. All other effects exerted by thrombin appear to be mediated through cofactors(38). Exosite I binds and activates FXIII but the efficiency of this process is, indirectly, greatly increased by the presence of fibrin. The Exosite II binds heparin and thrombomodulin (see figures 10 and 11).
Platelets

Platelets are important in the formation of a plug, in combination with the web-like fibrin, to reduce blood loss. At the time of vascular injury, platelets present in the blood in a quiescent state, adhere to the site of injury, activate and aggregate (see figure 11). This is initially mediated by exposed subendothelial collagen and von Willebrand factor (vWF), a large multimeric protein found in the plasma and subendothelial extracellular matrix. vWF is normally cleaved by the metalloprotease, ADAMTS13, preventing platelet binding and microangiopathic thrombosis. This situation, however, changes at times of shear stress and endothelial damage when the exposed, collagen-anchored vWF is able to act as a bridge between the platelet glycoprotein surface receptor, GPIb,
and collagen. Platelets are also capable of binding directly to collagen through GP1bα and GPVI receptors(37, 39). Thrombin is capable of binding to the Gp1bα receptor using exosite II. This interaction greatly influences the magnitude of platelet activation and also facilitates the activation of factor XI, which can also be directly activated by exosite I(38).

Activation and aggregation are amplified by thrombin interactions, through cleavage of G-protein coupled receptors, termed protease activated receptors (PAR)-1 and 4, and upregulation of the adhesion molecule P-selectin and PARs in platelets and endothelial cells. PAR-1 is the predominant thrombin receptor and requires only small amounts of thrombin to be activated while PAR-4 requires high concentrations(35, 40). The platelet receptor GpV is also activated by thrombin exosite II and Gp1bα interactions(41).

Platelets produce specific prostaglandins which are important in the formation of thromboxane A2, a potent vasoconstrictor of blood vessels. This allows vascular smooth muscle, local to the injury, to contract and reduce the lumen size of the blood vessel. This reduces blood flow to the area of damage. It is also an inducer of the platelet release reaction. Activated platelets secrete mediators from alpha granules and dense bodies (vWF, adenosine diphosphate (ADP), serotonin, P-selectin) that promote their own aggregation and activation(42).

ADP activates the most abundant platelet receptor, GPIIb/IIIa (an integrin receptor), which aids in the binding of vWF and fibrinogen by interacting with the extracellular matrix. As more platelets are recruited from the circulation and aggregate, a plug is formed over the injury anchored by the fibrin mesh, creating an occlusive platelet thrombus(42).
1.5 **Endogenous anticoagulation**

The coagulation process is tightly regulated within the body by physiological feedback mechanisms to target areas of injury and potential blood loss, and avoid uncontrolled global coagulation. The body’s natural anticoagulation processes are upregulated for this purpose and dissolve the localised thrombus once the injury has healed sufficiently. The four major endogenous anticoagulant processes involve (see figure 12):

1. Antithrombin (AT)
2. Thrombomodulin (TM) and activated protein C (APC) pathway
3. Fibrinolysis.
4. Tissue factor pathway inhibitor (TFPI)
1.5.1 Antithrombin

Antithrombin (AT) is a 58kDa glycoprotein synthesised in the liver. It is an important endogenous anticoagulant, belonging to the serpin family of protease inhibitors, and is a specific inhibitor of thrombin in the circulation along with another serpin termed heparin cofactor II (HCII). It circulates in the plasma at a concentration of approximately 125mg/L (2.3µm) with a half life of 65 hours. Most AT is thought to be concentrated on the vascular endothelium, where it is activated by glycosaminoglycans (GAGs) such as heparan sulfate produced by mast cells, chondroitin sulfate and the anticoagulant heparin(38, 43). GAGs interact with exosite II on the thrombin molecule and act as cofactors for interactions with thrombomodulin, AT and HCII.

AT exists in many isoforms and has four identified glycosylation sites. The Asn167 site is glycosylated in the major form of AT, termed AT alpha, but approximately 5-10% of AT exists as AT beta, which is not glycosylated at this site. AT beta has a higher affinity for heparin than AT alpha, which may be clinically relevant(43). Initially it had been thought that AT II (fast-acting) and III (slow-acting) were distinct molecules, but now it is understood that they are the same substance, termed antithrombin, which can exhibit variable activities depending on the presence/absence of exogenous heparin. Heparin increases AT activity by more than 1000-fold. The interaction appears to not
only involve exosite II but also the sodium binding site and part of the active site known as the γ loop on thrombin(38).

AT and thrombin combine to form a stable 1:1 complex that is removed by the reticuloendothelial system. AT inhibition of thrombin’s actions requires thrombin to bind to heparins of a high molecular weight (≥18 saccharide units). AT, when complexed with tissue factor, is also able to inhibit the actions of factors Xa, IXa, XIa, XIIa and VIIa (see figure 13)(43).

**Figure 13 The anticoagulant interactions of exosites I and II of the thrombin molecule**
1.5.2 Thrombomodulin

Thrombomodulin (TM), first described by Esmon & Owen in 1981, is an endothelial cofactor required for thrombin to activate protein C (PC)(44) (see figure 13). It is also referred to as CD141, fetomodulin and blood dendritic cell antigen 3 (BDCA3). TM is present on the endothelium of all blood vessels and its expression is particularly high in the capillary endothelial cells of the lung alveoli. It is also expressed in lymphatic endothelial cells, human placental syncytiotrophoblast cells, monocytes, neutrophils, chondrocytes, synovial lining cells, mesothelial cells, keratinocytes, astrocytes and dendritic cells(45).

The TM gene is found on chromosome 20p12 and can be transcriptionally upregulated by thrombin as well as downregulated by shear stress and other haemodynamic forces(46). TM has a profound effect on thrombin when they are bound together in a 1:1 complex, by changing its substrate from fibrinogen to the plasma glycoprotein, protein C. TM-bound thrombin, through exosite I and II interactions, renders thrombin incapable of cleaving procoagulant compounds. Instead thrombin recognises and aids efficient conversion of protein C to activated protein C (APC)(37, 38). APC inactivates Factors Va and VIIIa, and consequently is instrumental in the anticoagulation process by downregulating thrombin production. The activation of protein C is enhanced 20-fold when it is bound to the endothelial cell protein C receptor (EPCR). This receptor is similar in structure to the MHC class I/CD1 molecular family, which aids T-lymphocytes in protecting the body from bacterial invasion. EPCR also appears to act as a protein C/APC-dependent, inflammatory regulator when its soluble form is released from the endothelium by metalloproteinases(47).

TM-bound thrombin no longer cleaves fibrinogen or activates platelets but does activate thrombin activatable fibrinolysis inhibitor (TAFIa) at low plasma levels, inhibiting fibrinolysis early in the coagulation process. TAFI is a metallocarboxypeptidase glycoprotein, synthesised in the liver as a prepropeptide and generated during the coagulation process. Activation to TAFIa is achieved through cleavage of the amino acid Arg92 by thrombin, trypsin (a similar molecule to thrombin), plasmin and meizothrombin. During fibrinolysis, the cleavage of fibrin leads to the generation of new carboxy-terminal lysine residues which, in turn, increase plasmin production by positive feedback. TAFIa carries a zinc ion which is important for its function in hydrolysing basic amino acids and downregulating fibrinolysis by removing
these carboxy-terminal lysine and arginine residues from fibrin. This results in reduced plasmin production and a stable thrombus. It is thought that, at low plasma levels, thrombomodulin will prompt production of TAFIa, whereas at higher levels it decreases TAFI activation suggesting a dual role for this molecule in coagulation and anticoagulation through regulation of fibrinolysis(48).

Thrombin has a high affinity for TM. At sites “downstream” or “distal” to a site of endothelial damage and thrombus formation, this high affinity usually acts to restrict the thrombus from extending distant to the site of injury(37).

### 1.5.3 Fibrinolysis

The gel-like fibrin web is broken down by the process of fibrinolysis, initiated by feedback mechanisms. This helps to control the extent of thrombus production and later aids in its dissolution. Tissue plasminogen activator (tPA) is released from the endothelium and has specific affinity for fibrin, making it clot specific. Plasminogen also has specific affinity for fibrin and is converted to plasmin by tPA-catalysed cleavage at the arginine560-valine561 amino acid bond. Urokinase type plasminogen activator (uPA) also performs the same job in excretory ducts. The binding of plasminogen and tPA to fibrin results in a complex which upregulates plasmin formation because bound plasminogen is a better substrate for tPA than free plasminogen in the blood. This binding also makes fibrinolysis more efficient because plasmin bound to the fibrin is protected from rapid inactivation by alpha 2 antiplasmin.

The resulting plasmin catalyses the breakdown of fibrin to degradation products which circulate for a period of time in the blood. One such fibrin degradation product (FDP) is the D-dimer, which results from lysis of fibrin but with the maintenance of the cross-linked D domains(36, 49).

The fibrinolytic system is subsequently controlled by the release of plasminogen activator inhibitors (PAI) 1 and 2, which inactivate tPA and uPA, and alpha2 antiplasmin which is able to neutralise any free plasmin not bound to fibrin and thrombus.
1.5.4 Tissue Factor Pathway Inhibitor (TFPI)

TFPI is a lipoprotein-bound plasma protease inhibitor which controls the extrinsic pathway through inhibition of the VIIa:TF:FXa complex. Heparin can also induce TFPI release from endothelial cells and platelets (50).

1.6 Summary

The integrity of the body’s vascular system is carefully guarded by complex homeostatic mechanisms which respond quickly and efficiently to tissue injury and minimise morbidity and mortality from blood loss. The coagulation mechanisms are finely balanced with the physiological or endogenous anticoagulant mechanisms to control the extent of thrombus formation in the presence and absence of injury. Many of the components have multiple roles to play which are, as yet, not fully understood. Some of these roles are not just related to coagulation, and ongoing research continues to shed more light on how important these components are in many life-sustaining processes.
1.7 Introduction
The homeostatic processes which finely balance physiological coagulation and anticoagulation can be disrupted. This may be due to genetic and/or environmental factors. If that imbalance is in favour of coagulation (i.e. a procoagulant or hypercoagulable state) then thrombi may develop anywhere within the vascular system and potentially reduce the quality of life, performance status, functional ability and survival of a patient. The term venous thromboembolism (VTE) encompasses thrombi that pathologically develop anywhere in the venous system and the pulmonary arteries. VTE can be considered in three broad categories:

- Pulmonary embolism (PE) or pulmonary thromboembolism (PTE) - thrombi of the pulmonary arteries.
- Deep vein (or venous) thrombosis (DVT) - thrombi of the deep venous system.
- Superficial vein (or venous) thrombosis (SVT) - thrombi of the superficial venous system. These events have not always been considered clinically significant and treatment approaches are variable.

The incidence of VTE is approximately one per 1,000 population per year in Westernised countries. In New Zealand (total population of 4.47 million, http://www.dol.govt.nz/immigration), it has been estimated that approximately 4,000 people per year will experience VTE with more than 1,500 events associated with hospitalisation according to the National Policy framework for VTE prevention in New Zealand committee (http://www.hqsc.govt.nz/our-programmes/other-topics/publications-and-resources/publication/654/).

1.8 Pulmonary embolism
Pulmonary embolism (PE) is the third most common cause of death from cardiovascular disease worldwide, after myocardial infarction and stroke in adult patients. Published all-cause mortality rates range from 8.6-17% at three months. This high mortality rate may be due to the direct effect of a large central PE causing significant cardiorespiratory compromise and cardiac arrest. More commonly, however, PE may contribute to a patient’s functional decline and mortality in combination with
pre-existing comorbidities such as cardiovascular disease, respiratory disease and cancer or through complications such as chronic thromboembolic pulmonary hypertension and recurrent thrombosis (51) (see figures 14 and 15).

**Figure 14 Pulmonary artery anatomy**
The term pulmonary embolism was coined by Rudolf Virchow in the late 19th century (14). The name is not entirely accurate, as thrombus may develop de novo within the pulmonary vasculature as well as embolise from veins in distant parts of the body, prompting some clinicians to use the term pulmonary thromboembolism instead (56). Embolisation occurs when a thrombus (or part thereof), which has developed outside of the pulmonary arteries, breaks away from its point of origin and travels to the pulmonary arteries through the venous system and the right side of the heart. The size of the thrombus will determine the size of the vessel within which it becomes lodged, with larger thrombi halting in the larger calibre central and/or lobar pulmonary arteries and smaller thrombi becoming trapped in the smaller calibre, more peripheral segmental and subsegmental vessels. These thrombi can embolise from the valves, atrium or ventricle of the right side of the heart as well as any part of the venous system (see figure 16).
1.9 Deep vein thrombosis

Deep vein thrombosis (DVT) can be subclassified into four groups depending on where the thrombus forms:

- Lower extremity DVT- involving the lower limbs
- Upper extremity DVT- involving the veins of the upper limbs, neck and chest.
- Abdominopelvic DVT- involving the venous system of the abdomen and pelvis including the portal venous system, the inferior vena cava (IVC) and iliac vessels and their branches.
- Cerebral vein thrombosis including the venous sinuses.

Most research on DVT, to date, has focused on lower extremity DVT, but there is now increasing awareness of the rarer forms of DVT at other sites and the necessity for new research to improve our understanding and management of them.

1.10 Lower extremity deep vein thrombosis

DVT of the lower extremity is common with an incidence in the United States of America of 0.5-1/1000/year. Although not life threatening in itself, part of the thrombus
Venous thromboembolism in cancer patients undertaking chemotherapy

may embolise to the pulmonary vasculature leading to PE(57). Embolic risk is significantly higher when the thrombus is located in the lower limb vessels proximal to and in the popliteal fossae (popliteal veins), compared with distal subpopliteal vein thrombosis(58, 59) (see figure 17).

Figure 17 The major superficial vessels (the greater and short saphenous systems) the major proximal deep veins of the lower limb.

DVT can result in disability if pain and swelling occur due to venous obstruction. Left sided DVT is more common than right sided DVT of the lower limb which is most likely a result of anatomical differences(60). On the left side, the common iliac vein can travel between the right common iliac artery and the underlying vertebral body which may compress it, impairing venous flow and increasing the risk of DVT. This anatomical variant accounts for left sided DVT in May-Thurner Syndrome(61, 62).
1.11 Superficial venous thrombosis and thrombophlebitis of the lower extremity

Superficial vein thrombosis (SVT) of the lower extremity is a common disease found in 3-11% of the population, a prevalence double that of DVT and PE combined (63) (see figure 17). It is most commonly diagnosed in women with raised BMI and/or a history of varicose veins with a mean age at diagnosis of 60 years (63). It is most commonly found in the great saphenous vein and can occur bilaterally in approximately 5-10%. Three-month mortality is documented at less than one percent (64).

Historically, SVT was deemed of little concern with regards to complications affecting patient morbidity and mortality. We now know that DVT and PE complicate the diagnosis of superficial thrombosis in up to 36% of patients with 4-8% of cases being symptomatic. They may be present at the time of diagnosis of the superficial thrombosis or subsequently develop over a period of weeks to months secondary to thrombus propagation through the perforator veins and into the deep system (65).

The POST study (n=844) showed that 25% of patients with superficial lower limb thrombosis also had DVT and/or symptomatic PE, at diagnosis, with 9.7% diagnosed with proximal vein DVT and 3.9% diagnosed with symptomatic PE (64). Of the patients with isolated superficial thrombosis 2.8% developed DVT and 0.5% developed symptomatic PE by three months from study inclusion, despite treatment of more than 90% (of 597 evaluable) with at least one anticoagulant (low molecular weight heparin/vitamin K antagonist) in combination with graded compression stockings and more than 50% with topical or oral nonsteroidal anti-inflammatory drugs (66). A high rate of concurrent DVT was also noted in the OPTIMEV study (67).

Although most patients present with warmth, tenderness, erythema and swelling in the region of the affected vein, superficial thrombosis can be asymptomatic. It is sometimes possible to palpate the affected vein as a swollen, cord-like structure on examination. Varicose veins have a strong association with the development of superficial thrombosis, being present in 80-90% of cases, but the presence of malignancy, autoimmune disorders and thrombophilias are also risk factors (68).
1.12 Abdominopelvic thrombosis
Thrombosis can develop in the larger veins of the abdomen and pelvis (the inferior vena cava (IVC) and iliac vessels) leading to marked swelling distal to the obstructing thrombus. One area of increasing interest is splanchnic vein thrombosis which encompasses thrombosis of the portal venous system, hepatic veins (Budd-Chiari syndrome or BCS), mesenteric veins and splenic veins. Splanchnic vein thrombosis is rare and so only low level clinical evidence is available, using case series and observational studies, to guide clinical management. It can lead to significant organ impairment or failure with resultant morbidity and mortality. Thrombotic events may involve multiple sites in the splanchnic venous system (38.5%) or be isolated to one area (portal vein 39%, hepatic vein 8.5%, mesenteric vein 9.1% and splenic vein 7.4%)(69).

1.12.1 Budd-Chiari syndrome (BCS)
BCS, or hepatic vein thrombosis, occurs in approximately 0.5-1 per million people(70). Thrombosis causes occlusion of the hepatic venous outflow, IVC or both, leading to a spectrum of clinical manifestations from the asymptomatic patient to fulminant organ failure and death. Thrombus may occur with or without identified predisposing factors. Membranous webs (congenital or acquired as a complication of IVC thrombosis) within the hepatic vein can contribute to the development of BCS(71). Recurrent bacterial infections and filariasis are the most common causes of BCS in the developing world (south East Asia and Africa) where there is a slight male predominance, but in the Western world women make up over two-thirds of the diagnoses(72-74).

In the Western world, myeloproliferative neoplasms (MPNs) are associated with almost half of all cases(75-77). The Janus Kinase 2 (JAK2) V617F gene mutation, which induces a gain-of-function in the resulting tyrosine kinase enzyme is strongly associated with the development of MPNs. It is found in almost all patients with polycythaemia (rubra) vera and approximately 50% of patients with essential thrombocythaemia. The development of splanchnic vein thrombosis (predominantly BCS and extra hepatic portal vein obstruction) is so strongly associated with this mutation that some clinicians screen affected patients for JAK2 mutations associated with an underlying MPN. Of note, the mutation can be found in the endothelial cells of patients with BCS and
polycythaemia vera, which suggests endothelial dysfunction may contribute to the hypercoagulable state as well as activation of leukocytes and platelets (78-80).

Up to 20% of paroxysmal nocturnal haemoglobinuria (PNH) sufferers develop BCS with arterial and venous thromboses (often in unusual sites such as the splanchic veins or cerebral venous system) the leading cause of mortality (81). Thrombophilias (Factor V Leiden and prothrombin G201210 mutations) and the oral contraceptive pill appear to increase BCS risk in a synergistic fashion in women (82). Pregnancy and puerperium also appear to put women at increased risk (82). Solid tumour malignancies can very rarely cause secondary BCS if present within the lumen of the blood vessel by interrupting flow or extrinsically compressing the vessel and markedly narrowing its calibre (69).

1.12.2 Portal vein thrombosis (PVT)
A recent autopsy study reported a one percent prevalence of thrombosis of the portal veins, which is considerably higher than previous epidemiological data suggested (70). Cancer (secondary to tumour within the vessel or extrinsically compressing the vessel) and cirrhosis are the most commonly associated underlying diseases (approximately 30% each) with 20-30% idiopathic. Surgery, infection and inflammation within the abdomen contribute to up to 20% of cases. Inherited thrombophilic mutations in the Factor V Leiden and prothrombin G201210 genes, connective tissue disorders and cytomegalovirus infection have also been associated with PVT. Extrahepatic portal vein obstruction (not associated with solid tumours or chronic liver disease) is also associated with the JAK2 V617F mutation with one third of these patients having an underlying MPN (70, 80).

1.12.3 Mesenteric (MVT) and splenic (SpVT) vein thrombosis
MVT affects approximately 2.7/100,000/year and its presence is associated with a significant risk of bowel ischaemia and infarction. Cancer, abdominal infection and inflammation and abdominal surgery are associated with its development in most cases. MPNs are found in up to 16% of cases (70).

The incidence or prevalence of SpVT have not yet been documented but the most prominent associated risk factors are cancer, acute pancreatitis and splenectomy (70).
1.13 Cerebral sinus and venous thrombosis

Due to the rarity of cerebral sinus and venous thrombosis (CSVT) epidemiological knowledge is limited, but it affects mainly young adults and children with estimated incidences of 3-4/million/year and 7/million/year respectively(83). In approximately two-thirds of cases, more than one sinus is involved with the superior sagittal and transverse sinuses most frequently affected(83). Symptoms relate to anatomical position but headache occurs in over 90% of cases and papilloedema in 30% as a consequence of raised intracranial pressure. Focal and generalised seizures (40%), neurological deficits (e.g. abducens nerve palsy) and altered consciousness are also common(84). Dysarthria and aphasia are, however, uncommon. Infarction and haemorrhage can complicate the patient’s condition further with intracranial haemorrhage reported to occur in up to 40% of thrombotic events. Clinical symptoms, signs and prognosis are significantly worsened in the presence of associated haemorrhage with dependency and death three-fold that of patients without haemorrhage(85).

Symptoms usually develop subacutely over days to weeks, which may be the only factor that differentiates the clinical presentation from stroke. Two-thirds of adults affected are female. This female preponderance is attributed to the use of oral contraceptives (six-fold increase in risk) and the increased risk during pregnancy and the puerperium (first three weeks). Factor V Leiden and prothrombin G20210A mutations also increase risk and have a synergistic effect in combination with oral contraceptive use. Hormone replacement therapy has not been shown to impact on risk to date. Patients with MPNs may also present with cerebral sinus and vein thrombosis as well as splanchnic vein thrombosis with JAK2 V617F mutations again associated(83, 86).

Six month mortality rates range from eight percent in more recent studies to 50% in older studies with the improving outcomes likely related to better understanding of the condition and better diagnostic techniques. The earlier the diagnosis is made the better the outcome(87, 88).
1.14 Upper extremity venous thrombosis

The upper extremity venous system consists of the superficial veins (the cephalic and basilic veins) and deep veins (the radial, ulnar, brachial, axillary, subclavian, internal jugular and brachiocephalic veins) (89) (see figure 18).

Figure 18 Venous anatomy of the upper extremities.

Much less is known about upper extremity thrombosis than lower limb DVT (89-91). Thrombosis of the deep veins of the arm is uncommon and accounts for up to 11% of all DVT events diagnosed (90). The subclavian vein is most commonly affected, but thrombus is usually found in more than one vein (92). At least two-thirds are secondary to an identified predisposing factor, such as the presence of a central venous access device or cancer, and usually affect older patients. Primary thrombosis of the upper extremity, occurring without an identified predisposing factor, includes idiopathic and effort thrombosis (Paget-von Schroetter syndrome). Effort thrombosis usually occurs in young and otherwise healthy individuals with thoracic outlet syndrome (external compression of the neurovascular bundle at the thoracic outlet) being a major predisposing factor. The dominant arm is normally affected following strenuous or repetitive exercise (89, 90, 93).

1.15 Risk factors for VTE

A number of risk factors for developing venous thrombosis have been identified with most of the current evidence derived from lower limb DVT and PE epidemiological research (see Table 1).
Table 1 Published risk factors for pulmonary embolism and deep vein thrombosis

<table>
<thead>
<tr>
<th>Demographics</th>
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<tbody>
<tr>
<td>Increasing age (&gt;65 years) (94, 95)</td>
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<tr>
<td>Hospital inpatient/ nursing home residency (95)</td>
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<tr>
<td>Ethnicity(96)</td>
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<td>Chronic obstructive pulmonary disease (98)</td>
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<td>Thrombophilia (94)</td>
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<tr>
<td>Hypertension (95)</td>
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<tr>
<td>Acute medical illness e.g. pneumonia (94, 95)</td>
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<tr>
<td>Trauma (94, 95, 99)</td>
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<tr>
<td>Pregnancy (94)</td>
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<td>Major surgery (94, 95)</td>
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<td>Neurological disease (95)</td>
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<tr>
<td>Immobility (94)</td>
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<tr>
<td>Limb paresis/ paralysis (95)</td>
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<td>Spinal injury (95)</td>
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<td>Cancer (94, 95)</td>
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<td>Varicose veins (68)</td>
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<td>Autoimmune conditions (68, 94)</td>
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<td>Oral contraceptive and hormone replacement therapy use (94, 95)</td>
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<tr>
<td>Idiopathic (94, 95)</td>
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<tr>
<td>History/ Family history of VTE (94)</td>
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<td>Long-haul travel (95)</td>
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<tr>
<td>Air pollution (94, 95)</td>
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1.15.1 Demographics

1.15.1.1 Age
VTE development is more likely as an individual ages but it is challenging to conclude that age itself is a factor when it is also associated with deteriorating mobility and comorbidities, such as cancer, which may confound observational cohort findings (103).

1.15.1.2 Ethnicity
Africans and Europeans appear to have the highest risk of VTE of all ancestries. African-Americans have a five-fold increase in VTE incidence compared with Asian ancestry populations who are low risk. Hispanic populations are moderate risk. These variations are currently not fully explained by genetic and environmental risk factors. Little epidemiological research has taken place outside of North America and Europe to validate previous findings although emerging data from the Asian continent following orthopaedic surgery, suggest that VTE rates may not be as low as once thought. This suggests that clinical suspicion, surveillance and access to healthcare may be confounding factors (96). A retrospective study, carried out in a northern region of New Zealand, also found higher rates of VTE in European patients compared with Maori, Pacific Island and Asian patients (see table 2).

Table 2 VTE rates and ethnicity in a New Zealand population

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Number of patients</th>
<th>Age adjusted annual rate of diagnosis per 100000 population</th>
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</thead>
<tbody>
<tr>
<td>European</td>
<td>315425</td>
<td>101.7</td>
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<tr>
<td>Maori</td>
<td>42579</td>
<td>51.2</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>30405</td>
<td>31.6</td>
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<tr>
<td>Asian</td>
<td>66273</td>
<td>25.3</td>
</tr>
</tbody>
</table>

Adapted from Liao et al. JTH 2014(104)
1.16 Comorbidities

1.16.1.1 Medical interventions and Comorbidities

The Worcester venous thromboembolism study investigated risk factors associated with VTE development in their patient population. Immobility for >48 hours in the preceding month (45%), hospitalisation in the last three months (39%), surgery in the past three months (34%), infection in the past three months (34%), cancer in the past three months (34%) and current hospitalisation (26%) were the most commonly associated risk factors in the 587 patients diagnosed with VTE. Most patients described multiple risk factors with over half of the cohort describing more than three and only 11% not describing any. Other risk factors associated with admission to hospital with VTE within 90 days include fractures, erythropoietic stimulating agent use, reception of a blood transfusion and chemotherapy treatment.

Data from the Rochester Epidemiology Project were analysed by Heit et al. who identified trauma, institutionalisation with recent surgery, neurological disease with extremity paresis, central venous catheterisation, pacemaker insertion, cancer with and without chemotherapy treatment and patients younger than 70 years with varicose veins at increased risk for VTE. Controversially, above normal BMI, tobacco smoking, congestive heart failure and liver failure were not as associated with increased VTE risk, with liver failure associated with a 90% reduction in risk. This retrospective study was limited to what was documented in the medical records which may not have contained all the required information. In contrast, other studies, such as the large MEGA case-control study, have found heart failure and liver disease to be associated with increased VTE risk along with renal disease, rheumatoid arthritis, multiple sclerosis, haemorrhagic stroke and arterial thrombosis. This study also found that the risk increased further when the illness was combined with immobility and an inherited thrombophilia.

1.16.1.2 Renal disease

In all patients with chronic kidney disease thrombotic risk is higher than the general population with the estimated glomerular filtration rate and the urinary albumin-creatinine ratio associated with VTE risk. Arterial and venous thrombotic events can occur in up to 40% of patients diagnosed with nephrotic syndrome. For patients undergoing renal transplantation there is approximately a seven percent risk of
developing a VTE and, for those patients, a 50% recurrence rate at two years following withdrawal of oral anticoagulation (median treatment time 6.5 months). Higher levels of homocysteine, D-dimer and prothrombin fragments 1 and 2 were seen in patients who experienced VTE recurrence compared with transplant patients who did not develop VTE and patients with VTE but without renal disease(110).

1.16.1.3 Respiratory disease
PE may exacerbate the symptoms of chronic obstructive pulmonary disease (COPD). In a systematic review and meta-analysis, one in four inpatients with COPD were diagnosed with PE, although the studies examined were heterogeneous with regards to design and selection criteria(98).

1.16.1.4 Cardiovascular disease
Conflicting results have been published regarding cardiovascular disease and VTE risk. A Scandinavian study found a short term increase in VTE risk after stroke and myocardial infarction(111). A meta-analysis showed that obesity, hypertension, diabetes, smoking and hyperlipidaemia were also associated with increased risk of VTE(112). The Longitudinal Investigation of Thromboembolism Etiology, however, did not find any link between VTE and hypertension, hyperlipidaemia, physical inactivity, smoking and alcohol consumption but did associate obesity, diabetes mellitus and black ethnicity. Findings were also in agreement with the Tromsø study in which male sex and increasing age were associated(113). Only the Tromsø study found an association between family history of myocardial infarction and VTE development. Cardiac failure can be complicated by VTE and intracardiac thrombi, and is a prominent risk factor for death as an inpatient and within 30 days of a VTE event(113, 114).

1.16.1.5 Obesity
DVT and PE are both increased in obese patients (two-fold) with the impact greater in individuals younger than 40 years of age(112, 113, 115, 116). This risk may be exacerbated by smoking, immobility, the use of oral contraceptives and inherited thrombophilias(117).

1.16.1.6 Smoking
The contribution of smoking to VTE is controversial with many large studies reporting conflicting results. It is possible that smoking, in combination with other risk factors
such as age and use of oral contraceptives, may have a synergistic effect on VTE risk(112, 114, 118).

1.16.1.7 Surgery and trauma
Major vascular, orthopaedic, neurosurgical and cancer surgery appear to carry the greatest risk of venous thrombotic complications. Older age and anaesthesia were also shown to be associated with VTE(119, 120).

There are high prevalences of VTE following major trauma to the head, lower limb and pelvis(99). Even minor injuries requiring minimal medical intervention are associated with increased risk(121). Intravenous drug users are also prone to lower limb DVT if they regularly inject into their femoral veins(122).

1.16.1.8 Inherited thrombophilias
Approximately 60% of cases of VTE in patients with inherited thrombophilia are associated with Factor V Leiden and the prothrombin gene G20210A mutation. A family history of thrombosis or recurrent miscarriage may or may not be evident. The lifetime probability of developing thrombosis compared with those with no defect in a study of 150 pedigrees was 8.5 times higher in patients with protein S deficiency, 8.1 with antithrombin deficiency, 7.3 in protein C deficiency and 2.2 in Factor V Leiden deficiency(123). Two thrombotic defects may be detected together and are most commonly found in patients with Factor V Leiden mutations. Although this can further increase thrombosis risk markedly not all carriers of two defects are at higher risk(124, 125). Family history, even in the absence of an identifiable genetic abnormality, is an important risk factor for VTE development(126). This emphasises the understanding that the current thrombophilia test repertoire is incomplete(127).

1.16.2 Pregnancy, systemic contraceptives and hormone replacement therapy
Age-adjusted incidences of VTE are significantly higher in pregnant women than in non-pregnant women. They are a pathological complication of physical as well as physiological changes in the body. Oral contraceptive use increases VTE risk within four months of commencement. This risk returns to baseline approximately three months following discontinuation of the drug. The risk may also be associated with transdermal contraceptive patches(128, 129). Hormone replacement therapy doubles
the risk of VTE which is most marked in the first year of use(130). Oestrogen has been shown to lower protein C and S levels(131).

Antiphospholipid antibodies may be found in a primary disorder (antiphospholipid syndrome), associated with systemic lupus erythematosus (where it is part of the diagnostic criteria) or other rheumatological conditions. Patients can develop both venous and arterial thrombosis, thrombocytopenia and recurrent miscarriage(132).

1.16.3 Other risk factors
While venous stasis associated with bed rest or immobility is a well recognised risk factor for VTE, it is also an issue in people who sit for prolonged periods of time (e.g. thesis writing PhD students, computer workers) and those undertaking long distance travel where an approximate three-fold increase in risk is estimated(133).

Air pollution as a VTE risk factor is controversial, but shows no association in the only published prospective study(134, 135).

1.17 Treatment of VTE
Evidence for the management of VTE is predominantly obtained from studies of PE and lower limb DVT. International guidelines have been published by a number of medical organisations, including the American College of Chest Physicians (ACCP), to guide clinician decision making(20).

1.17.1 Upper and lower extremity superficial thrombosis/DVT and pulmonary embolism
Acute DVT causes partial or complete obstruction to venous outflow. This requires treatment to prevent propagation which allows the body’s own anticoagulation processes to dissolve the thrombus. Fifty percent of lower limbs will still demonstrate residual thrombus/fibrous scarring at three years from the index event, causing partial obstruction(136). The rate of recanalisation of a vessel is related to thrombus load and site with distal DVT more likely to undergo efficient and complete dissolution(137). The underlying physiological processes involved with recanalisation are not fully understood but they are likely to involve the fibrinolytic pathway(138).

Overlapping administration of low molecular weight heparin, unfractionated heparin or fondaparinux with vitamin K antagonists is the recommended treatment approach for
Venous thromboembolism in cancer patients undertaking chemotherapy

haemodynamically stable medical patients. These treatments are relatively labour intensive to administer because they are given parenterally and require skilled input or require regular monitoring with routine blood testing. The new ACCP guidelines give recommendations for the use of the new oral anticoagulants, rivaroxaban and dabigatran etexilate, but have shown preference for more established treatments until further research is completed(139).

Treatment of superficial vein thrombosis is not standardised and ranges from no treatment to nonsteroidal anti-inflammatory drugs and/or anticoagulants for variable lengths of time. The CALISTO study has shown that fondaparinux was effective in preventing extension of lower limb superficial vein thrombosis into the deep venous system and reducing resultant DVT and PE by 85%(66).

1.17.2 Thrombolysis

High risk PE is life-threatening and causes haemodynamic instability from pulmonary arterial obstruction. These situations require urgent restoration of pulmonary blood flow and reduction in pulmonary arterial pressure and right ventricular dysfunction. Unfractionated heparin followed by thrombolysis (streptokinase, urokinase or recombinant tissue plasminogen activator) is recommended to reduce mortality and recurrence(140). Surgical intervention is recommended if thrombolysis is unsuccessful or is contraindicated(20).

Thrombolytic agents have, historically, not been recommended in the setting of submassive PE without haemodynamic instability(140). Subsequent research has found significant reductions in post thrombotic pulmonary hypertension and recurrent thrombosis with its use at traditional therapeutic and lower doses(140-142). A large, double-blinded, placebo-controlled study involving 1006 patients, however, found significant increases in major extracranial bleeding (6.3% v 1.2%) and stroke (2.4% v 0.2%) when tenecteplase (versus placebo) was used in combination with low molecular weight heparin(143). Thrombolysis, therefore, should be used with caution in this patient group.

In patients with lower extremity DVT, thrombolysis or surgical intervention may be considered in situations of imminent limb loss due to venous occlusion. In a study of iliofemoral vein thrombosis, catheter-directed thrombolysis was superior to
anticoagulation alone in significantly increasing vein patency at six months and reducing post thrombotic syndrome at two years. As seen in the PE study, increased bleeding complications were seen and there were no improvements in recurrence rates or mortality compared with the standard arm(144). No thrombolysis studies have been published in patients with upper extremity DVT(59, 145).

1.17.3 DVT at other sites
Cerebral sinus and venous thrombosis is initially treated with low molecular weight or unfractionated heparin followed by conversion to an oral anticoagulant despite a meta-analysis finding no benefit from heparin compared with placebo(146, 147). These treatments should be used with caution when significant haemorrhagic transformation of the infarcted area is seen. The duration of anticoagulation depends on the clinical situation and perceived recurrence risk(83).

Splanchnic vein thromboses are managed in the same way as extremity DVT as there is little evidence to guide individualised treatment. Duration of treatment is usually three to six months unless there is a perceived high risk of recurrence when longterm treatment can be considered (e.g. JAK2 mutation or Paroxysmal Nocturnal Haemoglobinuria). Catheter-directed thrombolysis may be effective in acute or partially occlusive thrombosis in Budd-Chiari syndrome, extrahepatic vein thrombosis and mesenteric vein thrombosis. A transhepatic shunt in patients with BCS improves morbidity and mortality. Treatment, however, has to be balanced against the risk of catastrophic bleeding from varices(70, 148).

1.17.4 Vena cava filters
Filters are usually employed when there is contraindication to anticoagulation in patients with lower limb DVT or PE. Thrombi may develop at the level of the filter and so anticoagulation is recommended if the contraindication is reversible or transient. Filters have only been shown to improve survival in combination with anticoagulants in patients with DVT and PE who are haemodynamically unstable or post thrombolysis treatment but can increase the risk of recurrent DVT (149). Further research is required to clarify the role of vena cava filters in VTE management.
1.18 Complications of VTE

The occurrence of VTE can lead to many complications and ultimately death. For patients who survive the initial VTE event the following are major issues that require ongoing research to understand the pathophysiology, prevention and treatment.

1.18.1 Recurrent VTE

Up to 30% of patients with VTE will experience recurrence in the following 10 years. Eight percent of patients are affected by early recurrences within six months, often associated with cancer(150). Patients with idiopathic VTE or VTE associated with chronic, incurable conditions are at higher risk of recurrence than patients who develop their first VTE in association with reversible risk factors, such as pregnancy or surgery(151). Patients with antiphospholipid antibodies may be at high risk of recurrence with indefinite anticoagulation recommended but the strength of this evidence has been questioned in a recent systematic review(152).

Recurrences are also more likely to occur at a similar site as the initial VTE event with patients experiencing PE as their first thrombotic episode more likely to develop recurrent PE, especially if symptomatic, and those with lower limb DVT more likely to develop recurrent lower limb DVT although in the contralateral leg from the index event(153, 154).

1.18.2 Chronic thromboembolic pulmonary hypertension (CTEPH)

Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare complication of PE defined as a persistently raised mean pulmonary artery pressure, of at least 25mmHg at rest, following PE caused by pulmonary artery obstruction despite anticoagulation for at least three months(155, 156). More than half of all treated patients with PE have residual perfusion defects at six months but only a small fraction go on to develop CTEPH (0.1-4% at two years)(157). Approximately one in four patients with this complication have not been diagnosed with clinically overt PE and prognosis is poor(158).

Median survival is less than two years in those with mean pulmonary arterial pressures greater than 30mmHg at diagnosis, with patients experiencing fatigue, syncope, haemoptysis and right sided cardiac failure(159).
1.18.3 Post thrombotic syndrome

Post thrombotic syndrome (PTS), a complication of valvular reflux in veins damaged by thrombus, may affect up to 79% of patients who experience lower extremity DVT and may be more likely in patients with a BMI $\geq 30$ kg/m$^2$(138, 160). Severe PTS affects 5-10% of those with symptomatic DVT and can lead to swelling, discomfort, increased susceptibility to infection and venous ulceration (4-6%)(59, 138, 161). Patients who experience PTS report a poorer quality of life compared to patients with chronic lung disease, arthritis and diabetes mellitus and those with severe PTS have a quality of life comparable to patients with congestive cardiac failure or cancer(162, 163).

The risk of PTS increases with the length of time it takes to recanalise (if this occurs at all) the affected vein (s) and if the patient experiences recurrent thrombotic events. Anticoagulation alone may not be effective enough to prevent this complication and it may require thrombolysis or more acute invasive approaches to remove the thrombus(59). Recurrent DVT in the same limb increases the risk of PTS by up to six-fold(164). There is evidence suggesting that the wearing of knee-length graded compression stockings, immediately after DVT diagnosis and for a year afterwards, may reduce the PTS risk by 50% at five years although this is not widely practiced(164).

Due to a lack of clear diagnostic criteria and risk factors, PTS patients are often diagnosed at a late stage when irreversible damage is done. Prevention of primary and recurrent DVT in at risk populations and treating DVT with the aim of rapid clot dissolution may minimise chronic venous damage.

1.19 Quality of life (QoL)

Research on the impact of PE on the physical, mental and social functioning of Dutch patients has recently been published using the standardised Short Form 36 (SF-36) and disease–specific Pulmonary Embolism Quality of Life (Pemb-QoL) instruments which assessed general well being over the preceding 30 days, and compared with the use of the SF-36 in patients with chronic obstructive pulmonary disease (COPD), congestive cardiac failure (CCF) and post acute myocardial infarction(165-167). Dutch patients with a history of PE experienced reduced quality of life compared with population norms. The time between study recruitment and PE was inversely related to QoL with determinants for poor QoL being prior PE, age, obesity, cancer and cardiopulmonary
comorbidities(168). Van Es et al. subsequently found that PE correlated with reduced social functioning, physical and emotional role functioning, vitality and general health when compared with the Dutch population norms, similar to patients who suffered an acute myocardial infarction. The PE cohort, however, exhibited significantly better quality of life than those with COPD and congestive cardiac failure(167).

1.20 Summary
Venous thrombosis can develop anywhere in the body and result in significant morbidity and mortality for those affected. Its prevention, prompt diagnosis and timely treatment after development are vital to minimise this. Despite extensive research our understanding of VTE at anatomical sites other than the pulmonary arteries and deep veins of the lower limbs is limited as are many of the pathophysiological mechanisms that lead to VTE development. Further research is required which will need to focus on specific subgroups of medical and surgical patients who are more likely to develop thrombotic complications. The remainder of this thesis will focus on how current evidence and knowledge of VTE specifically relates to patients with cancer.
1.21 Introduction
Venous thromboembolism (VTE) is a considerable problem in cancer patients.

- Cancer patients have at least a four-fold increase in risk of developing VTE compared with peers without cancer (95, 169).
- The thrombotic event may even precede the diagnosis of cancer by months to years and, in others, may be diagnosed on routine imaging or at post mortem when thrombosis was not clinically suspected (170-173).
- Up to twenty percent of all venous thromboembolic events are diagnosed in cancer patients (174, 175).
- Fifteen to twenty percent of all cancer patients will develop VTE before death (176, 177).
- Cancer patients are twice as likely to experience major bleeding complications on therapeutic anticoagulant therapy (23).
- Cancer patients are three times as likely to develop recurrent thrombosis compared with peers without cancer (23).
- Venous thrombosis is ranked as the second leading cause of mortality worldwide in cancer inpatients and is a leading cause of morbidity and mortality in cancer outpatients (13, 178-180).

1.22 VTE and cancer primary site
VTE risk varies with primary cancer site as well as tissue type further emphasising the importance of understanding tumour biology characteristics. State of California law, in the United States of America, requires all cancer patients to be identified and clinical information logged and reported. This California Cancer Registry (CCR) has provided important information with regards to VTE events in cancer patients. Wun et al. linked the registry to patient hospital discharge data and analysed all cases of cancer and VTE diagnosed between 1993 and 1999. Outpatient management of VTE with low molecular weight heparin was seldom used during this time period and so it was possible to
minimise case ascertainment bias and allow the investigators to link virtually all cases of cancer associated thrombosis to the relevant patient’s cancer stage and clinical outcome(181).

High grade gliomas, such as glioblastoma multiforme, and upper gastrointestinal cancers, such as gastro-oesophageal junction, gastric and pancreatic cancers had a high risk for VTE occurrence(181). Haematological malignancies, such as acute myeloid leukaemia were also ranked as high risk whereas lower incidences of VTE occurred in cancers of the colon, breast, prostate(182). Similar findings have also been reported in other studies including a large Dutch database(169, 183, 184) (see table 3).

**Table 3 Venous thromboembolism associated with specific types of cancer**

<table>
<thead>
<tr>
<th>Cancers associated with high rates of VTE(169, 181, 185, 186)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High grade gliomas</td>
</tr>
<tr>
<td>Gastric and gastroesophageal</td>
</tr>
<tr>
<td>Ovary and Uterus</td>
</tr>
</tbody>
</table>

Although important, analysis of epidemiological data from large registries is limited to what is reported and documented. Most databases will not have collected detailed enough information to correlate findings with anticancer treatments in order to compare the impacts of cancer biology alone versus cancer biology and anticancer treatment.

### 1.23 Tumour histology and VTE

Adenocarcinoma, especially mucinous adenocarcinoma histology, appears to correlate with thrombus development in cohorts of patients with colorectal but not breast cancer (187, 188). In patients with lung cancer, adenocarcinoma histology was significantly associated with symptomatic PE but not DVT(189). Ovarian, gastrointestinal and prostate adenocarcinomas have also been associated high incidences of VTE with patients diagnosed with pancreas adenocarcinoma faring worst of all(169, 183). A recently published retrospective analysis of 641 patients with epithelial ovarian, fallopian and primary peritoneal cancers, however, did not associate mucinous tumours with VTE but found that serous, clear cell and high grade undifferentiated histologies were associated with the highest VTE risks. Transitional cell histology was also associated with VTE but patient numbers in this subgroup were small(190). In breast
cancer, undifferentiated carcinoma histology, is associated with increased two-year mortality in those with VTE(188).

### 1.24 Cancer stage

Studies, to date, have provided conflicting opinions on the association between cancer stage and VTE risk. Patients with advanced cancer (metastatic or stage IV cancer) were at higher risk for VTE than their peers with localised disease according to a large Dutch database(169). Their risk was also higher when hospitalised or undergoing surgery(191, 192). Metastatic disease was also found to be a strong risk factor for VTE in retrospective analyses of colorectal, breast and ovarian cancer patients(187, 190, 193). Those patients developing VTE within one year of cancer diagnosis were more likely to have metastatic disease which was an independent risk factor for VTE development in that first year. A positive correlation between cancers with high one-year mortality rates (e.g. pancreas, lung) and VTE incidence has also been reported suggesting biological aggressiveness (e.g. tumour cell doubling time or rate of metastatic spread) rather than cancer stage may influence VTE risk(169, 182, 187).

Two studies (one retrospective and one prospective) investigating ovarian cancer patients have, in contrast, not found an association between VTE and advanced stage of disease(194, 195). No association between advanced stage and VTE was also found in a prospective study of ambulant patients undergoing chemotherapy treatment(196). Most patients were of good performance status (ECOG 0-1) and receiving outpatient treatment unless they became unwell suggesting that mobility, performance status and surgical intervention may confound the effect of cancer stage on VTE risk.

### 1.25 VTE and survival of cancer patients

Sorensen et al. first described the negative impact of VTE occurrence on survival in cancer patients. The one year survival reduced from 36% in cancer patients without VTE to 12% in those that had developed VTE(177). This study did not stratify for cancer stage but a retrospective analysis of the California Cancer Registry did and concluded that VTE was indeed an independent predictor of poorer survival in cancer patients(181, 193). Development of VTE was also an independent risk factor for poorer survival in the first few months of chemotherapy treatment regardless of cancer type, cancer stage, age, gender, ethnicity, performance status, body mass index (BMI),
Venous thromboembolism in cancer patients undergoing chemotherapy and comorbid conditions(197). This has been shown in a cohort of irresectable locally advanced and metastatic pancreatic cancer patients(198).

Levitan et al. retrospectively reviewed the Medicare Provider Analysis and Review Record (MEDPAR) database in the US involving over eight million patients hospitalised between 1988 and 1990. The probability of death within 183 days of hospitalisation was three-fold higher for cancer patients with VTE compared with non-cancer patients with VTE and two-fold higher than cancer patients without VTE(186).

The prospective, ongoing RIETE registry assessed the 30-day outcome, post acute VTE diagnosis, in all women with cancer(199). Of the 2474 enrolled women with cancer and acute VTE:

- 329 (13%) died (71 (2.9%) from PE and 22 (0.9%) from bleeding) at 30 days from diagnosis.
- PE (2.9%) was the second most common cause of death behind progressive cancer (4.3%) at 30 days post diagnosis.
- Pancreas, stomach, lung cancers and carcinoma of unknown origin had higher rates of fatal PE than other primaries at 30 days post diagnosis.
- Death from PE heavily outweighed fatal bleeding on anticoagulation in women with breast and lung cancers(199).

The MASTER Oncology multicentre study prospectively assessed risk factors for VTE and compared survival between adult cancer patients with and without VTE(200). Clinical data was collected for:

- Patients with diagnosed suspected and unsuspected VTE (cases) within two months of consent to the study.
- Patients without VTE (controls) who were enrolled in the same cancer centre as the cases. These patients did not undergo radiological imaging to exclude VTE.

Participants (237 cases and 339 controls) were seen at baseline (consent and inclusion) and followed up over a 10-month period or until censored. Patient characteristics were well balanced in both cohorts and VTE findings are summarised in table 4.
Table 4 Breakdown of VTE cases in the Master Oncology study

<table>
<thead>
<tr>
<th>VTE (N=237)</th>
<th>Symptomatic (%)</th>
<th>Asymptomatic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVT</td>
<td>132 (76.3)</td>
<td>41 (23.7)</td>
</tr>
<tr>
<td>PE</td>
<td>26 (32.9)</td>
<td>53 (67.1)</td>
</tr>
</tbody>
</table>

All cases were treated with antithrombotic medication and nearly 90% received low molecular weight heparin (LMWH). Survival analysis, after an average follow up of 8.3 months involved 136 (57.4%) cases and 127 (37.5%) controls who had died, as shown in table 5.

Table 5 Median survival in the Master Oncology study

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Median survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=127)</td>
<td>14.3</td>
</tr>
<tr>
<td>VTE Cases (n=136)</td>
<td>8.7**</td>
</tr>
<tr>
<td>Symptomatic VTE</td>
<td>7.6**</td>
</tr>
<tr>
<td>Asymptomatic VTE</td>
<td>11.4*</td>
</tr>
</tbody>
</table>

* p (compared with controls) <0.05; **p<0.01

On multivariate analysis the hazard ratio (HR) for death was 1.55 for patients with VTE compared with patients without VTE.

Patients treated by the Regional Oncology Centre in Auckland, New Zealand are referred to a Thrombosis Unit if newly diagnosed with VTE. Prestidge et al. (2010) retrospectively reviewed cancer patients with first episode VTE between 1997 and 2006. All patients, without contraindications, were treated with extended LMWH for at least 18 months (201). Only five of the 559 patients investigated had unsuspected VTE. The overall one year survival was 53%. Table 6 summarises the survival data.
Table 6 Auckland survival data stratified by VTE site

<table>
<thead>
<tr>
<th>VTE (n=559)</th>
<th>Median survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>13.5</td>
</tr>
<tr>
<td>Lower limb DVT (54%)</td>
<td>16.0</td>
</tr>
<tr>
<td>PE (23%)</td>
<td>12.3</td>
</tr>
<tr>
<td>Iliocaval/abdominal DVT (15%)</td>
<td>10.1</td>
</tr>
<tr>
<td>Upper limb DVT (8%)</td>
<td>31.1</td>
</tr>
</tbody>
</table>

The differences in median overall survival from VTE diagnosis were not statistically significant between the VTE subgroups but study numbers were too small to adequately power subgroup analyses. Almost all deaths were due to progressive cancer although few post mortem examinations of cancer patients were done(201).

1.26 Clinical presentation of VTE

Symptoms and signs of DVT and PE can be non-specific or absent and clinician suspicion can be clouded by the presence of medical comorbidities, especially in elderly patients(202). Clinical presentation ranges from those who are asymptomatic or clinically unsuspected to those who are critically ill with shock and haemodynamic instability, requiring urgent and potentially life saving treatment. Pulmonary embolism can be a cause of cardiac arrest and death in patients whom have significant clot burden and obstruction of their pulmonary arteries resulting in complete obstruction to blood flow or raised pulmonary and right heart pressures leading to right heart strain with dilated pulmonary vessels and right heart chambers proximal to the thrombus. These sequelae are more likely in patients with large, obstructive central thrombi (e.g. a saddle embolus affecting the main pulmonary artery and straddling the proximal aspects of the left and right pulmonary artery branches) and/or those with poor performance status due to chronic medical comorbidities and little pulmonary and/or cardiac reserve which is complicated further by the development of PE.
1.26.1 Symptoms and signs of pulmonary embolism

In patients presenting with clinical concern for PE, no single symptom or sign is diagnostic, although sudden onset dyspnoea (breathlessness) is associated with its presence(203). The full triad of chest pain, dyspnoea and haemoptysis, commonly described in medical textbooks, is identified in only a minority of patients. In the PIOPED (Prospective Investigation Of PE Diagnosis) study, chest pain (66%), dyspnoea (73%) and cough (37%) were the most frequently described symptoms and tachypnoea (70%) and tachycardia (51%) were the most commonly documented signs but their prevalences did not significantly differ between patients eventually diagnosed with PE compared with those who were not(204, 205).

The PIOPED and PISA-PED studies have suggested that the respiratory physician’s clinical acumen does have predictive value in diagnosing PE(204, 206). Clinical judgement changes with the experience/seniority of the assessing clinician when applying unstructured clinical probability estimation to routine data collected at the bedside. Junior doctors are more likely to agree with their peers as to the probability of PE being present, by this approach, than their senior colleagues but it is inconsistent and leads to more conservative practice(207). A recent meta-analysis, of over 25,000 patients in 19 studies, found that clinical impression alone had a sensitivity of 85% and specificity of only 51% when making the diagnosis of acute PE. This has confirmed the need for additional tools such as clinical decision rules in the diagnosis of acute venous thromboembolic events(21).

1.26.1.1 Electrocardiography

The electrocardiogram (ECG) cannot be used alone to diagnose PE. Acute PE can be associated with findings, such as atrial arrhythmias, right bundle branch block and ST-segment changes, but interpretation is frequently clouded in patients with pre-existing cardiovascular disease where these changes may be chronic(208). Tracings consistent with the S1Q3T3 pattern and right ventricular strain are uncommon unless massive PE and cor pulmonale are present(209). ECG assessment may be useful in assessing the extent of vascular occlusion as was shown in one retrospective study performed in Christchurch, New Zealand(210).
1.26.2 Clinical presentation of lower extremity deep and superficial venous thrombosis

Patients with lower extremity DVT may also be asymptomatic but most who present to hospital with acute DVT experience calf tenderness and lower limb swelling. The leg may appear erythematous or cyanotic with prominent superficial veins evident on examination of the skin. Iliofemoral DVT which cause complete obstruction to outflow can present with blanching of the skin (phlegmasia alba dolens) and pitting oedema. A number of non-specific clinical assessments for DVT have been described in the literature but are of limited value in the clinic to help differentiate thrombosis from other lower limb pathologies. They are(211):

- Michaeli’s sign (fever without other cause) in association with Mahler’s sign (increase in heart rate).
- Pratt’s sign- tenderness of veins in the popliteal region.
- Sigg’s sign- Pain elicited in the popliteal region on knee extension.
- Homan’s sign- calf pain is elicited by passive dorsiflexion of the foot on the affected side.
- Loewenberg test- quantification Homan’s test by using a pneumatic pressure cuff and measuring the pressure that elicits pain.

Although most patients with superficial vein thrombosis present with warmth, tenderness, erythema and swelling in the region of the affected vein, the event can be asymptomatic. It is sometimes possible to palpate the affected vein as a swollen, cord-like structure on examination. Varicose veins have a strong association with the development of superficial thrombosis being present in 80-90% of cases but the presence of malignancy, autoimmune disorders and thrombophilias are also risk factors(68).

1.26.3 Laboratory tests and PE diagnosis

Routine laboratory tests such as arterial blood gas measurement and elevated brain natriuretic peptide (BNP) levels are also non-specific in the diagnosis of acute PE(205, 212, 213). BNP and/or its precursor NT-proBNP may, however, have a prognostic role to play in patients with PE as elevated levels appear to correlate with subsequent
complications and length of hospital stay. Serum troponins I and T, markers of myocardial damage, are elevated in up to half of patients presenting with moderate to large PE, likely related to right heart strain and are also associated with poorer prognosis but are not useful in the diagnostic process (214, 215).

1.26.4 Clinical decision rules in PE and lower extremity DVT and D-dimer assays

Over the last 15 years a number of clinical decision tools have been published to determine the pre-test probability of a patient’s hospital presentation being related to VTE (21). The Wells criteria (one devised for DVT and a second for PE) and the Geneva score (for PE) are the most validated and commonly employed rules in clinical practice today (216-218) (see table 7).

Table 7 Wells criteria for DVT and PE and revised Geneva criteria for clinical probability of PE in symptomatic patients

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Points</th>
<th>Risk score guide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wells score for DVT</strong>(216)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>+1</td>
<td>0 low</td>
</tr>
<tr>
<td>Paralysis/recent plaster cast</td>
<td>+1</td>
<td>1-2 intermediate</td>
</tr>
<tr>
<td>Bed rest&gt;3days or surgery&lt;4 weeks</td>
<td>+1</td>
<td>≥3 high</td>
</tr>
<tr>
<td>Pain on palpation of deep veins</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>Swelling of entire leg</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>Diameter difference on affected calf &gt;3cm</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>Pitting oedema on affected side only</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>Dilated veins affected side only</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>Alternative diagnosis at least as probable as DVT</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td><strong>Wells score for PE</strong>(217)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous PE/DVT</td>
<td>+1.5</td>
<td>0-1 low</td>
</tr>
<tr>
<td>Heart rate &gt;100 beats per minute</td>
<td>+1.5</td>
<td>2-6 intermediate</td>
</tr>
<tr>
<td>Recent surgery or immobilisation</td>
<td>+1.5</td>
<td>&gt;7 high</td>
</tr>
<tr>
<td>Clinical signs of DVT</td>
<td>+3</td>
<td>or</td>
</tr>
<tr>
<td>Alternative diagnosis less likely than PE</td>
<td>+3</td>
<td></td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>+3</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>+1</td>
<td>&lt;4 PE unlikely</td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>≥4 PE likely</td>
</tr>
</tbody>
</table>
Geneva score for PE (218)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Score</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; 65 years</td>
<td>+1</td>
<td>0-1 low</td>
</tr>
<tr>
<td>Previous PE/DVT</td>
<td>+3</td>
<td>2-6 intermediate</td>
</tr>
<tr>
<td>Surgery (under general anaesthetic) or fracture of lower limb &lt; 1 month</td>
<td>+2</td>
<td>≥6 high</td>
</tr>
<tr>
<td>Malignancy (solid or haematological, active or in remission within the last 1 year)</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>Unilateral leg pain</td>
<td>+3</td>
<td></td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 75-94 beats per minute</td>
<td>+3</td>
<td></td>
</tr>
<tr>
<td>- ≥95 beats per minute</td>
<td>+5</td>
<td></td>
</tr>
<tr>
<td>Pain on deep vein palpation in leg and unilateral oedema</td>
<td>+4</td>
<td></td>
</tr>
</tbody>
</table>

Symptomatic adult medical patients who present with clinical concern for VTE can be classified by these rules as low, intermediate or high probability for having a DVT or PE. Both PE scores have been further simplified with cut-offs designating PE likely or unlikely.

The presence of elevated fibrin degradation products (FDPs), as a result of fibrinolysis, in the blood of patients with PE was first documented in 1971 (219). D-dimer is one such FDP which maintains the D domain cross linking found in intact fibrin and can be routinely detected in the serum of blood samples obtained at the bedside on clinical presentation. A number of assays are available which have laboratory turnaround times ranging from minutes to hours depending on the technique used. The assays can be quantitative (providing a numeric, continuous variable result) or qualitative (providing a binary, positive or negative result). Research undertaken in medical patients has shown that D-dimer assays have a high sensitivity and negative predictive value but low specificity and positive predictive value making them useful tools in ruling out the presence of PE/ lower limb DVT but not for making the diagnosis in combination with clinical decision rules (220).

The interpretation of probability depends on the clinical decision rule and D-dimer assay combination employed. Using the recommended quantitative rapid or semi-quantitative latex agglutination D-dimer assays (approximate turnaround time of 30-40 and 15 minutes respectively) and the Wells criteria for PE, patients who present with a
D-dimer result within the laboratory-stipulated normal range and are classified low probability for PE do not require further investigation as they have a low likelihood of having or developing PE over the following three months (0.5-3%)(221, 222). Those patients that are classified as intermediate or high risk and/or have a D-dimer result above the normal range require further investigation(217). This approach has efficiently managed medical patients, minimised unnecessary investigations and reduced health provision costs with approximately 30% of the suspected PE population having been safely withheld from further investigation(213).

For clinically suspected lower limb DVT, patients with a D-dimer ELISA within the laboratory-stipulated normal range or a negative SimpliRED assay and low probability of DVT on Wells score the likelihood of having or developing DVT over the next three months is up to three percent. Moderate and high pretest probabilities can predict for DVT in 17% and 75% of patients respectively(216). The efficacy of the Wells criteria was confirmed in a review of 15 studies which showed that patients in the low pretest probability category had a median NPV of 96% which was improved to 99% with a negative/normal D-dimer result indicating how useful this approach is in ruling out DVT without the need for further investigations. The positive predictive value in patients with high probability reached 75% meaning that the criteria alone could not confirm the diagnosis of DVT and further investigations are warranted in this situation(223).

1.27 The diagnosis of VTE
Patients assessed as intermediate or high probability and/or with a raised D-dimer result, require further investigation to look for PE and/or DVT. Venography and pulmonary angiography remain the most definitive procedures for diagnosing VTE but are invasive and have largely been replaced by more accessible and efficient, non-invasive radiological imaging techniques(224).

1.27.1 Pulmonary embolism
Chest radiography will neither safely rule in nor rule out the diagnosis of PE as abnormalities, such as parenchymal changes and pleural effusions, seen in patients with PE are also commonly seen in patients without PE(205). Westermark’s sign (prominent central pulmonary artery appearance with decreased peripheral pulmonary markings)
and Hampton’s hump (peripheral wedge-shaped opacity indicating pulmonary infarction) are non-specific signs associated with PE on chest radiograph(56).

The ventilation/perfusion scintigraphy (V/Q) scan was, for many years, the non-invasive test of choice for the diagnosis of PE. A normal scan excluded PE and a high probability scan demonstrated high specificity for PE diagnosis. This was shown in the prospective investigation of pulmonary embolism diagnosis (PIOPED) trial which compared V/Q scanning with traditional invasive pulmonary angiography. In this trial, however, only 14% of patients had a normal V/Q scan and 13% had a high probability scan leaving 73% of the study cohort with low to intermediate probability results. Only 41% of the patients with PE, found on pulmonary angiography, had a high probability VQ scan with the remainder found to have low to intermediate probability scans(204).

The diagnostic uncertainty of V/Q scan results in these “middle ground” situations led researchers to look at the merits of computed tomography angiography in improving the diagnostic accuracy of PE. Two years later the first prospective study comparing single-detector computed tomography scanning with pulmonary angiography was performed on 42 patients and suggested that CT scanning (100% sensitivity and 96% specificity) may be useful in the diagnosis of PE(225). Subsequent studies have published sensitivities between 53-100% and specificities between 78-100% and a direct comparison study also showed that single detector CT pulmonary angiography was more accurate than VQ scanning with a higher sensitivity, specificity and negative predictive value and equivalent positive predictive value(226, 227). VQ single photon emission computed tomography (VQ SPECT) is, however, being evaluated in patients unable to undergo contrast enhanced CT scanning(228, 229).

The PIOPED II study, published in 2006, was designed to assess the ability of multidetector (4-, 8- or 16-row) CT angiograms to diagnose and rule out PE, whether CT venography improved the ability to rule out PE and whether the addition of the Wells clinical decision rule improved a clinician’s ability to diagnose or rule out PE(230). A sensitivity of 83-90% (+/- CT venography) and specificity of 96% were calculated from the results. When incorporated with the Wells criteria, PPVs for PE in patients with positive and negative CT angiograms were as listed in Table 8.
Table 8 Positive and negative predictive values for CT angiograms performed in the PIOPED II study

<table>
<thead>
<tr>
<th>Probability of PE</th>
<th>Positive predictive value +ve CTA (%)</th>
<th>Negative predictive value -ve CTA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>96</td>
<td>60</td>
</tr>
<tr>
<td>Intermediate</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>Low</td>
<td>58</td>
<td>96</td>
</tr>
</tbody>
</table>

The computed tomography pulmonary angiogram (CTPA) has now superceded V/Q lung scintigraphy as the preferred imaging modality for PE diagnosis in medical and surgical patients but is only useful with the appropriate level of radiological expertise. It enables the radiologist to visualise the pulmonary anatomy and thrombus with PE diagnosed by the finding of an intraluminal filling defect consistent with thrombus in the pulmonary arterial tree(213, 228).

CT scans may show alternative or additional intrathoracic pathologies responsible for the patient’s presentation, such as fibrosis, tumour, and infection(227, 231, 232). Sohns et al. (2008) retrospectively analysed 200 adult medical patients who underwent contrast-enhanced 64-slice MDCT of the chest, pelvis and thigh over 14 months for suspected PE and found that 60 (30%) had PE. One hundred and twenty incidental findings were, however, found in the chest, abdomen and pelvis including previously undiagnosed malignancies, pneumonia, pulmonary nodules, aortic aneurysms and pleural and pericardial effusions(233).

1.27.2 Deep vein thrombosis

Compression ultrasonography is a direct, non-invasive approach for the diagnosis of DVT. Prospective studies have shown that the lack of compressibility of a vein with the ultrasound probe as an indication of the presence of DVT in the proximal lower limb carries a sensitivity and specificity of >95%(234). This approach, however, can be challenging in obese patients due to poor visualisation of veins and difficulties in being able to adequately compress the deep veins. It is also complicated in assessment of the upper limb by limited ability to compress the brachiocephalic and subclavian veins.
underneath the sternum, ribs and clavicles although sensitivity and specificity >95% have been reported (235).

The costs of tests for investigating VTE in the Christchurch public medical system (as of June 2014) are:

- CTPA- $NZ 350.00. Same day report.
- V/Q scan- $NZ 800.00 (with SPECT). Same day report.
- D-dimer- $NZ 22.43. Result within 60 minutes but must analyse within four hours.
- Compression ultrasound scan of lower limbs- $NZ 95.66 (unilateral) or $NZ 118.00 (bilateral). Same day report.
- Compression ultrasound scan of upper limbs- $NZ 146.00 (unilateral) or $NZ 290.00 (bilateral). Same day report.

1.28 Clinical decision rules and the cancer patient

Although these tools (table 7) have been widely validated in general medical patients this is not the case in cancer patients. In fact, the Wells criteria and D-dimer assay have been shown to be of limited utility in cancer patients because:

- the presence of malignancy is included in the score.
- more than 80% of patients present with a D-dimer above the normal laboratory-stipulated range due to the impact of their cancer and/or treatments.
- many patients present with symptoms and signs that prompt radiological investigation (22).

In a subgroup analysis of the CHRISTOPHER study involving 474 cancer patients, who presented to hospital with clinically suspected PE, only 49 (10.3%) of the patients had both a normal D-dimer result and a low probability score on Wells criteria ruling out the requirement for further investigations. Using this approach 10 patients needed to be tested to rule out one PE. Four hundred and seventeen spiral CT scans of the chest were subsequently performed on these cancer patients, of which 286 (68.5%) were PE
negative and 130 (31.5%) were PE positive. Using the CT approach three patients needed to be tested to find one PE(213).

As was found in the Carrier et al. analysis, the combination of a normal D-dimer assay and a low probability score using a validated clinical decision tool could reliably rule out VTE in a symptomatic cancer population(22, 213). Unfortunately this comprised only a small proportion of the patients and so further investigation with CT scanning was required in approximately 89% of participants. The utility of the probability scoring and D-dimer assay, using current guidelines, appears to be limited in cancer patients and prevents only a small number of patients from being investigated further. It is also of importance to note that in cancer patients, other pathology may be affecting the lungs and would potentially be identified on a CT scan providing a diagnosis for presenting symptoms. In the CHRISTOPHER study 42% of cancer patients were noted to have alternate diagnoses, including consolidation and pleural effusions(213).

1.29 The cancer patient and upper extremity venous thrombosis

The presence of cancer increases a patient’s risk of upper extremity thrombosis by up to 18-fold compared to peers without cancer and is a more common finding in patients with distant metastases than in patients with localised disease. Cancer patients with an underlying prothrombotic mutation are at an increased risk of thrombosis which is increased 6-fold with the insertion of a central venous catheter (CVC)(236, 237).

Thrombosis risk in cancer patients is compounded by the increasing use of central venous access devices (CVADs) in oncology clinical practice which have significantly improved the experience and management of cancer patients by facilitating the administration of intravenous chemotherapy and supportive therapies(90, 237, 238). In a recent meta-analysis Chopra et al. reported a 6.67% frequency of peripherally inserted central catheter (PICC)- related DVT in 18 studies on cancer patients (n=3430). Only one of the studies explicitly stated that screening for unsuspected DVT took place and one study reported use of thromboprophylaxis(239).

The prospective RIETE registry is an international initiative designed to record clinical data relating to clinically suspected/symptomatic acute DVT or PE confirmed by radiological imaging. Cancer patients made up 38% (n=196) of the RIETE cohort with the most commonly associated primary sites being lung, breast and colorectal. CVAD
related DVT was more common in cancer patients than those without cancer (53% vs 39%, OR 1.7) as was the diagnosis of bilateral upper limb DVT (7.7% vs 1.6%, OR 5.1)(240). The Malmö Thrombophilia Study in Sweden has also prospectively reported on 63 patients with upper extremity DVT and found that 30% (n=19) of the cases were associated with malignancy (13 malignancy alone (21%), 6 (9%) malignancy and PICC or port-a-cath in situ)(91).

1.29.1 CVAD-associated upper extremity DVT in the cancer patient

CVAD-associated upper extremity DVT is often clinically unsuspected or asymptomatic. Rates of thrombosis range from 27% to 66% when routine venography is performed in cancer patients with CVADs in situ with reported rates of symptomatic DVT varying between 0.3% and 28.3%. Most studies have reported symptomatic DVT rates of four to eight percent. The rate of asymptomatic upper extremity DVT associated with CVADs is approximately 20% with the use of venography(237, 241). Within 24 hours of CVAD insertion a fibrous sheath develops around the device and appears to persist until removal. Thrombi can develop within the lumen of the device which manifests with an inability to aspirate blood from the device. This is now treated by instilling a small amount of thrombolytic agent within the device. Upper extremity DVT, however, develops outside of the device and is likely caused by a combination of vessel endothelial lining damage at device insertion and venous stasis due to occlusion of the venous lumen(90, 241).

Oncology treatments may add to the risk. The ETHIC study identified chest radiotherapy as an independent risk factor for DVT presumably secondary to endothelial activation but this has not been shown in other published studies(237). Chemotherapy has also increased the risk with bolus doses of antileukaemic drugs appearing to increase the rate compared with more dilute infusions(241).

When patients present with symptoms and/or signs of upper limb VTE:

- 80% develop oedema of the affected arm.
- 30-50% complain of pain.
- 15% develop erythema(89).
1.29.2 Risk factors for secondary upper extremity deep venous thrombosis

Indwelling central venous access devices (CVADs) confer the greatest risk for developing upper extremity thrombus. Increasing use in many medical and surgical specialties has increased the occurrence of this complication which not only depends on factors related to the type of device used but also on the patient(90).

1.29.2.1 The device

A number of device-related risk factors have been identified that may increase the likelihood of thrombus. They are:

- Left-sided insertion(90, 237).
- More than one insertion attempt(90, 242).
- The use of anatomical landmarks rather than ultrasound guidance for insertion(243).
- The type of material the device is made from with silicon and polyurethane associated with lower rates of DVT(237, 241, 244).
- Increasing numbers of lumens within the device increasing the device calibre(90, 241, 244).
- Previous device insertion at the same puncture site(242, 245).
- Devices that remain in situ for more than two weeks(90, 245).
- Infection of the line and sepsis (241).
- Device tip situated proximal to the atriocaval junction or misplaced in the upper half of the superior vena cava(237, 241, 245).

1.29.2.2 Other risk factors

A history of lower limb thrombosis, family history of VTE, immobilisation of an upper limb with a plaster cast and recent arm surgery are also important factors to consider in upper extremity venous thrombosis risk. At this stage obesity and hormone replacement therapy do not seem to confer an increased risk and the risk of oral contraceptive use is not clear and may only be relevant in women with underlying inherited coagulopathies(236).
A recent systematic review of the literature and meta-analysis investigated the risk of VTE associated with peripherally inserted central catheters (PICC lines). Sixty four articles involving a total of 29,503 patients were included, of which 12 were comparison studies with central venous catheters (CVCs) and 52 were non-comparison studies. The investigators included 22 articles published in abstract form and contacted the authors for additional information. Only five of the studies screened for clinically unsuspected upper extremity DVT, 20 did not report on whether tip position was checked radiologically post insertion and 37 did not report on the use of thromboprophylaxis(239).

It was concluded that patients with a personal or family history of VTE, diagnosed with cancer or critically ill in an intensive care setting were most at risk for developing PICC associated thrombosis. PICCs were also associated with significantly higher rates of thrombosis than CVCs (OR 2.55, 95% CI 1.54-4.23, p=<0.0001)(239).

The RIETE registry group compared 512 patients with upper limb DVT with a much larger cohort of patients diagnosed with lower limb DVT (n=11,564). Patients diagnosed with upper limb DVT were more likely to be male, younger in age, more likely to have an underlying diagnosis of cancer (OR 2.46 (CI 2.04-2.95, p=<0.001) and less likely to go on to develop a symptomatic PE(240). Monreal et al., however, reported a 16% incidence of PE (symptomatic and asymptomatic) in their prospective study, on performing mandatory ventilation/perfusion scintigraphy within 24 hours of the diagnosis of an upper extremity thrombosis in a total of 86 patients(246-248).

In an analysis of RIETE registry data on upper extremity DVT, Munõz et al. found that cancer patients were more likely to experience major bleeding on anticoagulation, develop recurrent VTE and have died by three months from DVT diagnosis. Cancer patients, however, were more likely to receive long term low molecular weight heparin (75%) and non cancer patients were more likely managed with warfarin (76%). These treatment approaches are consistent with current ACCP clinical guidelines and this database was not set up to compare interventions but to observe outcomes from “real world” clinical practice(20, 240, 249).
1.30 Summary

VTE risk is increased in cancer patients. The literature, to date, suggests that VTE at any site has the potential to negatively impact on morbidity and mortality in cancer patients and can complicate cancer treatment regimens. The risk factors are only partially understood. Existing clinical decision tools and D-dimer testing are of limited use in the diagnosis of clinically suspected cancer associated VTE meaning that most patients are referred for radiological imaging regardless of whether a clinical decision tool is used or not. The data currently suggests clinicians should refer symptomatic cancer patients with suspected VTE directly for imaging studies and avoid the use of existing clinical probability tools. Imaging is also useful in that CTPA has demonstrated other thoracic abnormalities in up to 42% of cancer patients. Our knowledge and understanding of venous thrombosis at sites other than the lower limb and pulmonary vasculature is limited although, with the increasing use of CVADs in clinical practice, the incidence of upper limb venous thrombosis is increasing.
Incidence, prevalence and clinical suspicion of VTE in patients with cancer

1.31 Introduction
Early studies investigating VTE will have underestimated its true incidence and/or prevalence because patients with clinically unsuspected VTE will have been missed (172, 184). VTE diagnosis currently relies on the:

- patient reporting symptoms.
- clinician demonstrating clinical signs.
- clinician’s index of suspicion for the presence of VTE.
- type and quality of radiological imaging used to diagnose VTE in combination with the expertise available to use the equipment and interpret and report the findings.

1.32 VTE incidence and prevalence in patients with cancer
Published incidences of clinically suspected VTE vary markedly from approximately 4 to 31% in cancer patients (250-252). This is higher than the general population where the age-adjusted incidence is approximately 2.5%(253). In patients with cancer, VTE incidence has appeared to increase over the last 20 years and is likely related to:

- improved clinician awareness worldwide of its association with cancer, lowering the threshold for initiating investigations looking for VTE.
- improved clinical and diagnostic techniques allowing more accurate diagnoses of VTE and the presence of malignancy.
- increasing research into VTE and cancer allowing large databases to be developed for more robust analysis.
- differences in tumour biology of cancer primaries/cell types.
- improved survival outcomes for cancer patients with newer therapies, some of which have been associated with arterial and venous thrombosis.
the aging population(170).

The incidence rate of VTE appears to be highest in the first six months following cancer diagnosis and decreases significantly over time(169, 254, 255). In a retrospective review of 68,142 patients with newly diagnosed colorectal cancer, 2,100 (3.1%) developed VTE within two years. The incidence rate dropped from 5% per 100 patient years in the first six months to 1.7% at 7-12 months and 0.6% in the second year following diagnosis(187).

Clinicians can only diagnose VTE at the bedside when symptoms and signs have developed and have already potentially impacted on quality of life, performance status, fitness for cancer treatments and survival. As discussed in section 1.27, published clinical decision screening tools for symptomatic adult medical patients with presumed VTE have limited utility in cancer patients(22, 213).

1.33 Unsuspected VTE in hospitalised patients with and without cancer

Unsuspected VTE are those that occur in patients who have not been prospectively identified as having symptoms or signs that raise sufficient clinical concern for the presence of VTE prior to the diagnosis being made. The term “unsuspected” has been interchanged with asymptomatic, silent and incidental. However, clinically unsuspected does not necessarily mean that the patient is asymptomatic (clinically silent) or that the VTE is an incidental finding without consequence for the patient. It may be that symptoms are not recognised or are too non-specific for the clinician to come to a conclusion that VTE may be present. Presenting symptoms may be attributed to known medical comorbidities (such as ischaemic heart disease, cardiac failure and chronic obstructive pulmonary disease) or, alternatively, the clinician may be unaware of the high VTE risk in the patient being treated(256). Unsuspected, therefore, appears to be the most appropriate term to use(202).

Stein et al. (1995) provided some insight into acute PE prevalence at post mortem in a general hospital setting(257). In this study, there were 51,645 admissions over a 21-month period including 2,235 patients who died (post mortem (PM) examinations were performed on 404 of these). The estimated prevalence of acute PE was one percent (526/51,645) based on imaging studies (V/Q scanning and pulmonary angiography) and review of the PM examinations undertaken. Fifty nine (14.6%) of the patients examined
at PM were found to have PE. PE was felt to be the direct cause of death in 20 (5%) patients, to have contributed to death in two patients (0.5%) and was an incidental finding in another 37 (9.2%) patients. On retrospective review of the medical records, PE was unsuspected ante mortem in 52 (88%) of the 59 patients, but most had advanced associated diseases such as a malignancy, chronic obstructive pulmonary disease or ischaemic heart disease, which may have influenced clinical suspicion. Seventy percent (14/20) of the patients who died from acute PE were unsuspected ante mortem, with 13 dying within 2.5 hours of their acute presentation to medical staff.

1.33.1 Unsuspected VTE in cancer patients

The diagnosis of unsuspected VTE occurs frequently on routine cancer staging CT scans. This is due to significant advances in radiological imaging resolution and increasing awareness of radiologists and other clinicians, that patients with cancer are at high risk for developing VTE(9, 258). Over the last 15 years, a number of studies have been published investigating the occurrence of unsuspected PE and DVT using multi detector computed tomography (MDCT) (see tables 9 and 10) and compression ultrasound scanning.

Gary et al. (2012) prospectively recruited 150 consecutive ambulatory cancer patients to their single centre cohort study, to investigate short term (nine-month) survival after diagnosis with an asymptomatic DVT or SVT. At baseline, the patients did not exhibit symptoms or signs suggestive of PE or lower limb DVT, had not been diagnosed with PE on staging CT scans of the chest, had no prior history of VTE and were not on anticoagulation. Each patient underwent compression ultrasound scanning at baseline by two specialised technicians to look for deep vein thrombosis (DVT) or superficial vein thrombosis (SVT) of the lower limbs. If a thrombus was diagnosed, the patient was treated with low molecular weight heparin (LMWH) and three month follow up monitoring for adverse events such as major bleeding. If the ultrasound scan was negative, patients were followed up for nine months as required by their treating oncologist and only investigated for VTE if clinically suspected(259).

- 27 (18%) patients were diagnosed with asymptomatic VTE at baseline assessment:
  - Seven (4.7%) with proximal DVT.
  - Nine (6%) with distal DVT (below knee).
13 (8.7%) with SVT of the saphenous veins.

- No clinical evidence of PE was found in any patient.
- Patients were not screened for unsuspected DVT/SVT beyond baseline or for unsuspected PE at any point in the study.

In the follow up period only two patients presented with symptoms/signs of DVT, but both had a negative ultrasound scan. No major bleeding events occurred in the patients who were treated with LMWH. Although this study was relatively small it gave important prospective prevalence data. It also showed poorer survival at nine month follow up for patients with asymptomatic DVT or SVT diagnosed at baseline compared with patients without, despite the use of anticoagulation. On multivariate analysis it was concluded that patients with asymptomatic DVT/SVT had a 2.4-fold increased hazard of dying by nine months compared to patients without (95% CI 1.2-5.3; p=0.03) (259).

### 1.33.2 Unsuspected PE and suspected lower limb DVT in adult patients with and without cancer

Using lung scintigraphy, three studies have described high prevalences of unsuspected PE (51%, 38% and 39.5-49.5% respectively) in cohorts of medical patients presenting with clinically suspected lower limb DVT(260-262). The patients did not exhibit signs or symptoms suggestive of PE presence(260-262). Meignan et al. also concluded that there was no correlation between the extension of the lower limb DVT and the presence of a high probability of PE on V/Q scan. This suggested that popliteal vein thromboses were associated with as many thrombotic events as more proximal thromboses(262).
Table 9 Prospective studies investigating unsuspected or incidental PE and/or DVT incidence or prevalence using CT scanning in adult patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Sample size</th>
<th>Number of cancer patients</th>
<th>Number of patients excluded</th>
<th>Unsuspected VTE in cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gosselin et al.(263)</td>
<td>Prospective</td>
<td>Consecutive adult patients</td>
<td>785</td>
<td>588</td>
<td>0</td>
<td>1.7% PE prevalence (8.6% inpatients, 0.6% outpatients)</td>
</tr>
<tr>
<td>Boswell et al.(264)</td>
<td>Prospective</td>
<td>Cancer patients</td>
<td>2085</td>
<td>2085</td>
<td>-</td>
<td>2.1% (result from citation, not actual publication)</td>
</tr>
<tr>
<td>O’Connell et al.(202)</td>
<td>Prospective</td>
<td>Consecutive cancer patients</td>
<td>4355 CT scans</td>
<td>-</td>
<td>-</td>
<td>1.2% 20 month incidence of PE</td>
</tr>
<tr>
<td>Sebastian et al.(265)</td>
<td>Prospective</td>
<td>Consecutive cancer patients</td>
<td>385</td>
<td>385</td>
<td>0</td>
<td>2.6% over 9 months</td>
</tr>
<tr>
<td>Ritchie et al.(17)</td>
<td>Prospective</td>
<td>Consecutive adult inpatients</td>
<td>547 inpatients</td>
<td>343 (suspected cancer)</td>
<td>60</td>
<td>5.2% inpatient PE prevalence in patients with suspected cancer</td>
</tr>
<tr>
<td>Browne et al.(266)</td>
<td>Prospective</td>
<td>Consecutive cancer patients</td>
<td>407</td>
<td>407</td>
<td>0</td>
<td>4.4% PE prevalence</td>
</tr>
</tbody>
</table>

Venous thromboembolism in cancer patients undertaking chemotherapy
Table 10 Retrospective studies investigating unsuspected or incidental PE and/or DVT incidence or prevalence using CT scanning in adult patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Sample size</th>
<th>Number of cancer patients</th>
<th>Number of patients excluded</th>
<th>Unsuspected VTE in cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winston et al.</td>
<td>Retrospective</td>
<td>Consecutive adult patients</td>
<td>1879</td>
<td>-</td>
<td>-</td>
<td>Not stated but 10/18 (56%) pts with PE had underlying cancer</td>
</tr>
<tr>
<td>Storto et al.</td>
<td>Retrospective</td>
<td>Consecutive adult patients</td>
<td>589</td>
<td>410</td>
<td>8</td>
<td>3.4% PE prevalence</td>
</tr>
<tr>
<td>Engelke et al.</td>
<td>Retrospective</td>
<td>Adult patients</td>
<td>600</td>
<td>600</td>
<td></td>
<td>4.3% PE prevalence over 11 months</td>
</tr>
<tr>
<td>Gladish et al.</td>
<td>Retrospective</td>
<td>Consecutive cancer patients</td>
<td>600</td>
<td>600</td>
<td></td>
<td>4% PE prevalence</td>
</tr>
<tr>
<td>Cronin et al.</td>
<td>Retrospective</td>
<td>Consecutive cancer patients</td>
<td>435</td>
<td>435</td>
<td></td>
<td>6.3% PE and DVT prevalence</td>
</tr>
<tr>
<td>Larici et al.</td>
<td>Retrospective</td>
<td>Cancer patients</td>
<td>787</td>
<td>787</td>
<td></td>
<td>1.9% (2.1% in inpatients)</td>
</tr>
<tr>
<td>Hui et al.</td>
<td>Retrospective</td>
<td>Consecutive adult patients</td>
<td>1168</td>
<td>765</td>
<td></td>
<td>2.2% over 1 month</td>
</tr>
<tr>
<td>Douma et al.</td>
<td>Retrospective</td>
<td>Cancer patients</td>
<td>838</td>
<td>838</td>
<td></td>
<td>2.5% over 1 year</td>
</tr>
<tr>
<td>Bach et al.</td>
<td>Retrospective</td>
<td>Cancer patients</td>
<td>3270</td>
<td>3270</td>
<td></td>
<td>3.9% unsuspected PE</td>
</tr>
</tbody>
</table>

Venous thromboembolism in cancer patients undertaking chemotherapy
1.33.3 Unsuspected VTE on single detector computed tomography (SDCT) scans

Gosselin et al. (1998) described a 1.5% prevalence of clinically unsuspected PE detected using helical single detector CT scans of the chest, performed on 785 adult patients being investigated for other potential pathology(263). This study found a higher prevalence of unsuspected PE (1.7%) in cancer patients, with hospitalised patients with cancer (8.6% unsuspected PE prevalence) identified as a high risk group (see table 9)(263). The 1.5% prevalence overall was higher than that previously reported in the literature, using single detector CT scanners(267, 275).

1.33.4 Unsuspected VTE on multi detector computed tomography (MDCT) scans

1.33.4.1 4-slice MDCT

Storto et al. (2005) performed a single centre, retrospective analysis of 589 patients (480 inpatients and 109 outpatients) who underwent a contrast-enhanced, 4-slice multidetector CT (MDCT) scan of the chest. Four hundred and ten (69.6%) patients had a confirmed cancer diagnosis and were undergoing staging of their disease while the remaining 179 patients were scanned for other reasons. This study, however, did not clarify whether some of the non cancer recruits were subsequently diagnosed with cancer(268).

The images were analysed at dedicated radiology workstations. Five hundred and eighty one scans contained adequate contrast opacification of the pulmonary vessels, of which 20 (3.4%) showed PE (19/474 (4%) inpatients and 1/107 (0.9%) outpatients). The higher prevalence was attributed to advances in CT technology which allowed improved visualisation of the pulmonary arterial tree. Fourteen (70%) patients with PE had an underlying malignancy, making the prevalence of unsuspected PE in cancer patients 3.4% (14/410). On statistical analysis, the prevalence of unsuspected PE was significantly higher in patients with cancer than in those without cancer (64.7% v 35.3%; p < 0.05)(268).

1.33.4.2 16-slice MDCT

Ritchie et al. (2007), conversely, did not find a statistically significant association between an underlying malignancy and the presence of unsuspected PE in a cohort of 547 consecutive inpatients who underwent 4- or 16-slice MDCT scanning of the chest. The prevalence of unsuspected PE in all patients was 5.9% compared with 5.2% in cancer patients, although this cohort also included patients with a presumed diagnosis of (not histologically confirmed) cancer(17).
Hui et al. (2008) retrospectively investigated 1168 consecutive adult patients, including 765 with cancer, undergoing 16-slice MDCT scanning of the chest over a one-month period in January 2005 (272). Of the 21 (1.8%) patients diagnosed with unsuspected PE:

- 17 (2.2%) had cancer (n=765).
- 11 (3.3%) patients had progressive cancer (n=332) (p=0.035).
- Four (2.5%) had stable cancer (n=162) (ns).
- Two (0.74%) had no evidence of cancer post treatment (n=271) (ns).
- Nine (4.7%) were undertaking chemotherapy (p=0.019).
- Eight (1.4%) were not on chemotherapy.

1.33.5 Studies investigating unsuspected VTE in cancer patients

A number of prevalence studies, many retrospective, have focused on unsuspected VTE in cancer patients and have analysed potential VTE risk factors as well as looked at the merits of CT scanning in this patient group.

In a retrospective review, Engelke et al. (2006) found 111 (6.1%) PEs in 1869 consecutive cancer patients that underwent 4- or 16-slice MDCT scans of the chest. Of the 91 patients with accessible and complete medical records, 56 (61.5%) had clinically unsuspected PE and 35 (38.5%) had documented symptoms consistent with PE. The authors documented a 4.3% incidence of unsuspected PE over the 11-month period. They also noted a high frequency of false negative reports in patients undergoing oesophageal and standard chest CT protocols, compared with computed tomography pulmonary angiogram (CTPA) protocols performed for patients presenting with clinically suspected PE (269).

Gladish et al. (2006) performed a retrospective study on 600 consecutive oncology patients who, within a 10-day period, underwent contrast-enhanced MDCT of the chest for clinical indications other than investigation for PE. Seventy nine patients were excluded, due to poor contrast opacification of the pulmonary vessels (69), respiratory motion artefact (eight) or because they did not have a malignancy (two). This left 31 (7.7%) inpatients and 372 (92.3%) outpatients for analysis. Sixteen (4%) patients had unsuspected PE (two (6%) inpatients and 14 (3.8%) outpatients). They found no significant difference in prevalence between inpatients and outpatients in contrast to previous studies, and chemotherapy treatment was not associated with presence of PE (270).
Cronin et al. (2007) performed a retrospective analysis investigating the prevalence of unsuspected DVT and PE in 435 consecutive oncology patients who underwent MDCT staging CT of the chest, abdomen and pelvis over a two-month period at a single centre in Ireland. Two hundred and thirty two patients had early stage disease and 203 had advanced stage disease. After assessment for motion artefact and adequate opacification of vessels, 397 patients were eligible for the PE study and 339 for the DVT study.

The overall prevalence of unsuspected VTE in this cohort was 6.3% (25/397) (outpatients 3.3% and inpatients 6.8%):

- 23 patients had unsuspected iliofemoral DVT.
- 18 (6.8%) had iliofemoral DVT only.
- Four (1.2%) had iliofemoral and common iliac DVT.
- One (0.8%) had iliofemoral, common iliac and inferior vena cava DVT.
- 13 patients had unsuspected PE.

A retrospective chart review was undertaken on the 25 unsuspected VTE cases and comparison of clinical data was made with a random selection of 25 patients without VTE in the study cohort. On logistic regression analysis:

- Patients with advanced disease were more likely to have a PE (p=0.017; odds ratio (OR) =5.8) or DVT (p=0.001; OR=6.3) than those with earlier stage disease.
- Inpatients were more likely to have a PE (p=0.004; OR=22.5) or DVT (p=0.002; OR=4.8) than outpatients.

The relative risk (RR) of VTE in an inpatient was 1.4 (p=0.047) and for a patient with advanced stage disease was two (p=0.001). Gender and chemotherapy use were not significantly associated with unsuspected VTE.

Four (17.8%) patients with unsuspected DVT (three with unsuspected DVT and PE and one with DVT only) subsequently presented with symptomatic PE within two months of the study scan.

### Missed PE diagnoses

In their retrospective review, Gladish et al. found that only four (25%) of the 16 patients with unsuspected PE, were actually diagnosed at the time of initial reporting of the CT scan. These
patients had developed multiple emboli including central or lobar thrombi. Of the 12 (75%) patients with a missed diagnosis of PE, six demonstrated solitary or multiple PEs in segmental or subsegmental vessels. These 12 patients were followed up and four were found to have DVT using other imaging modalities and were referred to appropriate clinicians for management. The remaining eight patients did not show clinical evidence of DVT or thrombus propagation and were managed by their supervising clinicians(270). Hui et al. also found that 14 (67%) of the 21 unsuspected PEs identified were missed at original reporting(272).

1.33.6 Unsuspected PE and symptoms

O’Connell et al. (2006) performed a prospective case-control study and retrospective chart review on 59 (incidence 1.2%) cancer patients diagnosed with unsuspected PE and 92 cancer patient controls (age- and stage- matched) without PE who underwent contrast-enhanced CT of the chest using a 16-slice multidetector CT over a 20-month period (May 2003-January 2005). Seven patients with PE were excluded because they were being treated with anticoagulation for a previously diagnosed VTE, and six were excluded as the investigators were unable to find appropriate controls scanned during the same time period(202). Unsuspected PE case patients were more likely to:

- have had a prior history of VTE (20% v 3%; p =0.007).
- complain of fatigue (54 v 20%; p = 0.0002).
- complain of shortness of breath (22% v 8%; p = 0.02).
- have a higher mean platelet count (240 x 10^9/L v 220 x 10^9/L; p = 0.04)(202).

There were no significant differences between cases and controls, documented in the medical records, with regards to chest pain, tachycardia, palpitations, limb pain or swelling, immobilisation, central venous access device use, chemotherapy administration within the last 30 days, thalidomide administration, oestrogen therapy administration, median haemoglobin levels or erythropoietin administration.

Two patients with PE did not commence anticoagulation after consideration by their treating oncologists. Both developed multiple thrombi a few months later, with one developing symptomatic central PEs(202).

The investigators concluded that cancer patients perceived as asymptomatic, with unsuspected PE, may actually be symptomatic and clinicians should have a lower threshold
of clinical suspicion for investigating PE. Seventy five percent of the patients with unsuspected PE had documented symptoms suggestive of PE on retrospective note review and were, in fact, symptomatic according to the research team. The clinical record review was, however, limited by what was documented. It was acknowledged that this study did not necessarily reflect clinician suspicion of PE, and that the researchers were aware of CT results prior to medical record review introducing a potential bias in the assessment of symptoms(202). It can also be argued that fatigue and dyspnoea are common complaints in cancer patients. Their aetiologies are multifactorial and, therefore, their presence does not automatically prompt the clinician to suspect a diagnosis of PE over cancer progression affecting the thorax, infection, medication/chemotherapeutic toxicity and/or other cardiovascular complications(276).

1.33.7 Prospective CT pulmonary angiography and unsuspected PE prevalence in cancer patients

Browne et al. (2010) were the first to publish a prospective, longitudinal cohort study in cancer patients which employed a CT pulmonary angiogram (CTPA) imaging protocol to diagnose PE. They demonstrated an unsuspected PE prevalence of 4.4% (6.4% amongst inpatients and 3.3% amongst outpatients) among 407 patients referred for staging CT scans at a single centre over a 10-month period(266). The scans were performed using a 64-slice multidetector CT scanner using 100-150mL of intravenous iodinated contrast administered through a cannula inserted into the antecubital fossa. At the point of maximal pulmonary arterial enhancement a spiral CT scan of the thorax was obtained from the lung apices to adrenal glands. The scan was then paused, to allow contrast to pass into the portal venous enhancement phase, at which point images of the abdomen and pelvis were obtained as per usual CT scan protocols for cancer staging(266).

Demographic data, cancer history, recent treatments, blood tests and reason for CT referral were recorded with the aid of the hospital records but not by direct patient contact. Anyone with a pre-test suspicion of PE or not undergoing the standardised departmental CT chest, abdomen and pelvis protocol for cancer staging was excluded from the study. Eighty three (20%) patients had received chemotherapy within 30 days of the scan being performed. Two hundred and eighteen (54%) patients were classified as having localised disease and 189 (46%) metastatic disease(266).
Eighteen (4.4%) patients were diagnosed with unsuspected PE with 14 (78%) having metastatic disease. Only two patients had documented symptoms associated with the diagnosis. PE was found most commonly in colorectal cancer patients (five patients).

Patients who had received chemotherapy in the last 30 days had a significantly higher incidence of PE than those who had not (11% v 3%). This was the only significant predictive factor for the likelihood of incidental PE on logistic regression analysis (p=0.008, 95% CI 1.05-12.44). Seventeen of the 18 patients did not have contraindications to anticoagulation or inferior vena cava (IVC) filter insertion. The remaining patient with thrombocytopenia and proposed bleeding risk represented with symptomatic multiple bilateral PEs five weeks later(266).

Study patients were followed up for six months with chart and imaging reviews. A further four patients were diagnosed with unsuspected PE on routine staging CT scans, six others presented with symptomatic PE and two others presented with symptomatic DVT. These 12 patients did not have PE on their baseline CTPA study. Overall, 22 of the 30 patients (5.4%) diagnosed with VTE were clinically unsuspected. Thirty nine percent of the PE diagnoses would not have been made without the additional CTPA images used in this study. Thrombi, in these patients, were found in the segmental and subsegmental pulmonary vessels(266).

1.33.8 Unsuspected PE and outcome

The development of unsuspected VTE has caused anxiety amongst clinicians with regards to management(256). There is controversy as to the clinical importance of thrombi, whether propagation will impact on a patient’s quality of life and function, or be life-threatening if left untreated.

Sahut D’Izarn et al. addressed this as part of a retrospective case-control study of adult cancer patients with unsuspected PE(277). Three groups were identified who underwent a 64-slice MDCT scan of the chest:

- Cancer patients with unsuspected PE- The case cohort.
- Cancer patients without unsuspected PE- Two cancer patients were recruited for each case of unsuspected PE. They were scanned within one week of the case patient but were not matched to the case in any other way.
Patients with haematological malignancies, with cancer in remission or on therapeutic dose anticoagulation at the time of the CT were excluded from the analysis. The medical records of patients with PE were reviewed by two clinicians.

Sixty six patients with unsuspected PE, 132 patients without PE and 65 patients with suspected PE consented to analysis. Of the 65 patients with suspected PE, 12 patients were included whose diagnosis fell outside of the three month window but were within six months of the case diagnosis. Of the 66 patients with unsuspected PE, 27 (41%) described symptoms which could be associated with the presence of PE prior to their MDCT. Patients with unsuspected PE, in comparison to patients without (multivariate analysis) were significantly older, more likely to have advanced cancer, more likely to have adenocarcinoma versus other histology and more likely to have had chemotherapy in the month prior to MDCT scanning. They were also more likely to have a WHO performance status >2 and to have a previous diagnosis of VTE.

In comparison to patients with symptomatic PE, the unsuspected PE cases had a lower BMI, were more likely to have a long-term central venous access device and to have received chemotherapy in the last month, were less likely to have undergone surgery for their cancer or have been hospitalised in the last month. At six month follow-up there was no difference in:

- survival between patients with PE and those without.
- risk of death at six months between patients with unsuspected and suspected PE.
- time to recurrent VTE in patients with unsuspected PE versus patients with suspected PE. All patients with unsuspected or suspected PE were anticoagulated with over 80% in both groups receiving low molecular weight heparin (LMWH).
- bleeding complications on anticoagulation between the PE groups.

In a retrospective matched cohort study, O’Connell et al. (2011) reviewed 70 cancer patients with unsuspected PE and compared their survival to 137 controls. The controls were matched to the cases for age (+/-five years), cancer and stage at the time of their CT scan which had to have taken place within a year of a case’s CT scan. All scans were performed using a 16-slice CT scanner.
MDCT producing 2mm image slices. Performance status, cancer grade and treatments were not included in the review. Survival was calculated from the date of the CT scan, not cancer diagnosis, in both groups. Not all of the control group fell within the preplanned parameters so the investigators expanded the age range for matching, and compared some cases to controls with a more advanced stage of disease (278).

Fifty patients (80%) were diagnosed with proximal PE. Four patients with segmental/subsegmental PE were not anticoagulated, due to contraindications, but the thrombi had resolved by the next CT scan. One patient subsequently developed a new unsuspected PE (278).

Median survival was eight months in the unsuspected PE group compared with 12 months in the control group (hazard ratio (HR) for death 1.51, 95% CI 1.01-2.27, p=0.048). When position of clot was analysed, proximal clot was associated with poorer survival (HR for death 1.70, 95% CI 1.06-2.74, p=0.027), but subsegmental clot was not compared with controls. When taking into account the use of anticoagulation the proximal PE group exhibited poorer survival. Patients with proximal PE were twice as likely to die at six months compared to controls. Patients with unsuspected PE were more likely to have received surgery in the last two months or had a prior history of VTE. They were also more likely to complain of dyspnoea or fatigue than controls (278).

These results indicate that unsuspected PE of the main, lobar or segmental pulmonary vessels is associated with poorer survival at six to twelve months from PE diagnosis, but clot isolated to the subsegmental vessels is not. It is unlikely that the acute VTE was directly related to death in most of these patients given the timeframe between diagnosis and death in the majority of cases. These results are hypothesis generating rather than definitive due to the retrospective nature of the study and the small numbers analysed, but they do suggest that VTE may be a surrogate for more aggressive cancer biology and hence associated poorer survival. VTE development may, therefore, be an important prognostic marker for survival, and an important complication to diagnose in clinical trials regardless of clinical suspicion (278).

1.34 CT scans and protocols
The studies summarised above employed specific, institution-sanctioned imaging protocols and used different CT scanners. Table 11 summarises the scans and published protocols used.
With the advancement in technology it can be seen that the number of detectors have increased and the collimation and slice width have been reduced to improve image resolution(279). Radiologists in many centres also use computer workstations to scroll through CT slices rather than hard copies of scans for the reporting of imaging studies. This has improved detection of thrombus and other abnormalities(263). Most early studies using CT involved 4-slice multidetectors but currently most institutions are using 64- or 128-slice detector scanners. It would be reasonable to hypothesise that the more detectors a scanner possesses and the thinner collimation and image slice thickness achieved, PEs will be diagnosed more convincingly to the subsegmental vessel level, reducing false positives (from artefact) and false negatives (PEs missed with thicker imaging slices)(227, 268, 279, 280).

Larici et al. (communication, 2007) performed a similar study in 787 oncology patients who underwent studies on a 16-slice MDCT scanner but have not, to date, published the full data. Unsuspected PE prevalence was 1.9% with no cases found in outpatients. The prevalence was lower than that found in the Gladish et al. study despite the employment of thinner imaging sections. No outpatients were diagnosed with unsuspected PE in this case series making the inpatient prevalence 2.1%(271).

Sebastian et al. (2006) prospectively evaluated 385 consecutive cancer patients who underwent CT scans of the chest over a nine-month period. Ten (2.6%) were diagnosed with unsuspected PE with two requiring a further CTPA to confirm the diagnosis. All patients were receiving or had recently received chemotherapy. The image slices were taken every 5 to 8mm which will have reduced the likelihood of diagnosing peripheral PEs(265).
Table 11 Scans and protocols documented in published studies investigating unsuspected VTE

<table>
<thead>
<tr>
<th>Study</th>
<th>CT</th>
<th>Imaging slice width (mm)</th>
<th>Rotation speed (s)</th>
<th>Pitch (mm/rotation)</th>
<th>Collimation</th>
<th>Imaging exposure</th>
<th>Contrast</th>
<th>Delay of scan post contrast infusion (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gosselin et al. (263)</td>
<td>SDCT</td>
<td>2.5-8</td>
<td>-</td>
<td>1.2-2.0</td>
<td>5mm (94%) 7-8mm (6%)</td>
<td>-</td>
<td>90-100ml @ 2.5ml/s</td>
<td>20-30</td>
</tr>
<tr>
<td>Winston et al. (267)</td>
<td>SDCT</td>
<td>5-10</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100-140ml @ 1.4-4ml/s</td>
</tr>
<tr>
<td>Storto et al. (268)</td>
<td>4-slice MDCT</td>
<td>5</td>
<td>0.5</td>
<td>-</td>
<td>7</td>
<td>4x1mm 4x2.5mm</td>
<td>140kVp 120mAs</td>
<td>80ml @ 3ml/s</td>
</tr>
<tr>
<td>Gladish et al. (16, 270)</td>
<td>4-slice MDCT</td>
<td>3.75</td>
<td>0.5</td>
<td>-</td>
<td>11.25</td>
<td>3.75mm</td>
<td>120kVp 320mAs</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16-slice MDCT</td>
<td></td>
<td>0.7</td>
<td>-</td>
<td>11.25</td>
<td>3.75mm</td>
<td>120kVp 320mAs</td>
<td>-</td>
</tr>
<tr>
<td>O’Connell et al. (202)</td>
<td>16-slice MDCT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Engelke et al. (269)</td>
<td>4/16-slice MDCT, CTA, ACTA</td>
<td>0.7-5</td>
<td>-</td>
<td>5-12.8</td>
<td>4x1-2.5mm 16x.75mm</td>
<td>120kVp 90-200mAs</td>
<td>120-140ml @ 3-5ml/s</td>
<td>50-60</td>
</tr>
<tr>
<td>Sebastian et al. (265)</td>
<td>Single Slice CT and 4-slice MDCT</td>
<td>5-8</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>5 or 8mm</td>
<td>-</td>
<td>100ml @ 3ml/s</td>
</tr>
<tr>
<td>Cronin et al. (258)</td>
<td>MDCT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8mm</td>
<td>-</td>
<td>150ml @ 3ml/s</td>
<td>30</td>
</tr>
<tr>
<td>Study</td>
<td>CT</td>
<td>Imaging slice width (mm)</td>
<td>Rotation speed (s)</td>
<td>Pitch</td>
<td>Table speed (mm/rotation)</td>
<td>Collimation</td>
<td>Imaging exposure</td>
<td>Contrast</td>
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</tr>
<tr>
<td>Larici et al.(271)</td>
<td>16-slice MDCT</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ritchie et al.(17)</td>
<td>4-slice MDCT</td>
<td>2-3</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>120kVp</td>
<td>90ml @ 3-4ml/s</td>
</tr>
<tr>
<td>Ritchie et al.(17)</td>
<td>16-slice MDCT</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>23</td>
<td>-</td>
<td>120kVp</td>
<td>90ml @ 3-4ml/s</td>
</tr>
<tr>
<td>Hui et al.(272)</td>
<td>16-slice MDCT</td>
<td>2.5</td>
<td>0.5</td>
<td>0.935</td>
<td>18.75</td>
<td>2.5mm</td>
<td>140kVp</td>
<td>65ml @ 2ml/s</td>
</tr>
<tr>
<td>Browne et al.(266)</td>
<td>64-slice CTPA</td>
<td>1-1.5</td>
<td>0.5</td>
<td>0.9</td>
<td>-</td>
<td>32x0.6mm</td>
<td>100-120kVp</td>
<td>100-150ml @4-5ml/s</td>
</tr>
<tr>
<td>Bach et al.(274)</td>
<td>64-slice MDCT or CTPA</td>
<td>2.5mm (CTPA)</td>
<td>5mm (MDCT)</td>
<td>-</td>
<td>0.6-1.2</td>
<td>-</td>
<td>120kVp</td>
<td>60-140ml @2ml/s</td>
</tr>
</tbody>
</table>
1.35 Proximity of clot and clot burden in cancer patients with unsuspected PE

Table 12 below summarises the most proximal thrombi visualised in published studies. Of the seven with full information it appears that subsegmental PEs were more reliably seen on MDCTs with more than four detectors using a thinner image slice. The Storto et al. study, using a 4-MDCT with 1-2.5 mm image slices detected five central, five lobar and 10 segmental PEs as the most proximal site of thrombi, with 13/20 of the patients showing multiple filling defects. No subsegmental PEs were seen(268). In the study by O’Connell et al., using a 16-MDCT (image slice thickness not described), 14 (27%) cases involved subsegmental PEs(202). The Browne et al. study, using a 64-MDCT/CTPA protocol with 0.6mm image slices, demonstrated three (17%) subsegmental PEs. Fifteen patients (83%) demonstrated PE in more than one pulmonary artery(266). There have not been any published data on PE prevalence in cancer patients using 128-slice or above MDCTs to date.
Table 12 Studies that documented the proximal extent of thrombus in all patients diagnosed with unsuspected PE

<table>
<thead>
<tr>
<th>Study</th>
<th>Most proximal location of pulmonary artery emboli in patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Main (%)</td>
</tr>
<tr>
<td>Winston et al. (267)</td>
<td>-</td>
</tr>
<tr>
<td>Gosselin et al. (263)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Storto et al. (268)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Gladish et al. (270)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>O’Connell et al. (202)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Ritchie et al. (17)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Hui et al. (272)</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>Browne et al. (266)</td>
<td>4 (22)</td>
</tr>
</tbody>
</table>
1.35.1 Subsegmental PE

Advances in multidetector CT scanning have improved our ability to detect segmental and subsegmental pulmonary emboli and offer the possibility of detecting alternative causes for presenting symptoms and signs(279). It is not uncommon for patients to be diagnosed with a single subsegmental PE (SSPE) on CT (30% Carrier et al.) and no evidence of thrombus elsewhere(281). The clinical outcome for adult medical patients with subsegmental PE and the clinical benefit from anticoagulants in these situations is unknown. The current American College of Chest Physicians’ (ACCP) guidelines recommend anticoagulation, at this stage, but many clinicians feel uncomfortable with this approach when considering the likelihood of bleeding complications on treatment(20). Carrier et al. (2010) performed a systematic review and meta-analysis of management outcome studies in adult medical patients diagnosed with subsegmental PE on CT. They found that MDCT detected more SSPE than SDCT but the three-month thromboembolic risk for those left untreated in each group were not significantly different suggesting SSPE was not clinically significant(281).

O’Connell et al. associated unsuspected PE of the main, lobar or segmental pulmonary vessels with poorer survival at six to twelve months from PE diagnosis, but also found that PE isolated to the subsegmental vessels was not. In this study it was unlikely that the acute VTE was directly related to or solely caused death in most of these patients, given the timeframe between diagnosis and death(278).

1.36 Summary

VTE development is a significant issue for cancer patients and clinicians alike. Currently, there are many unanswered questions that require further investigation. Unsuspected VTE is a particular area of concern as patients are currently managed as per symptomatic patients in international clinical guidelines. This may not be appropriate for all patients and may not influence important outcomes such as VTE recurrence and survival. Anticoagulants may also be harmful in some circumstances leading to adverse events such as haemorrhage which may be life-threatening, especially in elderly patients with
There is a school of thought that malignancy is not associated with a higher prevalence of PE but it just appears so because cancer patients are scanned more often than patients without cancer (256). Cancer patients are, therefore, more likely to make up a large proportion of the subjects analysed in observational studies leading to an inherent bias in accrual. Patients without cancer may, conversely, not comprise a large enough cohort in some studies to allow adequate statistical power for analysis of unsuspected PE in cancer patients versus patients without cancer.

More focused research has taken place over the last 15 years in cancer patients where tumour biology and/or cancer treatments may increase thrombosis risk. Most of the research, to date, has been retrospective and limited to clinical record review as opposed to patient assessment to evaluate clinical presentation. The need for prospective studies correlating clinical findings with radiological findings are paramount to provide robust data on VTE prevalence and outcome. This will provide health care workers and patients with more reliable information about VTE development associated with cancer and help to assess patient quality of life, survival and the impact of anticoagulants on outcome. Research will also continue to validate possible ways to predict VTE risk, help tailor prophylaxis to those who require it and minimise haemorrhagic complications.
Current understanding of the pathophysiology of cancer associated VTE

1.37 Introduction
Although not fully understood, it is recognised that a cancer may exert effects on its host that, in turn, induce responses from that host. These effects and responses may involve the endogenous coagulation and anticoagulation processes of the body, with the increased risk of VTE attributed to a net procoagulant effect that may be enhanced by the administration of certain chemotherapeutic agents. Possible mechanisms include induction of increased levels of procoagulant molecules, reduction or impairment of endogenous anticoagulant levels and damage to endothelial cells leading to apoptosis and cytokine release(282). It has been shown that endogenous coagulation and anticoagulation mechanisms may influence inflammatory and angiogenic processes within the host as well as the biology of the tumour where promotion of tumour cell survival, growth, invasion and the development of metastases are supported(283).

1.38 Virchow’s triad
In the mid 20th century a triad, named after Rudolf Virchow, was described to explain the underlying processes contributing to the development of thrombosis(284). It identifies three important aspects:

1. The integrity of the blood vessel wall and vascular endothelial lining.

2. The flow of the blood within the blood vessel.

3. The constituents of the blood.

1.38.1 The vascular endothelium
The endothelial lining of blood vessels is the thin, single cell-thick ablumenal layer which comes into direct contact with the blood. It is anchored to the basement membrane of the surrounding tissue by pericytes and smooth muscle cells. These anchoring cells penetrate the basement membrane and make direct contact with the endothelial cells (ECs) allowing communication via signalling
messengers. Under physiological conditions ECs are stable, rarely undergo apoptosis (turnover rate of months to years) and form an unbroken layer separating the blood and its constituents from the subendothelial extracellular matrix (ECM)(285). The blood travels unimpeded over this layer and is not induced to undergo coagulation as is the case when blood contacts other surfaces. ECs form a barrier to the passage of substances through them by adherens and tight junctions (cell to cell contacts) consisting of transmembrane proteins including VE-cadherin, N-cadherin, claudins and occludin. These adherens and tight junctions also allow cell to cell communication and vary in different vascular tissue beds, meaning that there is variable permeability in different tissues. Brain vasculature has many tight junctions which significantly reduce permeability (the blood-brain barrier), while post capillary venules have relatively few tight junctions to allow increased permeability and leukocyte transmigration. Gap junctions allow the intercellular passage of small molecules(286).

The endothelial lining, therefore, allows smooth, laminar flow and regulates all substances, cells and pathogens that may potentially pass across from the blood into the extracellular matrix or vice versa. Stimulation of ECs by pro-angiogenic factors or cell damage will significantly alter this control and may induce thrombosis as well as support easier passage of substances between the blood and the ECM(285, 287, 288).

1.38.2 Blood flow

Blood flow is normally unidirectional and laminar due to the pumping action of the heart, the subendothelial muscle contractions of the blood vessel wall, the venous muscle pump of the lower limbs and the actions of venous and cardiac valves. Anything that disrupts flow may induce thrombosis. This may be due to the presence of an intrinsic or extrinsic mass/process narrowing or obstructing the vessel lumen leading to sluggish flow, turbulent flow or stasis. Reflux of blood through damaged valves may also have a similar effect(49).

1.38.3 Blood constituents

The blood contains many constituents which are vital for survival and maintenance of the body. At a macroscopic level it appears to be a liquid but, at
a microscopic level, is far more complex. The major components of the blood are:

The liquid component termed serum, a non-cellular liquid in which all blood constituents, including coagulation factors, flow.

The solid component made up of red blood cells and their precursors, which transport oxygen to the tissues, white blood cells and their precursors, involved in host immune and inflammatory responses, and platelets and their precursors, involved in coagulation and inflammation.

The numbers of mature cells circulating in the blood are usually tightly controlled in the healthy individual but can be upset in disease e.g. infection and cancer. Increasing absolute numbers of premature and mature cells can increase the solid component of the blood and increase the viscosity of the blood leading to sluggish flow and the increased potential for thrombosis (e.g. leukaemic and polycythaemic patients)(49). Functional changes in blood cells (e.g. platelets) will also impact on thrombosis risk even if the absolute number present in the blood appears normal(42).

1.39 The tumour microenvironment and coagulation

Tumour cells can independently induce the activation of the coagulation pathways at multiple levels (see figure 19). This may be influenced at the molecular level by oncogenes and tumour suppressor genes(289). Tumour cells can interact with the host’s vascular endothelium by physical adherence or remotely by the production of cytokines messengers(290). Physical adherence can take place directly between tumour and endothelial cells or indirectly through recruitment of macrophages and platelets(170).
Figure 19 Pathophysiological mechanisms involved in chemotherapy associated venous thromboembolism
1.39.1 Mutations in tumour cells affecting coagulation

Oncogenes and tumour suppressor genes have long been associated with the progression of cancers. These genes within cancer cells exhibit mutations which are not seen in the host’s normal cells allowing unmoderated tumour progression. By both direct (influencing the coagulation pathway) and indirect (influencing tumour aggression, invasiveness and spread) means transcription of these genes may promote a procoagulant state within the host (see table 13).

Table 13 Oncogene and tumour suppressor gene mutations associated with thrombosis

<table>
<thead>
<tr>
<th>Oncogene/ tumour suppressor gene study</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal Growth Factor Receptor antagonism(291)</td>
<td>Reduced tissue factor expression on tumour cells</td>
</tr>
<tr>
<td>JAK2V617F mutations (myeloproliferative neoplasms)(283, 292)</td>
<td>Thrombosis at unusual sites</td>
</tr>
<tr>
<td>KRAS (colorectal cancer)(293)</td>
<td>Increased TF expression</td>
</tr>
<tr>
<td></td>
<td>Increased TF microparticle release from tumour cells</td>
</tr>
<tr>
<td></td>
<td>Increased procoagulant activity</td>
</tr>
<tr>
<td></td>
<td>Increased proangiogenic activity</td>
</tr>
<tr>
<td>p53 (colorectal cancer)(293)</td>
<td>Increased TF expression hypoxic conditions</td>
</tr>
<tr>
<td>PTEN (glioma and non small cell lung cancer) inactivation(294)</td>
<td>Reduced TF expression</td>
</tr>
<tr>
<td></td>
<td>Successful treatment of disseminated intravascular coagulation</td>
</tr>
<tr>
<td>PML-RARα oncprotein antagonism in acute promyelocytic leukaemia (APL) by All-Trans- Retinoic Acid (ATRA)(295, 296)</td>
<td>Thrombo-haemorrhagic syndrome secondary to increased plasminogen activator inhibitor (PAI)-1 and cyclooxygenase-2</td>
</tr>
</tbody>
</table>

At a molecular level, PTEN tumour suppressor gene inactivation has been linked to Tissue Factor (TF) gene induction in human astrocytoma cells and in non-small cell lung cancer cells. Both TP53 and PTEN mutations have been shown to increase TF mRNA levels. TP53 inactivation and KRAS activation
have also resulted in increased TF expression and increased release of cell-derived microparticles containing TF (TFMPs) in a colorectal cancer model.

In a recently published retrospective case control study of non small cell lung cancer patients, VTE occurred in 57 patients and were compared with 102 matched controls to assess the associations between VTE and KRAS or EGFR mutations. The majority of the patients presented with adenocarcinoma and a history of smoking. Mutations in KRAS codons 12 or 13 were associated with VTE development (OR 2.67; p=0.014) but mutations in EGFR exons 19 or 20 were not (OR 0.99; p=0.99)(298). TF has been shown to be upregulated in vitro in KRAS mutant colorectal cancer cell lines, and in non small cell lung cancer mRNA levels of TF and VEGF were significantly higher in patients with KRAS mutation codon 12. Prospective validation of KRAS status and VTE development is awaited as smoking history and adenocarcinoma are established VTE risk factors that may confound retrospective findings(298).

1.39.2 Murine studies
Although murine studies have been helpful in this area it has been found that there may be species-specific effects that do not translate from the mouse to the human and vice versa. Mice will rarely develop extremity deep vein thrombosis and have been shown in vascular studies to experience significant mortality from atherosclerotic plaque rupture. The significant disturbances of the haemostatic system seen in humans with APL, associated with the PML-RARα oncoprotein, have not occurred in mice. On the other hand, severe and lethal murine thrombo-haemorrhagic complications induced by a MET oncogene-promoted hepatocellular malignancy have not been observed in humans. Xenografts of procoagulant advanced human tumours in mouse models have also not affected the murine haemostatic mechanisms as severely. Human Tissue Factor (TF) is capable of efficiently initiating the coagulation cascade in mice, but murine TF does not have the same effect in humans(289).

1.40 The tumour cell and the host response
Tumour cells are capable of producing a number of components of the coagulation process as well as inducing the host to promote a procoagulant state.
1.40.1 Tissue Factor

Tissue Factor (TF), the principal activator of coagulation *in vivo*, is a 47 kDa transmembrane glycoprotein which is expressed in a tissue-specific manner with high levels in brain, heart, kidney and placenta(102). It is produced in the adventitial fibroblasts of collagen in the subendothelial extracellular matrix of the blood vessel wall, which facilitates coagulation after injury. TF is not normally induced in the smooth muscle and endothelial cells of healthy subjects but has been demonstrated on monocytes, macrophages and endothelial cells in pathological conditions such as sepsis and cancer when they are exposed to proinflammatory stimuli such as tumour necrosis factor alpha (TNFα), interleukin (IL)-1β, platelet-activating factor (PAF) and bacterial endotoxins(283, 299).

1.40.1.1 Tissue factor and cancer

TF is constitutively expressed on the tumour cell surface of many cancer types and promotes a procoagulant state. It is continuously available to bind circulating FVIIa leading to thrombin and fibrin generation locally and systemically (284) (102). Tumour expression of TF may correlate with circulating TF antigen and in glioma, breast and pancreatic cancer cells has been shown to correlate with tumour grade and/or progression(291, 300, 301). TF mRNA may be upregulated by progesterone in patients with breast cancer and TF expression may be induced by nitric oxide, TNFα via the nuclear factor kappa B (NF-κB) pathway and VEGF independent of NF-κB(302-304).

Very low levels of TF are present in the circulating blood of healthy individuals in the form of cell-derived microparticles (MPs) which measure between 0.1 and 1 micrometre in diameter, although not all microparticles detected in the blood contain TF. TFMPs are mainly derived from platelets and monocytes (>80%) in healthy individuals and this is increased in the presence of cancer. This increase is related to MPs released from tumour cells, macrophages and damaged endothelial cells as well as platelets. In patients with colorectal cancer, platelets produced double the amount of TFMPs compared with those from controls without cancer(305). Higher levels of platelet-derived MPs have been found in stage IV gastric cancer compared with earlier stages suggesting that MPs may predict for the development of metastatic disease. In gastric cancer
patients TF produced by tumours is associated with extravascular coagulation and, in mice, injection of tumour-derived TFMPs can induce a disseminated intravascular coagulation (DIC)-like syndrome(306).

TF not only contributes directly to thrombosis but may also provide feedback to tumour cells encouraging growth, angiogenesis and metastasis. *In vitro* models have shown that TF expression by vascular endothelial cells of breast cancer tissue can stimulate angiogenesis. This may, in part, relate to the promotion of vascular endothelial growth factor (VEGF) production and regulation of endothelial cell growth factors(290, 307). Conversely, VEGF appears to activate TF through mediation from the zinc finger transcription regulator Early Growth Response-1 (EGR-1)(302, 308) and through p38 and Erk-1/2 MAP kinases(309).

Many TF assays have been applied to assess plasma or serum levels and activity but there is currently no “gold standard” test. A promising test uses impedance flow cytometry to measure levels of TF-rich MPs in human venous blood and may be useful in predicting VTE in cancer patients(310). This, however, uses specialised equipment which still requires validation in further studies. Hugel et al. use a solid phase capture assay for the determination of MPs which is more easily performed and appears considerably less expensive to perform(311).

**1.40.2 Cancer procoagulant (CP)**

Cancer procoagulant (CP) is a 68kDa vitamin K-dependent cysteine protease which is secreted by various tumour cells but has not been detected in the normal cells of the body except amniochorionic cells. It interacts with factor X independent of the TF-FVIIa complex and at a different site to other known factor X activators(102, 290). It has been measured using antigen and functional assays (312, 313). CP has been detected in patients with acute promyelocytic leukaemia and becomes undetectable in patients responsive to All-Trans-Retinoic Acid (ATRA) (314). ATRA may also reduce hypercoagulability in breast cancer patients treated with tamoxifen(315).

**1.40.3 Thrombin**

Increased levels of thrombin will increase thrombosis risk through coagulation processes discussed previously. It is recognised that thrombin production is
increased in many cancers including malignant melanoma and small cell lung cancer(316, 317). Thrombin, however, has been found to perform a wide range of functions outside of the clotting process influencing cellular processes through its interaction with Protease-Activated Receptors (PARs) (see figures 20 and 21). These are a family of 7-transmembrane G-protein coupled receptors of which 4 members have been identified. PAR-1, PAR-3 (murine) and PAR-4 are activated by thrombin cleavage but PAR2 is not. Only low thrombin concentrations are required to cleave PAR1 whereas 10-100-fold higher concentrations are required to cleave PAR-4 suggesting PAR-1 is the major coordinating receptor in the early stages of coagulation and PAR-4 becomes more involved in the later stages and may be more sustained in its downstream signalling effects(35, 40). PAR-1 expression has also been associated with malignant melanoma progression(318).
Figure 20 Procoagulant substrates and cofactors of thrombin

**Exosite I – Procoagulant Pathways**

- Sodium (Na+) Receptor
- Exosite I
- A Chain
- B Chain
- Exosite II
- Factor V
- Factor VIII
- PAR 1
- PAR 4
- Fibrinogen
- Factor XII
- GpI b a
- Fibrin
- Factor XIII

**Exosite II – Procoagulant Pathways**

- Sodium (Na+) Receptor
- Exosite II
- A Chain
- B Chain
- Exosite I
- GpI b a
- Factor V
- Factor VIII
- PAR 1
- PAR 4
- Factor XII
- GpV
1.40.4 Platelets
Thrombin is the most potent activator of platelets and activates them through both PAR1 and PAR4. It also binds to the GPIb receptor which may play an important role in PAR cleavage, although the result of this interaction is not fully understood[39]. On activation, platelets change shape from the smooth appearance of the quiescent cells to the spikey appearance of the active cell. Integrin alpha IIb/beta 3 (αIIb/β3 also known as the GPIIb/IIIa receptor) undergoes a conformational change, exposing the RGD binding site that recognises the RGD (arginine-glycine-aspartate sequence) sequence in fibrinogen, the latter forming the bridge between aggregating platelets. This sequence is an important cell attachment site for many proteins in the blood, extracellular matrix and on the cell surface including integrins. It may also
allow binding to integrin alpha 5/ beta 3 (αV/β3) on tumour cells in the presence of vWF and fibrinogen (319-321). Activated platelets also synthesise and release substances such as PAF, ADP, serotonin, thromboxane A2 and P-selectin which promote further platelet aggregation and activation as well as tumour cell adhesion and vasoconstriction. Fibronectin and vWF expression are further stimulated to anchor platelets to tumour cells and support thrombin in this regard (299, 322). Murine studies have shown that the removal of circulating platelets results in reduction of metastasis formation. This may be due to reduced tumour cell protection from immune system destruction and reduced invasiveness as platelets produce heparanase to aid this process (323).

1.40.4.1 P-selectin

L-, P- and E-selectins comprise a family of carbohydrate-binding adhesion molecules expressed by leukocytes, platelets and the vascular endothelium. The glycoprotein P-selectin (also termed GMP-140, PADGEM or CD62P) is the protein product of the SELP gene located on chromosome 1q24-25 near the locus of the factor V gene. Various polymorphisms of this gene have been associated with increased thrombosis risk in medical patients (324). P-selectin is stored in the alpha granules of platelets and in the Weibel-Palade bodies of endothelial cells. When platelets are activated or the endothelial lining is damaged, P-selectin production is upregulated and released onto cell surfaces to aid interaction and adhesion with other platelets and leukocytes (325, 326). P-selectin release can, therefore, be induced by thrombin (327).

When P-selectin combines with an associated receptor known as P-selectin glycoprotein ligand-1 (PSGL-1), predominantly present on the surface of leukocytes but also platelets, it plays a part in stimulating the release of procoagulant MPs from leukocytes promoting fibrin formation and the development of thrombi. MPs are thought to be rich in phosphatidylserine and PSGL-1 as well as tissue factor (TF) (328). TF expression and phosphatidylserine exposure on monocytes have been linked to P-selectin upregulation amplifying the coagulation process (329). P-selectin can also bind to leukocytes through PSGL-1 to activate neutrophil integrins, upregulate TF production in monocytes and increase thromboxane A2 through the arachidonic acid pathway leading to vasoconstriction of the blood vessel and promoting...
thrombosis(330). Adenosine diphosphate (ADP) subsequently released from both tumour cells and platelets triggers a feedback mechanism increasing platelet aggregation and activation(15).

In cancer patients P-selectin is thought to also mediate the adhesion of tumour cells to platelets and leukocytes and support inflammation, thrombosis and cancer growth and metastasis through interactions with tumour cell CD24 ligand(325, 329). These interactions may be organ-specific, as shown in mouse models, with platelets promoting lung metastases but not liver metastases in B16F1 melanoma cells independent of natural killer (NK) cell function and P-selectin promoting both lung and liver metastases (331).

### 1.40.5 Mucins

Mucins are glycoproteins with clustered O-linked glycans(330). Carcinoma cells can upregulate the expression of mucin polypeptides (MUC1, MUC2 etc.) and shed them to circulate in the blood. It has been shown that the MUC1 epithelial mucin is present on microparticles circulating in the blood of pancreas and breast cancer patients. This has been associated with TF positive microparticles, thrombosis risk and poorer survival outcome(332).

Mucins are able to act as pathological ligands for selectin adhesion molecules. The selectin adhesion molecules recognise structurally related ligands containing sialic acid and fucose residues which are present on mucins. L- and P-selectins can bind to mucins which may contribute to the procoagulant state. L-selectin is expressed on neutrophils, monocytes and immature lymphocytes, and on binding mucin, is able to facilitate intercellular communication and activate circulating platelets promoting thrombus formation. P-selectin is likely to be involved in platelet aggregation on binding with mucin but also plays a part in thrombus propagation(330). P-selectin has been shown to bind to protein s-Le (x) on mucin-producing carcinomas(333).

### 1.40.6 Thrombin and inflammation, repair and angiogenesis

Protease activated receptors (PARs) induce proinflammatory effects on the endothelium(35, 327). Thrombin can act as a potent mitogen and predominantly supports inflammation and angiogenesis through PAR1 by promoting(39, 40):
- Cell growth and differentiation(33).
- Smooth muscle proliferation(35).
- EC proliferation(33, 40).
- Macrophage proliferation(334).

Thrombin also promotes a proinflammatory state through the release of growth factors, chemokines and extracellular proteins that promote proliferation and migration of neutrophils, monocytes and also tumour cells. Thrombin decreases the ability of ECs to attach to the basement membrane by reducing intracellular cyclic AMP levels and its controlling effects on EC barrier function. Although thrombin has been shown to also cause vasodilatation through nitric oxide release with the intact vascular endothelium, it has also been shown to induce vasoconstriction when the endothelium is not intact in vitro(40, 327, 335).

Subsequent to thrombin interactions, histamine release from mast cells, serotonin release from platelets and increased production of the smooth muscle relaxant prostacycline result in vasodilatation, vascular permeability, oedema and swelling. The increased vascular permeability results in transendothelial migration of neutrophils and plasma proteins and enhances adhesion between platelets, ECs and the extracellular matrix by mobilising adhesion molecules, such as P-selectin, to the platelet surface on platelets and ECs. These processes can also potentially increase adhesion between tumour cells in the same way and also facilitate invasion through the basement membrane by activating collagen type IV degrading enzyme and matrix metalloproteinase 2(40).

Angiogenesis is induced by thrombin through the promotion of vascular endothelial growth factor (VEGF) release leading to promotion of cell migration, EC proliferation and vascular tube formation(336). VEGF release from the alpha granules of platelets may also be increased by thrombin in non small cell lung cancer(337). This upregulation in a number of cancers may be due, in part, to the induction of reactive oxygen species and the expression of hypoxia inducible factor (HIF) 1 which promote VEGF production(338). AlphaV/beta3 (αVβ3) and alpha V/beta 5 (αVβ5) integrins are expressed on the
endothelium(339). Thrombin binds to αV/β3 and promotes the expression of the integrin at the mRNA and protein levels(340). Through PAR1 thrombin also plays a role in tissue repair by inducing connective tissue growth factor (CTGF).

**1.40.7 The functions of thrombin- in vivo animal studies**

The vast majority of the evidence supporting thrombin’s functions have been obtained from *in vitro* studies to date. Published *in vivo* studies, involving animals intravenously infused with thrombin have shown that thrombin can induce very different outcomes depending on the concentration of thrombin and the method of administration. When high doses are infused, as a bolus or by short infusion, the animals develop microthrombi in many parts of the body. Conversely, thrombin infused slowly results in bleeding suggesting that, in the presence of quiescent blood vessels with no endothelial damage, thrombin’s anticoagulant and fibrinolytic properties are favoured(35).

Infusions of thrombin in various animal models have:

- increased consumption of clotting factors V, VII, VIII, IX, X and XII and induced activation of protein C and haemolysis(341-344).
- resulted in thrombocytopenia(342, 343, 345-357).
- reduced plasma fibrinogen(342, 343, 345, 346, 348-352, 354, 357, 358).
- increased fibrin degradation products (FDPs)(344, 357, 359).
- Reduced antithrombin levels(346, 356, 360).
- decreased plasminogen activator inhibitor (PAI)(361).
- decreased the leukocyte count(344, 353, 356).
- prolonged the APTT, PT, TT and/or TCT(342, 343, 345, 346, 353, 354, 359, 361).
- increased levels of free Hb(352, 362).
- increased histamine and serotonin levels(347).
Thrombin infusions have been associated with changes in cardiorespiratory function independent of thrombosis development, perhaps mediated through PAR-1(35).

It is difficult to conclude from the above studies that similar interactions occur in humans, as the in vitro studies have been performed with supraphysiologic levels of thrombin. In vivo studies have used human or bovine thrombin infused into animal species that may have very different coagulation physiology and homeostasis. These animals may have reacted differently to thrombin harvested from different species. They do, however, show that thrombin can exert multiple effects within the body which are not necessarily related to coagulation. Very little is known about physiological levels of thrombin and its effect on human vasculature and tissues, as it has been challenging to measure in plasma and has a short half life(35, 363).

1.41 Fibrinogen and fibrin
The fibrin mesh is thought to have a number functions which aid tumour progression(364):

- A backbone or scaffold to allow angiogenesis in tumour vasculature and upregulate the production of interleukin (IL)-8 which further promotes angiogenesis.

- Sequester growth factors resulting in protection from degradation.

- Aid tumour cell adhesion to the endothelium and stabilise tumour associated emboli encouraging invasion and metastasis by upregulation of tissue factor.

- Sequester tumour cells from host immune cell destruction.

Fibrin is constantly formed and then broken down with mature connective tissue deposited in its place. If tumour cells are present in this stroma and are viable they can continue to grow and spread elsewhere. Fibrinogen deficiency in animal models has demonstrated reduced development of lung metastases suggesting that fibrin is required for metastasis adhesion and stability.
Fibrinogen and plasminogen can also leak into the extracellular matrix after endothelial cell disruption (365).

1.42 Endogenous anticoagulation and cancer
Tumour cells may impact on endogenous anticoagulation processes to induce a procoagulant state. This may be achieved by either inhibition and/or reduction in production of biological natural anticoagulant proteins.

1.42.1 Antithrombin
Antithrombin is the most potent inhibitor of thrombin and has been associated with thrombosis in deficient patients. Little has been researched around the effect of cancer on antithrombin aside from investigating the presence of congenital antithrombin deficiency in cancer patients through thrombophilia testing. It is currently not recommended that cancer patients routinely undergo thrombophilia screens unless there is a family history as these conditions are relatively rare.

1.42.2 Thrombomodulin
Thrombomodulin (TM) plays a role in inflammation and cell adhesion in vivo but an anti-inflammatory role has also been demonstrated (366). TM deficiency in murine studies induces a hypercoagulable state.

The TM gene is found on chromosome 20p12 and is transcriptionally upregulated by thrombin, VEGF, histamine, dibutyryl cAMP, theophylline, heat shock and statin drugs (46). Its transcription is downregulated by shear stress, haemodynamic forces, hypoxia, oxidized LDL and TGFβ. TNFα and IL-1β downregulate macrophage expressed TM (367).

1.42.3 TM structure
TM is a single-chain type 1 transmembrane glycoprotein and its structure can be split into 5 regions (see figure 22):

- N-terminal domain.
- Epidermal Growth Factor (EGF)-like repeats domain.
- serine-threonine rich region.
- transmembrane region.
- short cytoplasmic tail.

The structure of TM varies in other animals compared with humans and resembles the LDL receptor (45, 367).

The N-terminal is a globular domain that enables endocytosis and regulates tumour growth. It has some sequence similarity to C-type lectins and can downregulate the NF-kappa B and mitogen activated protein kinase (MAPK) pathways which are associated with endothelial cell activation and dysfunction (47). The EGF-like repeat region contains six EGF-like modules named EGF 1-6. These modules (or domains) have various roles to play, alone or together, in maintaining structural integrity, stimulating fibroblast growth (EGF 1-6), the binding of thrombin (EGF 5 and 6), protein C (EGF 4-6) and thrombin activatable fibrinolytic inhibitor (TAFI) (EGF 3-6). The EGF modules that bind thrombin increase thrombin-mediated inhibition of plasmin production by its cleavage of single chain urokinase-type plasminogen activator (45, 46).

**Figure 22 Thrombomodulin structure**

The serine-threonine rich region contains 7 sites specifically for the addition of a chondroitin sulfate moiety and is heavily modified by O-linked sugars. Chondroitin is not always bound to TM but its presence promotes stronger binding to thrombin, enhanced protein C activity, faster thrombin neutralisation.
by heparin-antithrombin and the protein C inhibitor and facilitates binding of platelet factor 4 (PF-4) to protein C to augment its activity(37, 38, 45, 47).

The short cytoplasmic tail is the intracellular aspect of TM immediately following the transmembrane region. It activates endothelial nitric oxide synthase 3 (NOS-3) and modulates G protein coupled signaling. Thrombin binding to TM stimulates PLC gamma and PI3K leading to an increase in intracellular calcium and its complexing with calmodulin (Ca2+-CaM). This triggers calmodulin kinase II (CaMKII) which, when phosphorylated, activates NOS-3. The NO produced, in turn, activates Src kinases by nitrosylation. This process plays a part in the inhibition of thrombin mediated PAR activation and cell proliferation through the MAPK pathway(368, 369).

1.42.3.1 TM functions

TM is multifunctional and is involved in anticoagulation, antifibrinolytic, cell adhesion, inflammatory and anti-inflammatory processes.

Soluble forms of TM, derived from proteolysis of the endothelial cell membrane bound protein, circulate in the blood and are excreted in the urine as cleaved fragments(370). Normal plasma levels have been documented between 3-50ng/mL but higher levels occur after vascular damage in many pathological states, including inflammation and sepsis, leading to soluble TM release predominantly from ECs by the action of neutrophil-derived enzymes and metalloproteinases in the extracellular matrix and intramembrane rhomboid proteases(371). The actual function of soluble TM is as yet unclear but it may be vasculoprotective. Elevated plasma TM levels not only indicate endothelial damage or increased endothelial cell turnover, but are inversely correlated with coronary artery disease and may be useful in monitoring organ dysfunction in sepsis and disseminated intravascular coagulation (DIC). Smokers have been found to have raised soluble TM concentrations which correlate with pack years of smoking and their risk of thrombosis(372). Soluble TM concentrations have been shown to be significantly higher in patients with advanced colorectal and pancreas cancers compared to local or locally advanced disease(373). There are, however, significant variations in levels among different cancer types likely due to differences in the underlying tumour biology(374).
Venous thromboembolism in cancer patients undertaking chemotherapy

TM was found to be associated with invasive malignancy approximately 30 years ago with subsequent research demonstrating its expression in several tumour types(370, 375). TM is not as prevalent in adenocarcinomas compared with squamous cell carcinoma and expression in well differentiated squamous cell carcinoma is considerably lower than in normal epithelium(376). Reduced or absent TM expression is an independent prognostic variable associated with shorter survival and increased metastatic potential in human tumours of embryonal, epithelial or lymphatic origin(377, 378). The presence of lymph node metastases also correlates with loss of TM expression in tumours(370, 379). Colon cancer tumour cells lacking TM may also lack significant anticoagulant activity(380).

TM can regulate tumour growth and metastasis by modulating proliferation and invasion and concentrates in regions of cell to cell contact and in the tumour cell cytoplasm (46, 366). TM expression is progressively lower when examining normal epithelium, dysplasia and carcinoma, respectively, in the same tissue type. It is, therefore, thought that the reduced TM may promote loss of differentiation and enhance metastatic behavior. This was shown in hepatocellular carcinoma where the presence of TM was associated with reduced intrahepatic spread, capsular invasion and also portal vein thrombosis(381). TM reduces tumour cell proliferation and invasion in vitro and TM expressing cells produce smaller tumours in vivo(366). This is partly attributed to TMs cell to cell adhesion role which is similar to the effects of E-cadherin(382).

In summary, loss of TM expression in a tumour correlates with a more invasive phenotype and a poorer prognosis for those patients, but TM measurements require validation as a surrogate test for tumour aggression, prognosis and thrombosis risk in cancer patients(366, 383). HMG CoA reductase inhibitors (statins) can induce TM expression independent of cholesterol function, but it remains to be seen if these drugs can impact on cancer growth and progression(370). A recent retrospective case-control study of 740 patients has, however, associated statin use with a significant reduction in the incidence of VTE (8% v 21%) in cancer patients. This finding will need to be validated in a
prospective, randomised, placebo-controlled trial to provide more evidence for the efficacy of statins in this situation(12).

TMpro mice are engineered to produce mutant thrombomodulin, which substitutes a proline for a glutamine at position 387, resulting in a significant reduction in thrombin binding affinity (100-fold) and protein C activation (1000-fold)(384). Experiments involving these mice have shown that endogenous thrombomodulin derived from host endothelial cells, rather than the tumour, can influence cancer progression and result in increased fibrin deposition(385, 386). In a Lewis lung carcinoma model, primary tumour growth did not appear to be influenced by TMpro in comparison to a wild-type mice cohort, but greatly enhanced metastatic potential through supporting early survival of tumour cells newly localised to the lung. This depended upon TF expression, circulating prothrombin, and thrombin activity on targets, such as platelets, and was not seen in a third cohort of mice producing TM without the lectin-like domain. This depended upon TF expression, circulating prothrombin, and thrombin activity on targets such as platelets. It may be that TM modifies metastatic potential by regulating thrombin’s procoagulant function either directly or indirectly through its interactions with protein C(386). TM may act through thrombin-independent processes, as yet, undiscovered. TMpro mice have a reduced ability to activate protein C which results in reduced anticoagulant, antiproliferative and anti-inflammatory processes which may also influence metastatic spread. Murine studies involving the administration of exogenous activated protein C (APC) have shown that it is associated with a modest limitation of metastasis development(387). This has also been seen in mice that overexpress the endothelial protein C receptor (EPCR)(387). Activation of PAR-1 by APC will logically be reduced in TMpro mice, reducing the production of secondary messengers, which may influence endothelial barrier function and anti-inflammatory effects and allow further support to metastatic spread(386).

1.42.4 Fibrinolysis
Genome-wide expression profiling of murine hepatocytes expressing the MET (Mesenchymal-Epithelial Transition) oncogene have shown upregulation of
cyclooxygenase-2 (COX-2) and Plasminogen Activator Inhibitor-1 (PAI-1)(289). Tumours cells may also secrete plasminogen activators and inhibitors (including tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA) and PAI-1 and -2) as well as annexin II, a co-receptor for tPA and plasminogen and have significant effects on the fibrinolytic system. Some components can be induced by VEGF which is able to activate prourokinase on binding to the VEGF receptor 2(388). uPA is the most widely expressed fibrinolytic promoter on cancer cells, which also possess the appropriate receptors (uPARs) on the cell surface to initiate fibrinolysis. This appears to not only impact on bleeding risk, as seen in patients with leukaemia, but also release of tumour cells from the primary site, tumour invasion, angiogenesis and metastasis formation(283, 290, 389). PAI-1, on the other hand, may increase thrombosis risk in solid tumours(15). UPA and PAI-1 may also be useful prognostic markers in breast cancer patients(390).

1.4.3 Angiogenesis

The more efficient a tumour cell is at promoting new blood vessel formation (angiogenesis) and developing metastases, the more aggressive the cancer is likely to be and the faster it is likely to grow. Vasculogenesis is the process by which embryonic progenitor cells develop into the primitive vasculature. The primitive vasculature then sprouts new vessels, remodels itself and can regress in areas (pruning) to produce a coordinated network of blood vessels throughout the developing body as it grows and matures. New blood vessel development from existing blood vessels, termed angiogenesis, is characterised by EC migration, proliferation, extracellular matrix proteolysis and capillary tube formation. Without a blood supply, containing nutrients and oxygen, living tissue cannot grow. Vasculogenesis and angiogenesis are, therefore, downregulated in the healthy adult, except in the tissues of the female reproductive system. Angiogenic activation of ECs in adults is associated with the physiological process of wound healing, pathological processes including cancer, thrombosis, infection and inflammation and is associated with obesity(285, 391).
Professor Judah Folkman was the first to suggest the concept of anti-angiogenesis treatment for cancer. He showed that angiogenesis is crucial to the growth of tumours beyond 2-3mm(392). Tumours initiated angiogenesis via signalling messengers, including Tumour-Angiogenesis Factor (TAF), which was not present in normal tissues apart from the placenta, and was identified after isolation from human and animal tumours. It was mitogenic to endothelial cells (ECs) and induced the formation of new capillaries in animals(392).

1.43.1 Vascular endothelial growth factors, their receptors and other pro-angiogenic factors

The Vascular Endothelial growth factor (VEGF) family are VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF). They are ligands for VEGF tyrosine kinase receptors 1, 2 and 3 (VEGFR-1, VEGFR-2 and VEGFR-3). VEGFR-2 is highly expressed in endothelial cells involved in angiogenesis and in circulating bone marrow-derived endothelial progenitor cells. It is unclear what role VEGFR-1 plays in angiogenesis. VEGF-A (also known as VEGF), a dimeric 34-50kDa protein, is a major mediator of angiogenesis and exerts its effect by interacting with VEGFR-2, also known as foetal liver kinase-1 (flk-1) in mice or kinase-insert domain-containing receptor (KDR) in humans. The predominant form of VEGF-A in the circulation is VEGF165 which is mostly bound to heparin(393). Its binding to VEGFR2 is potentiated by neuropilin-1 which is known to be highly expressed in pancreatic adenocarcinoma tissue, and may have a role in the development of this cancer(394).

Most types of human cancer cells (e.g. colon, breast, ovarian, hepatocellular, cervical, lung) have been shown to express VEGF, which may be induced both genetically (oncogene activation or tumour-suppressor gene inactivation) and by the local environment such as in the presence of increased levels of angiotensin 2 and thrombin, mediated through hypoxia-inducible transcription factors (HIF) 1α, in normoxic as well as hypoxic conditions(327, 338). VEGF may also play a role in cancer development as well as impact negatively on prognosis(306, 395, 396). VEGF may be produced by host cells (α granules of platelets, muscle cells), in association with tumour-associated stromal cells. It acts via paracrine, autocrine or intracrine mechanisms promoting endothelial cell proliferation,
through the mitogen activated protein kinase (MAPK) pathway, and survival, through the phosphatidylinositol3’-kinase (PI3K)-Akt pathway, as well as migration and vascular permeability. New blood vessels induced by VEGF are, however, hyperpermeable and require the influence of other factors to provide vessel stability. This provides an explanation for the microvascular permeability seen in many tumour types (290, 327). Src signalling facilitates disruption of the endothelial cell barrier in the presence of VEGF (397). Murine studies have shown that mice with a mutant VEGF gene in vascular endothelial cells have severe cardiac defects and are at high risk of gastrointestinal perforations and thromboembolic events.

Bevacizumab is a humanised monoclonal antibody targeting VEGF. Bevacizumab was approved for use in metastatic colorectal cancer more than three decades after Folkman first described the concept. Anti-angiogenic drugs have not yet had the impact on cancer that researchers hoped. Only in the treatment of renal cell carcinoma has the use of an anti-angiogenic receptor tyrosine kinase inhibitor improved survival by more than a small number of months in solid tumours. Bevacizumab is effective in improving survival in patients with metastatic colorectal cancer but has no effect on survival in the adjuvant setting (398, 399). These drugs can also have significant adverse effects. In human clinical trials, bevacizumab has been associated with serious complications such as spontaneous bowel perforation, venous and arterial thromboembolic events and significant hypertension.

VEGF is a chemotactic factor for macrophages and can induce macrophage production of tissue factor and TM as well as protein C production in endothelial cells (290, 370). TF is able to trigger angiogenesis independent of the coagulation cascade by phosphorylation of its cytoplasmic tail or, when complexed with FVIIa, can directly activate PARs allowing multiple downstream signalling pathways to be triggered and conferring a more invasive phenotype to tumour cells (400, 401).

1.43.2 The Angiopoietin-Tie2 pathway

The Angiopoietin (Ang) family is made up of four secreted glycoprotein growth factors known as Ang-1, Ang-2, Ang-3 and Ang-4 that are essential for blood

Venous thromboembolism in cancer patients undertaking chemotherapy
vessel formation. They are all ligands for the Tie-2 receptor. Ang-3 and Ang-4 are orthologs found in mice and humans, respectively, and have not been well characterised(402).

1.43.2.1 Angiopoietin 1 (Ang-1)
Ang-1(403) is expressed by pericytes, smooth muscle cells and other perivascular cells. It is involved in regulating normal cell-cell interactions between endothelial cells (ECs)(404) and between ECs and their underlying supporting cells (pericytes/smooth muscle cells), thus maintaining vascular stability(402, 405). Ang-1 induces angiogenesis when transgenically over-expressed in skin in vivo and normalises blood vessel hyperpermeability after VEGF-induced angiogenesis, suggesting essential interactions between these molecules(405). These processes take place through the binding of the Tie-2 receptor which it phosphorylates. Ang-1 is recognised as an activator of the PI3kinase/Akt pathway which inhibits tissue factor expression and may, therefore, protect against thromboembolic events(406).

1.43.2.2 Angiopoietin 2 (Ang-2)
Ang-2 is expressed almost exclusively in ECs and stored in Weibel-Palade bodies (WPB). Activation of the endothelium by cytokines, such as thrombin or histamine, stimulate rapid release of Ang-2 from the WPB(407, 408). Like Ang-1, Ang-2 binds to the Tie-2 receptor but appears to prepare and activate endothelial cells to respond to angiogenic factors such as VEGF thereby inducing vascular destabilisation, sprouting and branching(409). In the absence of VEGF, however, Ang-2 destabilises vessels but vessel regression occurs(410). Ang-2 was initially reported to compete with Ang-1 as an antagonistic ligand inhibiting Ang-1/Tie-2 signalling but more recent research has suggested Ang-2 can be a Tie-2 agonist as well. Little is known about the mechanisms of Ang-2 function on Tie-2(409, 411).

1.43.2.3 The Tie receptors
The Tie (Tyrosine kinase with Immunoglobulin and Epidermal growth factor homology domains) family are two tyrosine kinase receptors expressed on vascular and lymphatic ECs that are essential for blood vessel remodelling during embryonic angiogenesis(402, 404, 405). Tie-1 (previously known as Tie)
and Tie-2 (previously known as Tek) both have unique extracellular domains with two immunoglobulin-like loops flanking three epidermal growth factor-like cysteine repeats and three fibronectin-type III repeats. The cytoplasmic region of the molecules contains conserved tyrosine kinase domains that are interrupted by a short kinase insert(405). Both Tie receptors are structurally similar in the cytoplasmic region (76% sequence identity) but not in the extracellular part (33% similar)(402).

Murine Tie-1 can be detected early in development in differentiating angioblasts of the head mesenchyme, in the splanchnopleura and in the dorsal aorta, as well as in migrating ECs of the developing heart. It is proteolytically cleaved following VEGF stimulation, with the cleaved extracellular portion interacting with Tie-2 and involved with Tie-2 signalling. Although no specific ligand has been identified for Tie-1, at high levels Ang-1 is capable of binding to Tie-1 via integrins(410).

Tie-2 is expressed by haematopoietic cells, including endothelial precursor cells and mature ECs, with expression being more abundant in larger blood vessels. The receptor has also been detected on some tumour-associated monocytes and eosinophils(410). Ang-1 and Ang-2 bind to the same site on the extracellular domain of Tie-2 and have similar affinities to the receptor(410). Low level Tie-2 phosphorylation has been described in the literature in healthy adults, leading to investigators concluding that ongoing low level Ang-1/Tie-2 phosphorylation maintains vessel stability(412). Phosphorylation of Tie-2 by Ang-1 signals primarily through the PI3K-Akt (PKB) pathway, which promotes stabilisation and survival of cells and indirectly prevents Ang-2 expression as well as inhibiting Ang-2 secretion(413). The Nuclear Factor (NF)-κB pathway is also inhibited, preventing inflammation and apoptosis and maintaining an endothelial resting state. Ang-1 appears to inhibit VEGF-induced blood vessel formation and adhesion molecule expression to finish the wound healing process by stabilising the blood vessel lining and returning to quiescence. It is also thought to protect against radiation-induced endothelial damage(410) (see figure 23).
1.43.3 Dysfunction of the Ang-Tie-2 pathway

Ang-1 deficiency causes severe vascular defects including loss of periendothelial support cells\(^{(402)}\). Ang-1 deficiency in mice is not compatible with life and embryos are dead by day 12.5. Ang-2 overexpression, \textit{in vitro}, has a similar phenotype to Ang-1 loss, but has far more severe manifestations, suggesting that it antagonises the Ang-1/Tie-2 interaction. This has not been shown \textit{in vivo} to date\(^{(405)}\). Ang-2 deficient mice appear normal at birth and have been observed to develop normally to adulthood, but some have died within two weeks of birth from chylous ascites depending on the genetically modified mouse investigated\(^{(410)}\).

Tie-2 deficient mice embryos die due to vessel remodelling defects in the yolk sac, brain and heart. Blood vessels are poorly organised, have fewer branches and reduced pericyte coverage. Lack of Tie-2 function leads to endothelial cell apoptosis and subsequently haemorrhage showing that the Ang-Tie-2 system plays a role in vessel remodelling and maturation as well as vessel integrity maintenance\(^{(402)}\). Mice lacking Tie-2 versus mice lacking Ang-1 have a similar phenotype but the manifestations are far more severe in association with Tie-2
deficiency, which suggests that other ligands bind to this receptor and can activate some of the functions of Ang-1 in the very early stages of embryonic development(405). Tie-2 overexpression causes venous malformations and transgenic mice develop psoriatic-like lesions on their skin(414).

Mice lacking the Tie-1 gene, on chromosome 4, die due to loss of the structural integrity of endothelial cells leading to oedema and haemorrhage, but undergo normal developmental angiogenesis and haematopoiesis, suggesting Tie-1 is important for endothelial cell differentiation and regulation of vessel integrity(415) (see table 14).

**Table 14 Observed effects on tumour cells in published murine studies after manipulation of the Ang-Tie2 pathway**

<table>
<thead>
<tr>
<th>Parameter change</th>
<th>Effect on malignant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Pro cancer</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Anti cancer</strong></td>
</tr>
<tr>
<td>Ang-1 overexpression</td>
<td>Increased tumour growth (Glioma in rats)(416)</td>
</tr>
<tr>
<td></td>
<td>Upregulated tumour angiogenesis and inhibition of apoptosis (Cervical)(417)</td>
</tr>
<tr>
<td>Ang-1 deficiency/inhibition</td>
<td>Not compatible with life</td>
</tr>
<tr>
<td></td>
<td>Reduction in tumour size/weight</td>
</tr>
<tr>
<td>Ang-1 overexpression</td>
<td>Reduced tumour growth/proliferation (SCC, breast, colon)(418-421)</td>
</tr>
<tr>
<td>Ang-2 overexpression</td>
<td>Reduced vascular permeability and blood vessel number (Colon)(420, 421)</td>
</tr>
<tr>
<td>Ang-2 overexpression</td>
<td>Increased blood vessel maturity/stability (SCC, breast)(418, 422)</td>
</tr>
<tr>
<td>Ang-1 deficiency/inhibition</td>
<td>Not compatible with life</td>
</tr>
<tr>
<td>Ang-1 deficiency/inhibition</td>
<td>Not compatible with life</td>
</tr>
<tr>
<td>Ang-1 overexpression</td>
<td>More invasive hypervascular tumours (Glioma)(423)</td>
</tr>
<tr>
<td></td>
<td>Supports growth and proliferation (Colorectal, gastric)(424, 425)</td>
</tr>
<tr>
<td>Ang-2 overexpression</td>
<td>Supports angiogenesis and reduced apoptosis (HCC in combination with VEGF)(426)</td>
</tr>
<tr>
<td>Ang-2 overexpression</td>
<td>Tumour growth inhibition and reduction in blood vessel density of tumours (Glioma in rats)(416)</td>
</tr>
</tbody>
</table>
### 1.43.4 The Ang-Tie-2 pathway and cancer

In tumour angiogenesis, loosening of endothelial cell-cell contacts is a prerequisite for blood vessel sprouting and development of new tumour vessels\(^{(427)}\). Previous studies in our laboratory have shown that Ang-1 mRNA levels are significantly reduced in breast and colorectal tumours\(^{(428)}\), (M Currie, personal communication). Tie-2 expression has also been demonstrated on certain tumour cells such as Kaposi’s sarcoma and melanoma and expression is upregulated during tumour angiogenesis.

Ang-2 is only weakly expressed in endothelial cells under physiological conditions, but is dramatically increased in hypoxic conditions and markedly upregulated during tumour growth when vascular remodelling occurs. This has been shown in Kaposi’s sarcoma cells, glioblastoma cell small vessel endothelium and in areas of necrosis and hypoxia. Ang-2 is detectable in the blood of patients with SCC of the oesophagus, HCC, lung cancer, melanoma, stomach, colon and bladder carcinoma. Ang-2 expression correlates with tumour progression in melanoma and is expressed on the tumour cells of stomach, colon, bladder carcinoma, melanoma and non small cell lung cancer\(^{(402, 410)}\). Ang-2 induces tumour neovascularisation in combination with angiogenic factors, such as VEGF or bFGF, and has promoted breast cancer metastasis independent of the Tie-2 receptor by binding to integrin \(\alpha5\beta1\)\(^{(429)}\). This contrasts with Ang-2 overexpression in Lewis lung carcinoma cells and TA3 mammary carcinoma cells where tumour growth was suppressed, angiogenesis was disrupted and apoptosis was enhanced\(^{(430)}\).
Ang-1 overexpression leads to reduced tumour growth in several tumour types. Pericyte coverage of tumour vasculature is greatly increased and hence stabilised. However, Ang-1 has conversely been shown to promote tumour growth in rat gliomas and plasma cell tumours and Ang-1 downregulation in HeLa cells inhibited tumour growth and angiogenesis. Inhibiting and promoting functions of Ang-1 are likely dependent upon tumour cell type and location and the amount of Ang-1 present in relation to the amount of Ang-2 in the tumours (391, 402).

Ang-1 and Ang-2 (as well as VEGF) are also capable of promoting PAF synthesis in ECs, a potent mediator of platelet aggregation, inflammation and angiogenesis which has been associated with tumour growth, invasion and metastasis (299, 431).

1.43.5 The angiopoietin-1 to angiopoietin-2 ratio

Higher levels of Ang-1 mRNA expression have been detected in low grade compared with high grade renal cell carcinomas (432). In gastric tumours higher levels of Ang-2 mRNA expression are associated with advanced disease and increased vascularity of the tumour (425). Ang-2 positive tumour cells are also associated with the presence of metastatic disease in prostate cancer (433). Higher tumour-associated Ang-2 levels have correlated positively with lymph node involvement in oral squamous cell and breast carcinomas and have been associated with poorer prognosis in patients with hepatocellular carcinoma, bladder, prostate and non small cell lung cancers (433-439). It can, therefore, be hypothesised that lower levels of Ang-1 and higher levels of Ang-2 support a more aggressive malignant phenotype which will promote angiogenesis, growth, proliferation, invasion and metastasis. The underlying mechanisms for this switch from quiescence to a malignant microenvironment are, however, still to be understood.

The Ang-1:Ang-2 ratio, obtained by comparing circulating levels of both cytokines in venous blood samples, has been increasingly discussed in the literature as it may give a more accurate picture of the balance of signalling in the Ang-Tie-2 pathway in different disease states compared with the individual measurements of angiopoietin levels in the plasma or tumour. The plasma Ang-
1:Ang-2 ratio was found to be reduced in a study of relapsed or refractory multiple myeloma (MM) patients(440) and is also reduced in newly diagnosed, treatment naïve MM patients compared with healthy individuals of similar age and gender(441). Terpos et al. investigated 174 newly diagnosed MM patients and found that the ratio also correlated with survival with patients with a ratio below the median value of 6.03 having a significantly lower median survival (26.3 v 53 months, p=0.002). Their study found that this correlation with survival appeared to be associated with the biological therapies, bortezomib and thalidomide, rather than with conventional chemotherapy(441).

In both colon cancer and gastric cancer Engin et al. have found significantly higher plasma levels of Ang-2 and soluble Tie-2 (sTie-2) compared with healthy volunteers of similar age and gender(442, 443). Ang-1 levels were not different between the two groups. Ang-2 levels were also noted to be significantly higher in stage III colon cancer compared with patients with stage II disease as has been published by our own laboratory(442, 444). This was not the case with Ang-1 or sTie-2. There was also no correlation between stage of gastric cancer and angiopoietin or Tie-2 levels(443). Plasma levels of Ang-1, Ang-2 and sTie-2 as well as the Ang-1:Ang-2 ratio were also significantly higher in patients with cervical cancer compared with healthy volunteers(445). In a study of 71 patients with acute myeloid leukaemia plasma Ang-2, sTie-2 levels and the Ang-2:sTie-2 ratio were found to be significantly higher than in healthy volunteers with the Ang-2:sTie-2 ratio appearing to independently predict for poorer survival(446).

Vascular endothelial tissue factor (TF) expression has been associated with angiogenesis initiation in breast cancer patients and also correlates with the expression of VEGF(447). As previously discussed, this is likely to occur through coagulation dependent and independent mechanisms. In non-small cell lung cancer high tumour VEGF and Ang-2 levels have been associated with poorer five-year survival following potentially curative surgery(439). To date, there is no published research investigating the involvement of the Ang-Tie-2 pathway with VTE occurrence in cancer patients. As changes in angiopoietins are associated with endothelial and vascular disruption and they are known to interact with VEGF supporting angiogenesis and an inflammatory state, it is
logical to suspect that variation in the expression of these factors may impact on coagulation pathways. APC has been shown to upregulate the Ang-1 and Tie-2 expression in ECs and reduce Ang-2 expression. Thrombin, on the other hand, appears to downregulate this pathway(448).

Between 2006 and 2009 a pilot study was performed by the MacKenzie Cancer Research Group at the University of Otago Christchurch, New Zealand to determine differences in circulating proteins that could identify cancer in patients with VTE. Protein array data from 80 patients (Cancer n=40; (VTE n=20 and no VTE n=20) and non-cancer controls n=40; (VTE n=20, healthy volunteers n=20)) analysed using binary regression, indicated that low circulating plasma Ang-1/high circulating IL-29 could differentiate individuals with cancer with 87% accuracy. Due to the small numbers of patient samples analysed, no significant correlation/association was observed between Ang-1 levels and VTE presence (Gunningham, S. unpublished data).

1.44 Cytokines
Multiple pro-angiogenic and pro-inflammatory factors released from tumour cells and host cells, in response to the tumour, have been identified. TNFα and IL-1β can both induce TF production in vascular endothelial cells. IL-1β levels are increased following chemotherapy treatment in breast cancer patients resulting in increased endothelial cell activity to platelets(290, 449). Both cytokines also downregulate thrombomodulin and EPCR transcription which reduces activation of protein C(367). Numerous cytokines have been associated with tissue factor upregulation and inhibition of plasminogen activator inhibitor (PAI)-1(450, 451). These effects would logically promote a procoagulant state in cancer patients.

IL-1β and thrombin have been shown to stimulate metalloproteinases to release soluble forms of endothelial protein C receptor (EPCR) from the endothelium(452). Soluble EPCR, bound to protein C or APC, is thought to combat inflammation by binding avidly to leukocytes via an interaction between proteinase 3 (PR3) and the leukocyte integrin Mac-1, preventing the cell’s attachment to the endothelium during episodes of inflammation(453). This anti-inflammatory effect appears to be negated in the absence of protein C/APC(47).
IL-8, a potent proangiogenic cytokine, is upregulated by cross-linked fibrin on the surface of vascular endothelial cells. This appears to be a separate angiogenic pathway to that linking VEGF and tissue factor. IL-6 is also linked to thrombosis through endothelial damage(339).

1.45 Leukocytes
Neutrophils (and other granulocytes) appear to facilitate invasion and spread of tumour cells by adhering to them (β2 integrin CD11b/CD18 and selectins) and aiding the transendothelial migration process, as well as releasing matrix metalloproteinases which break down the extracellular matrix and allow tumour cell dissemination(454, 455).

Monocytes/macrophages circulate in the blood and travel to sites of inflammation. They do not normally express TF under quiescent conditions but can be stimulated to do so, in the presence of pathological conditions such as cancer, by inflammatory molecules, complement and immune complexes. This may provide a process by which monocytes invading tumours may interact with tumour cells and increase the procoagulant activity within the tumour microenvironment by promoting intratumoural fibrin deposition. Monocytes have also been shown to promote fibrinolysis(456, 457).

1.46 Summary
The underlying pathophysiological mechanisms leading to venous thromboembolic complications in cancer patients are complex and involve the upregulation of inflammatory and angiogenic processes as well coagulation. The environment is influenced by the patient and the cancer as well as by anti cancer treatments. Ongoing research is slowly piecing together the jigsaw of these dynamic interactions to identify important targets for future therapeutic agents which may not only prevent and treat thrombosis but may also impact as a cancer treatment itself as the processes of tumour survival, growth, proliferation and spread appear to incorporate aspects of the coagulation process.

Thrombomodulin has emerged as a potential marker of arterial thrombosis and cancer stage and progression but little has been published with regards to VTE risk and development in cancer patients. It is not known if soluble plasma levels
of thrombomodulin may aid in the prediction or diagnosis of cancer associated venous thrombosis. As this molecule is involved in anticoagulation as well as anti-inflammatory and anti-proliferative processes and that plasma concentrations may rise with endothelial damage, it would seem logical to investigate its association with VTE in cancer patients before and during chemotherapy treatment.

The Ang-Tie-2 pathway is altered in patients with cancer, with elevated levels of Ang-2 activating endothelial cells and inducing inflammation and angiogenesis in concert with VEGF and many cytokines. Ang-2 is produced by many tumours with raised concentrations detectable in the plasma. Ang-1 maintains vascular endothelial cell quiescence through interaction with its receptor Tie-2. Both Ang-1 and soluble forms of Tie-2 are detectable in the plasma. It would be reasonable to hypothesise that pathologically raised plasma concentrations of Ang-2 would correlate with prolonged endothelial cell activation and damage which may predispose cancer patients to increase risk of thrombosis whereas higher concentrations of Ang-1 may provide a more quiescent environment which may protect against vascular damage and thrombosis. Concentrations of these molecules have been investigated in cancer patients pre and post surgery but have not been monitored during chemotherapy treatment nor investigated for their association with VTE risk / diagnosis despite demonstrated interactions with APC and thrombin. Ang-1, Ang-2 and soluble Tie-2 may, therefore, be novel markers of VTE risk or diagnosis in patients with cancer and warrant investigation.
1.47 Introduction
Chemotherapy can significantly increase the risk of VTE in cancer patients, with an estimated annual symptomatic VTE incidence of 10% (458). The risk is likely to vary depending on the chemotherapeutic agents used as well as the previously mentioned risk factors such as cancer primary, stage and histological subtype. Early human research in this area was undertaken in breast cancer patients, and has provided us with the foundation of our epidemiological data on chemotherapy induced thrombosis and also thrombosis as a complication of hormonal manipulation (102, 290). Since then, VTE research has expanded into other cancers, but we are yet to clearly assign the underlying pathophysiological mechanisms and relative risks for VTE development to individual chemotherapeutic agents and/or regimens. This is due, in part, to the fact that chemotherapeutic agents are often given in combination, and published clinical trials, historically, have not routinely reported on venous thromboembolic adverse events (459). It is hypothesised that the pathophysiological mechanisms described in the previous section are exacerbated by chemotherapy and researchers have sought to clarify our understanding by studying in vitro effects of certain agents. However, those findings have not always translated to a prothrombotic phenotype in clinical practice.

A recent retrospective analysis of 27,479 patients with solid tumour malignancies associated with high VTE risk, registered on the IMPACT health claims database, showed that the overall incidences of VTE at 3.5 and 12 months after chemotherapy initiation were 7.3% (DVT 65.9%, PE 17.6% or both 16.5%) and 13.5% (DVT 62.8%, PE 17.0% or both 20.2%), respectively (460). During this 12 month period the patients with VTE also had a higher risk of major bleeding (19.8% v 9.6%), in line with previous research, and incurred higher health care costs (US $110,719 v US $76,804) than those without VTE (23, 460). A prospective study of over 4000 cancer patients undertaking chemotherapy also estimated a 47-fold increase in mortality associated with VTE compared to the general population without cancer (13).
1.48 Breast cancer chemotherapy and VTE

VTE occurs in up to 0.8% of patients with early stage breast cancer(461). The incidence is markedly increased with the introduction of chemotherapy treatment, with 5-10% of patients with early stage and up to 18% of those with advanced stage breast cancer developing a thromboembolic event, suggesting that VTE risk is attributable to chemotherapy as well as the cancer(462-465). Although mortality associated with this complication is low in early disease (0.2-0.5%), it is much higher in advanced disease (up to 9%)(463). Most VTE occur within three months of commencing treatment with the outcome for patients with VTE being inferior to those without(466).

1.48.1 CMF chemotherapy

Cyclophosphamide, methotrexate and 5-fluorouracil (CMF) combination chemotherapy is one of the oldest treatment regimens used to treat early and advanced stage breast cancer. A number of studies have investigated the effects of this treatment but in relatively small numbers of patients.

One cycle of CMF was associated with a significant reduction in the plasma levels of the endogenous anticoagulants antithrombin, protein C and protein S and an increase in the antifibrinolytic plasminogen activator inhibitor (PAI)-1. Although these results suggested the induction of a procoagulant state, the laboratory results have not correlated with increase d symptomatic thrombosis(467-469). Pretreatment levels of protein C and antithrombin were also noted to be higher in stage II disease compared with stage IV disease, suggesting that a tendency towards a procoagulant state may exist pretreatment in patients with more widespread disease(469).

Pretreatment levels of thrombin-antithrombin complex (TAT) were higher in patients with stage IV disease compared with stage II disease. Levels were within the normal laboratory range pretreatment in all stage II patients but were raised in 57% (n=17) of patients with stage IV disease, again suggesting a more procoagulant state exists with widespread disease. After one cycle of CMF, TAT was significantly raised in both patient cohorts compared with baseline, which would suggest an increase in thrombin generation(469).
1.48.2 CMFVP

Cyclophosphamide, methotrexate, 5-fluorouracil (5-FU), vincristine and prednisone (CMFVP) combination chemotherapy was associated with a 17.6% (n=24) incidence of symptomatic thrombosis or thrombosis found at autopsy in 159 women diagnosed with stage IV breast cancer. This was a composite of venous and arterial events (PE, DVT, renal vein thrombosis, disseminated intravascular coagulation, myocardial infarction and coronary thrombosis). All developed thrombosis within 3.5 months of commencing treatment. Nineteen (12%) patients were diagnosed with VTE which was associated with a seven percent mortality rate.

Twenty patients were prospectively analysed for markers of coagulation of which 10 received CMFVP and 10 did not. Of those not on CMFVP only three were not on a treatment (six received doxorubicin and one received testosterone). The results in both breast cancer patient cohorts were also compared to results obtained from a sample of pooled plasma obtained from 24 healthy males. The prothrombin time was found to be significantly shorter in patients on chemotherapy than those not treated, and the FVIII antigen:activity ratio was significantly elevated in the group receiving CMFVP compared to controls and the pooled plasma. Of note, there were no differences between the cohorts with regards to APTT, fibrinogen, platelet count, antithrombin, FV, fibrin degradation products or plasminogen levels. Although these results have shown procoagulant changes associated with cancer stage and chemotherapy treatment, this particular five-drug chemotherapy regimen is not normally given in routine clinical practice, the numbers in each arm were very small, the control group were heterogeneous with regards to their management and the pooled plasma was obtained from healthy males rather than females.

1.48.3 Anthracycline-based regimens and other chemotherapy agents

Subsequent research has focused on anthracycline-based chemotherapy which has now replaced CMF as the standard of care in most cancer treatment centres around the world. Despite the findings with CMF, adjuvant epirubicin and cyclophosphamide (EC) chemotherapy did not induce significant changes in protein C, antithrombin and PAI-1 post administration.
A randomised trial comparing cyclophosphamide, methotrexate, 5-FU, doxorubicin, vincristine and prednisone with and without tamoxifen in patients with stage II breast cancer found 14 (6.8%) clinically suspected thrombotic events in 205 patients, all of which occurred on chemotherapy. The chemotherapy-only arm exposed the patient to a considerably longer course of cytotoxic treatment (36 v 12 weeks) with this group experiencing more thrombotic events. The 12 week group did not develop thrombosis after the chemotherapy course had finished(464). A similar frequency (5.4%) of venous thromboembolic events was recorded in a retrospective analysis of seven Eastern Cooperative Oncology Group (ECOG) studies of adjuvant breast chemotherapy (combinations of cyclophosphamide, methotrexate, 5-FU, prednisone and tamoxifen) involving 2673 patients, but was confounded by the use of tamoxifen which has been shown to increase thrombosis risk (470). Clahsen et al. subsequently found that patients treated with only one cycle of adjuvant 5-FU, doxorubicin and cyclophosphamide were at three times the risk for developing a thromboembolic event compared with their peers who underwent surgery alone. Thrombosis risk was higher in postmenopausal women and in those that underwent mastectomy compared with wide local excision of their tumour, but was not related to nodal status or oestrogen receptor status(471). A recent retrospective analysis of 280 patients with breast cancer who had adjuvantly received four cycles of epirubicin followed by four cycles of CMF chemotherapy found that 21 (7.5%) developed clinically suspected arterial or venous thrombosis. Venous events were documented in 19 patients. Age greater than 60 years was associated with thrombosis with 27% (15/56) of this subgroup being diagnosed.(472).

Hoy et al. conducted a retrospective audit of breast cancer patients undertaking adjuvant breast cancer chemotherapy in the Australian Capital Territory over a two year period (473). The clinically suspected VTE incidence was almost 17% (54/325), which was considerably higher than previously published studies. This was because they included superficial vein thrombosis but importantly did not include arterial events. They found a significantly higher incidence (27% (47/176)) of VTE in patients treated with 5-FU, epirubicin and cyclophosphamide (FEC) compared with other chemotherapeutic regimens.
(including other anthracycline-containing regimens) (5% (7/149)) with the majority being superficial vein thromboses. It was concluded that the effect of epirubicin on VTE may be dose-dependent with a trend towards higher thrombotic events in those that received 100mg/m², and that 5-FU may also contribute through local vascular endothelial damage at the site of infusion(473).

Doxorubicin can induce endothelial apoptosis and damage. *In vitro* studies have shown that doxorubicin, but not epirubicin, reduces expression of the endothelial protein C receptor (EPCR) on the surface of human umbilical vascular endothelial cells (HUVECs). This impairs the conversion of protein C to APC and is thought to be mediated by free radical metabolites or radical oxygen species(474, 475). When defibrinated plasma is exposed to HUVECs treated with epirubicin or doxorubicin there is an increase in thrombin generation, which was not seen when 5-FU or methotrexate were administered. TF activity was induced by doxorubicin, cytarabine or vincristine *in vitro* using transitional cell carcinoma cells of the bladder resulting in increased thrombin generation(476, 477). It is thought that this is predominantly mediated by increased phosphatidylyserine exposure facilitating the action of TF:VIIa and prothrombinase complexes. It was also noted, to a lesser degree, that anthracycline may induce TF production in monocytes as well as endothelial cells. Of note, TF mRNA was not found to increase in endothelial cells and so the investigators hypothesised that pre-existing “cryptic” TF was decrypted by the chemotherapy and activated(475). Increased levels of plasma thrombin secondary to an increase in TF activity and phosphatidylyserine exposure have also been demonstrated in HUVECs exposed to anthracycline chemotherapy(475). Ma et al. found that chemotherapy promoted a procoagulant state in HUVECs and human microvascular endothelial cells (HMVEC’s)(478). These models may not reflect the *in vivo* effects of chemotherapy on the venous endothelium(477, 479).

The clinical effects of CMF, taxanes, vincristine and the anthracycline-based regimens AC (doxorubicin and cyclophosphamide) and FEC (5-FU, epirubicin and cyclophosphamide) were prospectively investigated in 134 patients undergoing neoadjuvant (n=11), adjuvant (n=87) and palliative (n=36) chemotherapy for a wide range of breast cancer subtypes. The follow up period
was planned for up to two years from recruitment or death. Patients in the palliative group tended to be older than those comprising the other groups, but there was no significant difference in the receptor status of the cancers for oestrogen and progesterone receptors and the human epidermal growth factor receptor-2 between the cohorts. Patients were screened for lower limb DVT, using duplex ultrasound scanning, one month into their treatment schedule and/or if they developed symptoms concerning for thrombosis (461).

The development of symptomatic VTE in 17% (Goodnough et al.), the increased association with advanced disease (17%) compared with earlier stage disease (eight percent) (Mandala et al., Nole et al., Goodnough et al.) and the eight percent mortality rate in patients with advanced disease (Goodnough et al.) were similar to other studies (461, 463, 480, 481).

Consistent with Goodnough et al., 10% (13) of the patients developed VTE of which 70% (nine) were symptomatic. VTE was more common in patients with advanced disease (17%) than in early stage disease (eight percent) which is consistent with previous and subsequent studies (463, 480, 481). Seventy percent of the patients developed VTE within three months of chemotherapy commencement, including all of the patients with advanced disease. The mortality rate in the patients with advanced disease was eight percent which is similar to Goodnough et al. (461, 463).

Potential biomarkers of coagulation were prospectively investigated in the 123 breast cancer patients undergoing adjuvant and palliative chemotherapy in the study by Kirwan et al., with 68 age-matched females with no history of cancer as controls (461). Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, D-dimer, platelet count and thrombin-antithrombin complex were measured using the standardised laboratory assays. TF and VEGF were measured using enzyme-linked immunosorbent assays (ELISA) with the latter being performed on platelet free plasma. Cancer procoagulant (CP) activity was measured by chromogenic assay. Blood was obtained at baseline, 24 hours, four days, eight days and three months (466).

Prior to chemotherapy commencement APTT, D-dimer and fibrinogen were prolonged in both cancer patient cohorts compared with controls. All other
variables were increased in advanced disease compared with the control cohort except TAT and PT. D-dimer, fibrinogen and VEGF were significantly higher in patients with advanced disease compared with early stage disease. Increased concentrations of fibrinogen, D-dimer, TF and VEGF were associated with the development of VTE at three months, and on further analysis the baseline fibrinogen and D-dimer results were felt to be predictors of VTE development, with every 1g increase in fibrinogen doubling the risk of VTE and every 1000ng/mL increase in D-dimer increasing risk 1.8-fold, consistent with previous findings(482, 483). Using a D-dimer cut-off value of 500ng/mL the negative predictive value for VTE development at three months was 97%. Thirty percent (37) of the cancer patient cohort had a D-dimer below this cut-off and could be deemed safe from VTE development over the next three months. This, however, has not yet been validated in other studies(466).

Despite changes in biomarkers, after chemotherapy administration, only the prolonged prothrombin time remained significant after eight days and independently associated with thrombosis after multivariate analysis. If this was not present, as was the case in almost half of the cancer patients in this study, the negative predictive value for VTE at three months was 100%. It is difficult to explain why this parameter alone would predict for VTE development, and it is hoped that validation studies can shed light on the usefulness of this finding and explain the underlying pathophysiology(466).

1.48.4 The influence of chemotherapy on cytokines and adhesion molecules that may induce coagulation

Interleukin (IL)-1β has been shown to be increased in the plasma of breast cancer patients treated with FEC. In vitro studies subsequently suggested that IL-1β induced increased reactivity of endothelial cells to platelets and so may contribute to the procoagulant state(449). VEGF and intracellular adhesion molecule (ICAM)-1 have also been shown to increase after anthracycline-based chemotherapy administration(484).

1.49 Thrombophilias and chemotherapy associated VTE

A prospective observational study investigating the development of suspected VTE in 381 patients with breast or gastrointestinal malignancy, who undertook
adjuvant chemotherapy, reported a rate of 7.9% (30 events)(480). Twenty-eight of the events occurred during chemotherapy treatment and were associated with a past history of VTE and prechemotherapy thrombocytosis (≥300x10^9/l). No symptomatic VTE events were diagnosed in patients diagnosed with pancreas (n=10) or rectal cancer (n=16) although numbers were small. Table 15 summarises the patients who developed VTE during chemotherapy treatment (n=28).

**Table 15 Breakdown of VTE events and chemotherapy treatments(480)**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Chemotherapy regimen</th>
<th>Number of VTE events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>CMF</td>
<td>8/108 (7.4)</td>
</tr>
<tr>
<td>Breast</td>
<td>Anthracycline-based</td>
<td>6/65 (9.2)</td>
</tr>
<tr>
<td>Breast</td>
<td>Taxane-based</td>
<td>2 (not stated)</td>
</tr>
<tr>
<td>Colon</td>
<td>Not stated but likely to be 5-FU</td>
<td>9/122 (7.4)</td>
</tr>
<tr>
<td>Gastric</td>
<td>Not stated</td>
<td>3/51 (5.9)</td>
</tr>
</tbody>
</table>

In this study, patients were screened for inherited VTE risk (protein C, protein S, antithrombin, homocysteine, G1691A factor V and G20210A prothrombin) prior to commencing chemotherapy, but this was not found to be associated with the development of VTE(480). Factor V and prothrombin mutations were not associated with thromboembolic events in a retrospective analysis of patients undertaking germ cell tumour chemotherapy(485).

Blom et al. found that a small number of VTE events in cancer patients may be attributed to thrombophilias with the most common forms, factor V Leiden or the G20210A gene mutations, estimated to cause 8-34 events per 100,000 patients(169). These studies argue that screening cancer patients for thrombophilias is not cost effective.
1.50 Other chemotherapy agents and biological therapies

VTE has been a notable complication of a number of cancers treated with specific chemotherapeutic or targeted therapy regimens.

1.50.1 Cisplatin

Cisplatin has been in use for over 40 years to treat many malignancies including lung, endometrial, head and neck and germ cell tumours. It is a DNA cross-linking therapy which has been associated with increased risk for VTE (Table 16).

**Table 16 Published incidences of VTE associated with chemotherapy regimens containing cisplatin**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Regimen</th>
<th>VTE incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroesophageal</td>
<td>Cisplatin, epirubicin and 5-fluorouracil or capecitabine</td>
<td>12.2 (486)</td>
</tr>
<tr>
<td>Germ cell tumours</td>
<td>Cisplatin and bleomycin</td>
<td>8.4 (485)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Cisplatin, epirubicin and cyclophosphamide</td>
<td>10.6 (102)</td>
</tr>
<tr>
<td>Non small cell lung</td>
<td>Cisplatin and gemcitabine</td>
<td>17.6 (487)</td>
</tr>
<tr>
<td>Cervical</td>
<td>Cisplatin chemoradiation</td>
<td>16.7 (488)</td>
</tr>
<tr>
<td>Urothelial transitional cell carcinoma</td>
<td>Cisplatin, doxorubicin, methotrexate and vinblastine</td>
<td>16.3 (489)</td>
</tr>
</tbody>
</table>

Reported VTE incidence rates are high in cisplatin-based regimens. A VTE rate of 8.4% was reported for cisplatin in combination with bleomycin for the treatment of germ cell tumours, 10% with epirubicin and cyclophosphamide in ovarian cancer, 17% with gemcitabine in non small cell lung cancer, 16% as
chemoradiation in cervical cancer and 7.8% with 5-FU and epirubicin in oesophagogastric tumours(459, 485, 487, 488).

A review of the REAL-2 study, which prospectively compared four regimens in the management of inoperable locally advanced or advanced oesophageal, gastroesophageal or gastric cancers, has provided insight into the impact of platinums and fluoropyrimidines on thromboembolic events(486). The overall rate of VTE was 9.4% (91/964). Of the 91 patients with VTE, 11.2% (54/484) had received 5-fluorouracil and 7.7% (37/480) had received capecitabine, but this difference did not reach statistical significance. The rate of capecitabine-associated VTE is consistent with other studies in advanced gastroesophageal cancer(490, 491). On analysis of the platinum agents, however, cisplatin was associated with significantly higher rates of VTE than oxaliplatin (12.2% (n=60) v 6.5% (n=31); p=0.002). CVAD-associated VTE was significantly higher in patients who received epirubicin, cisplatin and 5-FU (ECF) compared with epirubicin, oxaliplatin and 5-FU (EOF) (10.8% (27/249) v 3.0% (7/235); p=0.0007). These occurred despite the use of low dose warfarin prophylaxis. When CVAD-related events were removed from the analysis, the incidence of VTE in patients who received epirubicin, cisplatin and capecitabine (ECX) were significantly higher than in those receiving EOF (9.1% v 4.4% p=0.038)(486). This study reaffirms the importance of prospective data rather than retrospective review, as reported VTE incidences were markedly higher when recorded prospectively.

A review of pooled single patient data from three large prospective studies on advanced epithelial ovarian cancer found that 2.8% (76/2743) of the combined cohort developed clinically suspected VTE. Half of these events occurred within the first two months following debulking surgery and within the first two cycles of postoperative paclitaxel and platinum-based chemotherapy. On analysis, BMI >30 kg/m² and increasing age were associated with increasing VTE risk, but chemotherapy appeared protective against VTE(194).

In a systematic review and meta-analysis of 8,216 patients involved in 38 phase II and III trials investigating the management of cancers including lung, pancreas and gastroesophageal, cisplatin-associated VTE ranged from 0-17%
with the highest rate found in patients with urothelial tumours treated with cisplatin, doxorubicin, methotrexate and vinblastine(492). This study found a significant increase in VTE risk associated with cisplatin containing regimens (RR 1.67; p=0.01) and especially in those that received weekly cisplatin (RR 1.72; p=0.02). This was independent of cancer primary. This study, however, was unable to rule out the impact of other chemotherapeutic agents on VTE risk(492).

1.50.2 Gemcitabine
The pyrimidine antimetabolite gemcitabine is a potent inhibitor of DNA synthesis and repair and acts predominantly during the S phase of the cell cycle. Actively replicating cells, therefore, are affected including the normal gastrointestinal cells and bone marrow. It has been used to treat many cancers such as cholangiocarcinoma, lymphomas, pancreas, ovarian, non-small cell lung, breast and bladder cancers in combination with other chemotherapeutic agents or as a monotherapy. Gemcitabine has been associated with a number of vascular complications including venous and arterial thrombosis and thrombotic microangiopathy. Thrombocytosis and thrombocytopenia are both associated with gemcitabine use but the exact pathophysiology of venous thrombotic events secondary to this drug are not known. Clinical trials have not always reported on thrombotic events or have not been sufficiently powered to provide unequivocal evidence that gemcitabine alone induces vascular toxicity(493).

To date, haemostatic findings in clinical trials have been conflicting with regard to gemcitabine, as both a monotherapy and in combination with other agents. Gemcitabine infusion studies involving rats have not demonstrated significant damage to arterial or portal venous endothelial cells to provide an explanation for an induced procoagulant state(494). In a prospective, multicentre, randomised phase 2/3 clinical trial the triplet regimen of 5-FU, oxaliplatin and irinotecan (FOLFIRINOX) was compared to single agent gemcitabine as first-line treatment of metastatic pancreas adenocarcinoma. Eleven of 166 (6.6%) FOLFIRINOX patients experienced thromboembolic events over a median follow up period of 26.6 months compared with 7/169 (4.1%) of patients in the gemcitabine arm (NS). It must also be noted that over 40% of the
FOLFIRINOX group received granulocyte colony stimulating factor (G-CSF) injections compared with five percent in the gemcitabine group which may also have impacted on results(495).

Most clinical data have been obtained from trials in which cisplatin was also administered making it challenging to clarify the influence of gemcitabine on VTE risk. In a study of cisplatin-gemcitabine doublet chemotherapy, four out of 49 (8.2%) patients developed thrombotic events which were thought to be temporally associated with thrombocytosis at the time of chemotherapy administration(496). Following blood sample analysis, median homocysteine, D-dimer and fibrinogen levels at baseline assessment were higher than the stipulated normal laboratory range in these patients but did not significantly change during chemotherapy administration at days seven, 14 and 21 of the first cycle. All other measured coagulation markers except for the platelet count (including antithrombin, protein C, protein S and activated protein C resistance assays) were found to be normal and did not significantly change during chemotherapy treatment(496).

Conversely, a small study of six patients receiving cisplatin-gemcitabine chemotherapy alone did find a significant increase in prothrombin fragments 1 and 2 suggesting a potential increase in thrombin generation although other thrombin generation markers did not change significantly(497). A study involving 108 patients with advanced stage lung cancer who were treated with cisplatin and gemcitabine reported a 17.6% (n=19) rate of thrombotic events including 12 venous thromboses. The cumulative incidence of vascular events at one year was 22%(487). An increased procoagulant effect of gemcitabine in combination with cisplatin was not, however, seen in a landmark trial by Scagliotti et al. in comparison to other cisplatin chemotherapy doublets (vinorelbine or paclitaxel) in advanced non-small cell lung cancer, where vascular events were similar in all three arms but thrombocytopenia was more marked when gemcitabine was given(498). Although when given as a monotherapy gemcitabine has been associated with VTE, veno-occlusive disease, thrombotic microangiopathy, capillary leak syndrome and myocardial infarction, many suspect that it is the influence of the cancer itself and the platinum agent that significantly increase thrombosis risk(499-501).
1.50.3 5-Fluorouracil (5-FU)
This commonly used pyrimidine analogue is an established treatment, as a single agent or in combination with other drugs, for many solid tumour malignancies. The published incidence of VTE in colorectal cancer patients treated with 5-FU and leucovorin has been as high as 17% (458, 502). Patients receiving 5-FU infusions as a single agent or in combination with cisplatin have reduced levels of protein C and increased levels of fibrinopeptide A, a breakdown product from the conversion of fibrinogen to fibrin (503-505). Animal and in vitro studies have also confirmed that 5-FU can induce endothelial damage with the most profound effects taking place approximately 72 hours after drug administration commencement (506, 507). 5-FU is also associated with cardiotoxicity and coronary artery spasm (508).

1.50.4 Other agents
TF expression and activity is mediated by endothelial cells, monocytes and platelets (102). Endothelial damage has been demonstrated after treatment with mitomycin C, bleomycin and 5-fluorouracil (5-FU) and is associated with veno-occlusive disease complicating stem cell transplantation (102, 507, 509, 510). Intracellular adhesion molecule (ICAM)-1 and VEGF have also been shown to rise during anthracycline chemotherapy (102).

Despite the research undertaken, we are still unsure as to the relative effects of the tumour biology and chemotherapeutic agents on VTE risk (511).

1.50.5 Thalidomide, lenalidomide and pomalidomide
Thalidomide, an antiangiogenic agent, is not significantly associated with thrombosis as a monotherapy but, when given in combination with chemotherapeutic agents and/or steroids, incidence rates of up to 58% have been reported in myeloma. Thalidomide was initially used as an anti-emetic in pregnancy until the teratogenic effects were discovered leading to the drug’s withdrawal in 1962 (102). This synthetic derivative of glutamic acid inhibits TNF-α synthesis, blocks activation of nuclear factor (NF)-κβ kinase, downregulates expression of cell surface molecules and stimulates IL-2 and interferon (IFN)-γ (102). The risk of thrombosis is approximately five percent when thalidomide is used as a single agent in the treatment of myeloma but this
increases up to 20% in combination with dexamethasone and 40% with doxorubicin, gemcitabine and 5-FU.

VTE risk is significantly higher in patients with newly diagnosed disease compared to those with recurrent or refractory disease(511). Anthracycline, steroid and melphalan combinations appear to add significant risk. Low level evidence that prophylactic aspirin or low molecular weight heparin reduce the risk of VTE in patients on thalidomide treatment has been published(512-514). Thrombotic complications have also been observed in patients treated with newer antiangiogenic agents such as lenalidomide (7-75%) and pomalidomide (12-17%) in combination with high dose dexamethasone or chemotherapy(515-517).

D-dimer, prothrombin fragments 1 and 2, factor VIII, von Willebrand Factor and homocysteine have all been raised above normal concentrations at baseline assessments in myeloma patients but have not significantly changed following treatment with thalidomide or pomalidomide(518-520). No significant changes in soluble tissue factor and soluble P-selectin were observed on pomalidomide therapy when measured by ELISA. Reduced antithrombin levels have been reported at baseline, which returned to normal post thalidomide treatment, and thrombomodulin levels were reduced following one month of thalidomide therapy. Acquired activated protein C resistance independent of Factor V Leiden mutations have been recorded in patients on thalidomide(521).

Whilst concentrations of endogenous procoagulants and anticoagulants, at baseline, and acquired APC resistance and reduced thrombomodulin, on treatment, may increase VTE risk in myeloma patients, chemotherapy and steroids as well as myeloid growth factors are implicated as well. It has been suggested that thalidomide may target PAR-1 present on ECs and impact on coagulation via this G-coupled receptor(522).

1.50.6 Bevacizumab
The humanised monoclonal antibody bevacizumab targets vascular endothelial growth factor (VEGF) and has been investigated as a treatment for a number of cancers including non-squamous non-small cell lung and metastatic colorectal
cancers. A pooled analysis of 6,055 patients with colorectal, breast, pancreas, renal cell and non-small cell lung cancers from 10 studies were analysed (3,448 treated with bevacizumab and 2,607 controls). There was no significant increase in risk of VTE in the bevacizumab group compared with controls, but arterial events were more commonly associated with bevacizumab(523). The VTE findings contrasted with those previously published in a meta-analysis although that analysis was limited by summary rather than individual patient data, and the inclusion of studies that did not report all grades of VTE and/or did not distinguish between venous and arterial events(524).

1.51 Supportive care
Erythropoiesis stimulating agents (ESAs) have been used to reduce red cell transfusion need and improve quality of life in cancer patients but have also been associated with poorer treatment and survival outcomes(525-529). A meta-analysis has shown a significant association between the use of ESAs, such as darbepoietin, and VTE development (RR 1.67) in 9,353 patients involved in 57 trials (530). Further evidence has been provided in cervical cancer studies where significantly higher incidences of VTE are seen when ESAs are employed compared with management without ESAs(531, 532). This may be due to the inhibition of endogenous anticoagulants such as antithrombin and protein C as demonstrated in the blood of renal dialysis patients on an ESA, but it may also relate to the fact that erythropoietin is an inducer and regulator of angiogenesis(533-535). HUVECs proliferate and stimulate PAI-1 in response to both ESA and G-CSF exposure(536). Increased platelet reactivity, endothelial activation and platelet production in response to ESAs in healthy human subjects also suggests the development of a thrombogenic environment(537).

The use of granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) subcutaneous injections to reduce the length of the neutropenic nadir in patients treated with chemotherapy have been associated with thrombotic episodes in breast cancer patients(538).

1.52 Summary
Chemotherapeutic agents are associated with VTE but the effects of individual agents are difficult to evaluate given that most are administered in combination
with other agents. Myeloma treatments in combination with steroids (10-75%) and platinum agents (9-18%) appear to carry a higher risk for VTE but data for other agents are conflicting. Reporting of VTE complications in prospective clinical trials of chemotherapeutic agents is necessary to establish the association of VTE with particular therapies, and any associations with cancer primary and stage. As was shown in the REAL-2 study, higher incidences of VTE are recorded when data is collected prospectively. Currently there is little high level evidence to guide decision making on thromboprophylaxis during chemotherapy treatment.
**VTE risk factors, prediction and primary thromboprophylaxis in cancer patients undergoing chemotherapy**

**1.53 Introduction**

In cancer patients, VTE is often diagnosed late, and anticoagulation administration is associated with a two-fold higher risk of bleeding complications in patients with cancer compared with medical patients without cancer(23). A logical approach, therefore, could be to identify patients at high risk of VTE and provide them with prophylactic (low dose) anticoagulation in the hope that this will prevent VTE occurrence, improve patient outcomes and reduce bleeding complications.

Research performed in Christchurch has shown that there is a need for improved assessment of cancer patients to guide our decision making on VTE prophylaxis. This was echoed in a recently published prospective multicentre trial performed in five American academic hospitals(539, 540). The American College of Chest Physicians (ACCP) guidelines for thromboprophylaxis only recommend its use in cancer patients if they are hospital inpatients and immobile or perioperative without contraindications to anticoagulation. There is no recommendation for thromboprophylaxis in ambulant outpatients during chemotherapy treatment courses, on hormonal treatments such as tamoxifen nor to improve survival(20, 249). Researchers, therefore, are looking for ways to:

- diagnose VTE at an early/subclinical stage in the hope that timely initiation of treatment will reduce VTE-related morbidity and mortality.
- identify patients at high risk of VTE development to tailor prevention strategies.

**1.54 Primary prevention of VTE**

While primary prevention of VTE appears to be a sensible approach, the risk of significant bleeding on thromboprophylaxis is at least three to four percent in cancer patients, and so it would be prudent to tailor appropriate prophylactic
anticoagulation to those cancer patients at high risk for VTE and avoid exposing low risk patients to a potentially harmful intervention which may provide minimal benefit(541, 542).

Risk assessment for VTE in cancer patients is not routinely carried out in New Zealand, as has been the case in many other countries(8, 543). The FRONTALINE survey registered the responses of 3,891 clinicians from the fields of medical oncology, surgery and haematology and showed significant variation on perceptions of VTE risk in cancer patients and clinical practice in the use, type and duration of thromboprophylaxis. There was also variation in the choice and duration of treatment for established VTE and whether that treatment was initiated as an inpatient or outpatient. Although this study has limitations as to the conclusions drawn it does highlight that guidelines are required to assist clinicians with their decision making and that further trials are required to address treatment choice and duration both in prophylaxis and treatment.

The current guidelines followed in New Zealand for thromboprophylaxis in cancer patients are the 9th ACCP guidelines which do not recommend thromboprophylaxis during chemotherapy on current evidence(20).

1.54.1 Vitamin K antagonists

Data published, to date, on the primary prevention of VTE in cancer patients has been conflicting. Early studies were performed using vitamin K antagonists (VKA) and the first study showing a statistically significant reduction in VTE in ambulatory patients on chemotherapy with a VKA compared with placebo was a double-blind randomised trial using low dose warfarin in stage IV breast cancer patients(544). Three hundred and eleven patients were randomised to 1mg warfarin for six weeks followed by dose-adjusted warfarin to a target international normalised ratio (INR) of 1.3-1.9 (n=152) or placebo (n=159) while on chemotherapy and for one week after completion. Over the follow-up period seven (4.4%) patients in the placebo arm and one (0.6%) of the patients in the warfarin arm developed symptomatic VTE. No increase in bleeding was documented with warfarin and survival was similar in both cohorts. Despite this result, the use of prophylactic warfarin has not been adopted as a standard of care in this population(544).
Bern et al. showed a reduction in VTE events in cancer patients with central venous access devices (CVADs) in their study which randomised patients to 1mg of warfarin or placebo. The study was, however, small with 121 patients recruited and only 80 completing follow up. Nevertheless 37.5% of patients developed thrombosis in the placebo arm compared with 9.5% in the warfarin arm. Two larger trials were subsequently published investigating the effect of low dose warfarin on VTE risk in cancer patients with CVADs as its use had not been widely accepted. Couban et al. showed no reduction in risk in comparison to placebo if a fixed 1mg oral dose of warfarin is used(545). Young et al. also did not demonstrate a reduction in catheter-related thrombosis with fixed dose or dose adjusted warfarin to a targeted INR of 1.5 compared with no prophylaxis. They did, however, observe an increase in major bleeding events associated with dose adjusted warfarin(546).

1.54.2 Low molecular weight heparins

The Fragmin Advanced Malignancy Outcome Study (FAMOUS) was a double-blind, placebo-controlled, multicentre study administering 5,000 international units (IU) of dalteparin or 0.9% saline subcutaneously, for up to one year, to patients with stage III or IV cancers of the breast, lung, GI tract, pancreas, liver, genitourinary tract, ovary or uterus. The primary outcome of this study was one-year survival and symptomatic VTE events were a secondary endpoint. Of the 374 analysed patients recruited to this study only nine developed a symptomatic VTE (four (2.4%) in the dalteparin group and five (3.3%) in the placebo group, suggesting that this approach did not significantly reduce VTE and slightly increased major and minor bleeding risk. No survival advantage was seen with the use of dalteparin prophylaxis at one year in this study, although it was acknowledged that the study was underpowered to fully investigate this endpoint and a high proportion of patients died early in the study suggesting they may not have had time to benefit from the intervention(547).

The Prophylaxis Using Dalteparin in Patients with Glioblastoma Multiforme (PRODIGE) trial randomly allocated subcutaneous administration of 5000 units of dalteparin or saline placebo, for six months, to patients with newly diagnosed glioblastoma multiforme (GBM) or anaplastic astrocytoma. One hundred and
eighty six patients were recruited over four years, at which point the trial was discontinued due to poor accrual. Ninety nine received dalteparin and 87 received the placebo. Nine patients in the LMWH arm and 13 patients in the placebo arm developed VTE in the first six months of follow-up, but five patients in the LMWH arm experienced major intracranial bleeding versus one in the placebo arm. This study did not detect a significant reduction in VTE events but was underpowered to do so(548).

A prospective, randomised trial of thalidomide and chemotherapy versus chemotherapy alone was performed in patients with myeloma. The first 221 patients recruited received no anticoagulation, and the next 35 were given 1mg warfarin daily. In the first 87 patients who received thalidomide and chemotherapy 30 (34.5%) developed VTE and 11 out of the 35 (31.4%) patients on warfarin also developed VTE. In June 2001, prophylaxis with enoxaparin was introduced in the thalidomide arm. Enoxaparin 40mg was administered in a daily subcutaneous injection to 68 patients on thalidomide and 62 control patients. Fifteen percent of the thalidomide patients and 14.5% of the control group developed VTE(549). This trial has been criticised due to the fact that the enoxaparin group was not observed at the same time as the thalidomide groups not on enoxaparin and, therefore, comparisons may be subject to bias. There was also no central adjudication of thrombotic events. Subsequent studies have suggested benefit of using aspirin as prophylaxis in myeloma patients on thalidomide or lenalidomide, but these studies are limited either due to their lack of randomisation, lack of a comparative control group or because the two populations compared were from different points in time, introducing bias into the comparison(511, 550).

The PROphylaxis of ThromboEmbolism during CHemoTherapy (PROTECHT) study investigated the use of 3,800 IU nadroparin, administered daily as a subcutaneous injection, as primary thromboprophylaxis in adult cancer patients commencing chemotherapy for metastatic or locally advanced lung, gastrointestinal (stomach, colon, rectum), pancreatic, breast, ovarian or head and neck cancers(551). This prospective, multicentre, double-blind, randomised, placebo controlled study assigned 1150 patients in a 2:1 allocation in favour of nadroparin and followed them for up to four months(551).
In contrast to the findings of PRODIGE and FAMOUS, a statistically significant reduction in thromboembolic events was observed in favour of nadroparin (15/769 (2%) v 15/381 (3.9%) on placebo (one-sided p=0.02)). Eleven patients in each group (1.4% and 2.9% respectively) were diagnosed with venous events. Response to chemotherapy and development of superficial thrombophlebitis or asymptomatic thromboembolic events were similar in both cohorts. Five patients in the nadroparin cohort had major bleeding events compared to zero patients in the placebo group but this difference was not statistically significant. Minor bleeding was similar in the two groups.

Reported events in the placebo cohort were much lower than those estimated in the prestudy statistical planning. Antiplatelet agents were an exclusion criteria in this study, which may have significantly impacted on recruitment and ruled out patients with cerebrovascular, ischaemic heart and peripheral vascular diseases. Many patients died at home and were deemed to have died from progressive cancer, appropriately without the need for a post mortem examination, and so it would have been difficult for investigators to accurately identify those that died from thromboembolic events.

A subsequent randomised open-label study investigating nadroparin’s effect on survival was performed using weight-adjusted therapeutic doses for two weeks followed by four weeks of half dose followed by a cyclical course of two weeks at therapeutic doses and four weeks of washout period for up to six cycles. Five hundred and seven patients with stage IIIB non-small cell lung cancer, hormone-refractory prostate cancer and locally advanced pancreas cancer were randomised to nadroparin or no nadroparin in addition to their usual care. There were no significant differences in survival between the cohorts (nadroparin 13.1 months v control 11.9 months) nor VTE events (nadroparin 16 v control 15). These findings suggest that continuous use of nadroparin is more effective than the use of washout periods in thromboprophylaxis.

Following on from the PROTECHT study, SAVE-ONCO is the largest thromboprophylaxis study published to date. This was a prospective, double-blind, multicentre, placebo-controlled trial of an ultra low molecular weight heparin named semuloparin. This drug was designed to have high anti factor Xa
activity but minimal anti IIa (thrombin) activity. The primary endpoint for the study was symptomatic venous thromboembolic events (any limb DVT, PE, death related to VTE) in ambulatory adult patients with solid tumours undergoing chemotherapy occurring from randomisation to three days post completion of trial drug/placebo course. A secondary endpoint was overall survival one year post randomisation or at study end date, and the main safety analysis was clinically relevant bleeding over the same time period as the primary endpoint.

Patients recruited to the study had locally advanced or metastatic cancer of the lung, pancreas, stomach, colon, rectum, bladder or ovary, all highly associated with VTE on chemotherapy. Baseline risk factors were history of VTE, CVC use, obesity, age >75 years, chronic heart and respiratory failure, venous insufficiency or varicose veins and use of hormonal therapy.

Consented patients were randomised (1:1) to receive 20mg of semuloparin or placebo subcutaneously once daily commencing on the first day of chemotherapy. The treatment was to continue for a minimum of three months, unless chemotherapy was stopped prior to this, and a new chemotherapy regimen was not started. If the chemotherapy continued beyond three months, the treatment continued until the chemotherapy course was completed or stopped. Other anticoagulants and fibrinolytics were not permitted in the study but antiplatelet drugs and non-steroidal anti-inflammatory drugs (NSAIDs) were. Follow up took place at routine chemotherapy appointments.

It was assumed that there would be a four percent VTE event rate in the placebo arm. Demographic data were well balanced between the two cohorts, with two-thirds diagnosed with lung or colorectal cancer and two thirds with metastatic disease.

Median duration of treatment in both arms was 3.5 months and reasons for not completing treatment were similar in both cohorts. A statistically significant reduction in symptomatic VTE was observed in the semuloparin cohort (20/1608 (1.2%) v 55/1604 (3.4%) on placebo (HR 0.36; p=0.001)). There were no significant differences in survival, major or minor bleeding events or serious adverse events between the two cohorts. Cancer patients with three or more...
baseline risk factors were at higher risk of VTE in both cohorts, with 12.5% (5/40) in the placebo group versus 2.5% (23/932) with no risk factors (553).

Apixaban, an oral anti Xa inhibitor, was investigated in a smaller randomised study (ADVOCATE) at three different daily doses (5, 10 or 20 mg) compared with daily placebo in 125 patients with a variety of advanced cancers undergoing chemotherapy. Three of the 30 patients on placebo developed VTE versus none of the 95 patients on apixaban. Bleeding risks appeared similar between groups (554).

1.54.3 Pancreas cancer
Having established that VTE risk was very high in pancreatic cancer patients, two prospective thromboprophylaxis clinical trials have been undertaken. Charité ONKologie (CONKO) 004 investigated the use of 1mg/kg enoxaparin for 12 weeks followed by a daily 40mg dose for the duration of chemotherapy treatment versus no anticoagulation. Significant reductions in VTE were seen in the enoxaparin arm (1.9% v 9.9%) at three months and were maintained out to 12 months (555).

FRAGEM investigated the use of dalteparin (200 IU/kg (two weeks) followed by 150 IU/kg (four weeks)) versus no dalteparin. VTE events were significantly lower in the anticoagulated group (3% v 23.3%, p=0.002) at 100 days of follow up (556). Neither of these trials detected differences in survival but were underpowered to do so and, of note, there were no significant differences in major bleeding between treatment and non-treatment arms.

A subgroup analysis involving 254 patients with metastatic pancreatic cancer in the SAVE-ONCO study found that semuloparin reduced VTE events compared with placebo (3/126 (2.4%) v 14/128 (10.9%)) lending more weight to the value of thromboprophylaxis in patients with advanced pancreatic cancer (557).

1.54.4 Other studies
Low molecular weight heparin (LMWH) prophylaxis was assessed in the Thromboembolism Prevention in Cancer (TOPIC) trials. TOPIC I randomly assigned 353 patients with metastatic breast cancer to six months of certroparin 3,000 units daily subcutaneously or placebo. The patients were screened for
DVT using four-weekly ultrasound scans. Four percent of patients in both arms developed DVT but the incidence of bleeding was 1.7% in the certoparin arm and 0% in the placebo arm. TOPIC II was an identical study design but randomising 547 patients with stage III/IV non-small cell lung cancer. VTE developed in 4.5% of patients in the certoparin arm versus 8.4% in the placebo arm but this reduction was not statistically significant. Bleeding was recorded in 3.7% of patients on LMWH and 2.2% on placebo.

A combined analysis of the lung cancer patients recruited to the TOPIC II and PROTECHT studies (n=811) revealed a 3.2% (15/467) incidence of symptomatic VTE in the anticoagulated group and 5.5% (19/344) in the placebo group. Addition of asymptomatic thrombosis to the analysis increased the number of events in the anticoagulant group to 20 (4.3%) and 27 (7.8%) in the placebo group. Twelve of 472 (2.5%) patients on anticoagulation and six of 353 (1.7%) patients on placebo experienced major bleeding. The number needed to treat to prevent one VTE was 28 and the number needed to cause one major bleeding event on anticoagulation was 125.

A subgroup analysis of the lung cancer patients recruited to the SAVE-ONCO study revealed that semuloparin also reduced VTE events and/or VTE-related death (1.5% (9/591) v 4.2% (25/589)).

1.54.5 Thromboprophylaxis for upper extremity thrombosis in cancer patients

Treatment approaches for upper limb venous thrombosis have been extrapolated from lower limb DVT clinical management where risk factors can be quite different. The natural history of clinically unsuspected upper extremity thrombosis in cancer patients with and without CVADs is unknown.

The literature remains controversial as to the merits of routine thromboprophylaxis in this setting, even in the presence of a CVAD. Three randomised controlled trials do not support the use of daily low molecular weight heparin or low dose warfarin in preventing clinically manifest upper extremity thrombosis. However, if routine diagnostic imaging (venogram or ultrasound) is employed to screen for clinically unsuspected as well as clinically suspected thrombosis, significant reductions in CVAD-associated
thrombosis are seen. In the control groups the diagnoses of thrombosis were considerably higher with mandatory screening compared with the symptomatic end point studies (12-62% v 4-12%). This will likely be related to a significant number of clinically unsuspected thrombi found, heterogeneous referral criteria for imaging and/or differences in patient characteristics in each study.

In a prospective observational study investigating the effect of thromboprophylaxis on thrombosis-related complications and mortality, in 1,410 cancer patients with CVADs, one-third received thromboprophylaxis (low dose warfarin (76%) or low molecular weight heparin (24%)) with a six month median duration of treatment. Superficial venous thrombosis, but not DVT or PE rates, were significantly reduced in the prophylaxis cohort. However, 150 patients were lost to follow up, with significantly more lost from the no prophylaxis group (137 v 13). Advanced disease and not receiving thromboprophylaxis were associated with significantly poorer survival(561). Study accrual was stopped earlier than planned in 2005 after two randomised, placebo-controlled trials, reported no benefit from thromboprophylaxis(545, 562).

Balancing the benefit of clot prevention with a higher bleeding risk has led to international guidelines not recommending thromboprophylaxis to date(20, 563-567). Further prospective studies are required to provide more clarity for clinicians on the benefits and risks of prophylaxis and treatments. Studies concentrating on cancer patients will be important as the risk of VTE is likely to be increased further by chemotherapeutic agents, blood products and other treatments administered.

1.55 Anticoagulants and cancer survival

It has not yet been established whether anticoagulation prolongs the survival of patients with cancer. Small cell lung cancer has been associated with increased thrombin generation and fibrin deposition. Studies in patients with this cancer have shown promising results but were underpowered or were single arm studies.
1.55.1 Small cell lung cancer and anticoagulation

Zacharski et al. first reported an improvement in survival in small cell lung cancer when warfarin was used in combination with chemotherapy (568). This was not confirmed in a larger multicentre study but did show an improvement in response to treatment (569). Two subsequent underpowered studies in small cell lung cancer have reported survival advantages with the use of heparin in addition to chemotherapy. Le Beau et al. randomised 277 patients to receive two to three daily subcutaneous unfractionated heparin injections for five weeks (n=138) or no treatment (n=139) concurrent with chemotherapy and brain irradiation protocols. The heparin injections were to stop one week after the chemotherapy. Patients were also stratified by stage of disease (106 patients with limited stage and 143 patients with extensive stage disease) and further stratified by the chemotherapy regimen received (alternating v sequential), which made patient numbers very small in each subgroup and limited statistical analysis. Overall response rates were similar between the two cohorts, but complete response rate was higher in the anticoagulation arm (33% v 21% p=0.03) as was median overall survival (317 days v 261 days). Survival was significantly improved in limited stage patients treated with an alternating chemotherapy regimen but not a sequential regimen. The opposite was found in extensive stage patients (570). Altinbas et al. investigated the efficacy of low molecular weight heparin in a prospective randomised study of small cell lung cancer patients undertaking chemotherapy v chemotherapy and daily dalteparin (5,000 IU) for the 18 week duration of chemotherapy. Forty patients (25 limited stage and 15 extensive stage patients) received chemotherapy alone and 39 patients (23 limited stage and 16 extensive stage patients) received chemotherapy and dalteparin. In both limited and extensive stage disease progression free survival and overall survival were significantly prolonged in the anticoagulated cohort. This study again recruited small numbers to each subgroup but has again suggested that anticoagulation in small cell lung cancer may be important for outcome as well as thromboprophylaxis (571).

1.55.2 Studies with results awaited

FRAGMATIC is a phase III randomised trial currently accruing across the United Kingdom and investigating the effect of six months of dalteparin (5,000
IU) when added to standard therapy versus standard therapy alone in patients with lung cancer. The primary outcome measure will be overall survival and the secondary endpoints are venous thrombotic event free survival, serious adverse events, metastasis-free survival, toxicity, quality of life, levels of breathlessness, anxiety and depression, cost effectiveness and cost utility. This large study hopes to accrue 2,200 patients over three years followed for a minimum of one year post randomisation. It is powered to detect a five percent increase in one year survival.

GASTRANOX is enrolling patients with advanced gastric cancer and will randomise patients to standard chemotherapy alone or standard chemotherapy with six months of enoxaparin dosed at 1mg/kg. Primary end points are event free survival (EFS), defined as composite endpoint of overall survival and free of symptomatic VTE up to one year from start of treatment. Secondary end points are incidence of symptomatic VTE, overall survival, major and minor haemorrhages during chemotherapy and/or up to 30 days after the last dose is provided, serious adverse events, heparin induced thrombocytopenia (all recorded up to one year from the start of treatment). All VTE events will be adjudicated. No subcutaneous placebo will be offered to the group randomised to standard chemotherapy alone.

In summary, there is little consensus in the literature to show efficacy of primary prophylaxis for VTE in ambulatory cancer patients and to guide decision-making. Despite existing evidence for the efficacy of adjusted-dose warfarin in metastatic breast cancer patients, this is not standard of care due to concerns over bleeding complications and the need for laboratory monitoring of the INR. Dose adjusted warfarin or LMWH (and aspirin) are recommended by international guidelines for myeloma patients on thalidomide (and related agents) in combination with chemotherapy or dexamethasone but not in solid tumours such as small cell lung cancer.

1.56 Clinicopathological factors in cancer associated VTE
Risk factors and biomarkers of increased thrombotic risk have subsequently become an area of interest to identify patients at high risk of VTE development in order to maximise benefit (VTE prophylaxis and survival outcomes) and
minimise complications such as bleeding. A number of potential biomolecular markers and risk factors for VTE development in cancer patients have been published and are summarised in tables 17 and 18 below.

**Table 17 Risk factors associated with VTE in cancer patients**

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khorana et al.(185); Lin et al.(574)</td>
<td>Older age (≥65 yrs) for hospital inpatients</td>
</tr>
<tr>
<td>Agnelli et al.(192)</td>
<td>Older age (≥60 yrs) undergoing surgery</td>
</tr>
<tr>
<td>Khorana et al.(184, 185)</td>
<td>Female gender &gt;65 yrs</td>
</tr>
<tr>
<td>Cronin et al.(258); Ritchie et al.(17)</td>
<td>Older age associated with increased incidence of unsuspected PE</td>
</tr>
<tr>
<td>Chew et al.(193, 254, 255); Alcalay et al.(187); Rodriguez et al.(575); Khorana et al.(184); White et al.(576)</td>
<td>Ethnicity (African American high risk, Asian/ Pacific Islanders low risk) in certain cancers</td>
</tr>
<tr>
<td>Blom et al.(169)</td>
<td>Inherited thrombophilias</td>
</tr>
<tr>
<td>Prandoni et al.(23, 151)</td>
<td>Previous VTE</td>
</tr>
<tr>
<td>Numico et al.(487)</td>
<td>Poor performance status</td>
</tr>
<tr>
<td>Khorana et al.(185); Alcalay et al.(187); Rodriguez et al.(575); Chew et al.(254, 255)</td>
<td>Comorbidities</td>
</tr>
<tr>
<td>Agnelli et al.(192)</td>
<td>Prolonged immobility</td>
</tr>
<tr>
<td>Khorana et al.(24, 196)</td>
<td>Cancer primary site, BMI≥35, erythropoiesis stimulating agents</td>
</tr>
<tr>
<td>Shah et al.(577); Wun et al.(182)</td>
<td>Cancer primary site,</td>
</tr>
<tr>
<td>Blom et al.(183); Wun et al.(182)</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>Wun et al.(181)</td>
<td>Mucinous tumours</td>
</tr>
<tr>
<td>Agnelli et al.(551)</td>
<td>High BMI, poor ECOG performance status, analgesia and corticosteroid use, platinum and gemcitabine chemotherapy</td>
</tr>
<tr>
<td>Khorana et al.(184)</td>
<td>Infection, obesity, lung/kidney disease, anaemia, neutropenic complications.</td>
</tr>
<tr>
<td>Konigsbrugge et al.(578)</td>
<td>Varicose veins</td>
</tr>
<tr>
<td>Kroger et al.(579)</td>
<td>Inpatient treatment, family history of venous thromboembolism, Past history of DVT, chemotherapy</td>
</tr>
<tr>
<td>Blom et al.(169) Zangari et al.(514) Heit et al.(95) Haddad &amp; Greeno(102)</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Deitchez et al.(580)</td>
<td>Hormonal therapy</td>
</tr>
<tr>
<td>Bohlius et al.(530)</td>
<td>Erythropoiesis- stimulating agents</td>
</tr>
<tr>
<td>Kakkar et al.(581)</td>
<td>Major surgery</td>
</tr>
<tr>
<td>Heit et al.(95) Khorana et al.(196)</td>
<td>Hospital inpatient</td>
</tr>
<tr>
<td>Study</td>
<td>Type of study</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Khorana et al.(196)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Ay et al.(582-584)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Kroger et al.(585)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Satoh et al.(195, 586)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Vormittag et al.(587)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Simanek et al.(588)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Ferroni et al.(589, 590)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Zwicker et al.(591)</td>
<td>Prospective observational and interventional</td>
</tr>
<tr>
<td>Khorana et al.(592)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Thaler et al.(593)</td>
<td>Prospective observational</td>
</tr>
<tr>
<td>Thaler et al.(594)</td>
<td>Prospective observational</td>
</tr>
</tbody>
</table>
1.57 Candidate biomarkers for VTE prediction in cancer patients

1.57.1 C-reactive protein (CRP)

CRP is a non-specific inflammatory marker which is elevated in many cancer patients. It is normally synthesised in the liver and adipocytes, but production can be induced in tumour cells by the infiltration of lymphocytes and monocytes. CRP appears to support tissue factor and P-selectin production from monocytes and endothelial cells. It is also thought to be an activator of the complement system\(^2\). CRP concentrations in the blood have been associated with the development of cancer associated thrombosis and have been seen to reduce after tumour resection suggesting some correlation with tumour mass. In a prospective study of 507 patients with various malignancies, C-reactive protein was the only laboratory parameter to predict symptomatic VTE development over a six month follow up\(^5\).

1.57.2 CATS

The prospective Cancer and Thrombosis Study (CATS) is being conducted by the University of Vienna in Austria. This group recruits patients with solid tumours and follows them for up to two years to see if they develop symptomatic VTE. At consent, venous blood is collected and information on medical history, clinical examination and the cancer are recorded, but patients are not screened for VTE. Patient plasma is frozen until analyses of various candidate biomarkers for VTE risk are performed. Patients are followed up every three months to review their management and assess for the development of VTE. Each VTE event is assessed and confirmed by an independent review committee which convenes annually. To date, a number of potential biomarkers have been identified (see table 19).
Table 19 Potential biomarker predictors for VTE risk published by CATS

<table>
<thead>
<tr>
<th>Publication</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ay et al.(582)</td>
<td>Raised D-dimer and prothrombin fragments 1 and 2</td>
</tr>
<tr>
<td>Ay et al.(583)</td>
<td>High Soluble P-selectin</td>
</tr>
<tr>
<td>Vormittag et al.(587)</td>
<td>Raised Factor VIII levels</td>
</tr>
<tr>
<td>Ay et al.(584)</td>
<td>High Peak thrombin (Thrombin Generation Assay)</td>
</tr>
<tr>
<td>Simanek et al.(588)</td>
<td>Raised platelet count</td>
</tr>
<tr>
<td>Konigsbrugge et al.(578)</td>
<td>Varicose veins</td>
</tr>
<tr>
<td>Thaler et al.(593)</td>
<td>Low platelet count and high soluble P-selectin (in high grade gliomas only)</td>
</tr>
</tbody>
</table>

1.58 Predictive scores for developing VTE on chemotherapy treatment

In 2008, Khorana et al. published a risk score for symptomatic VTE in cancer patients undergoing chemotherapy to help identify those at high risk who may benefit from thromboprophylaxis(24). The patients had been enrolled in the Awareness of Neutropenia in Chemotherapy (ANC) Study Group Registry, which is a prospective observational study monitoring cancer patients initiating a new chemotherapy(24).

Two thousand seven hundred and one (n=2701) patients were randomly assigned to a derivation cohort where multiple clinical and laboratory variables were analysed to look for associations with symptomatic VTE development on chemotherapy. In a prior analysis, a haemoglobin level of <10g/dL (or the use of an erythropoiesis stimulating agent (ESA) and a platelet count of ≥350x10⁹/L had been associated with the development of VTE(196). Cut-off levels for other
Continuous laboratory variables, analysed during the derivation process, were obtained from the laboratory normal ranges published by the Massachusetts General Hospital (595).

Sixty (2.2%) patients developed symptomatic VTE in this cohort and, on multivariate analysis, risk factors were identified. These risk factors were then combined into a probability score and assessed using a separate validation cohort of 1,365 patients (see Table 20). Both cohorts of patients were well balanced on assessment of demographic data. In this cohort 28 (2.1%) patients developed symptomatic VTE.

Table 20 Khorana predictive model for chemotherapy-associated VTE

<table>
<thead>
<tr>
<th>Cancer primary</th>
<th>Gastric, GOJ, pancreas</th>
<th>2 points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung, lymphoma,</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>gynaecological, bladder, testicular</td>
<td></td>
</tr>
<tr>
<td>Other primaries</td>
<td></td>
<td>0 points</td>
</tr>
<tr>
<td>Hb &lt; 100g/L or use of ESA</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>WCC &gt; 11x10⁹/L</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>Platelet ≥ 350x10⁹/L</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>Body Mass Index ≥ 35kg/m²</td>
<td></td>
<td>1 point</td>
</tr>
</tbody>
</table>

In the validation cohort, the score was found to a have a negative predictive value of 98.5%, a positive predictive value of 6.7%, a sensitivity of 35.7% and a specificity of 89.6%. This suggests that the score is excellent in identifying patients who are low risk for developing symptomatic VTE on chemotherapy but cannot be relied on to identify those patients that will develop VTE. This score could, therefore, currently be used to guide clinicians in withholding anticoagulation prophylaxis in low risk patients.
Ay et al. expanded the Khorana score with the addition of soluble P-selectin, D-dimer and other tumour primaries, including high grade glioma, in their prospective observational cohort study of 819 patients consented to the CATS (see table 21) (596).

**Table 21: Khorana/Ay score for VTE prediction in cancer patients**

<table>
<thead>
<tr>
<th>Cancer primary</th>
<th>Gastric, GOJ, pancreas, high grade gliomas</th>
<th>2 points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung, lymphoma, kidney, myeloma, gynaecological, bladder, testicular</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>Other primaries</td>
<td>0 points</td>
</tr>
<tr>
<td><strong>Hb &lt; 100g/L or use of ESA</strong></td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td><strong>WCC &gt; 11x10⁹/L</strong></td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td><strong>Platelet ≥ 350x10⁹/L</strong></td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td><strong>Body Mass Index ≥ 35kg/m²</strong></td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td><strong>Soluble P-selectin ≥ 53.1ng/L</strong></td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td><strong>D-dimer ≥ 1.44 ug/L</strong></td>
<td></td>
<td>1 point</td>
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</tbody>
</table>

Sixty one (7.4%) patients had developed VTE at the time of analysis during a median follow-up of 656 days. With the addition of these two variables the performances of the new score versus the original score for high risk patients (score ≥5 v ≥3) at six months in this study were as listed in table 22.
Table 22 Comparison of the Khorana and Khorana/Ay scores

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Khorana score (original validation cohort) (24)</th>
<th>Khorana score (CATS cohort) (596)</th>
<th>Khorana/Ay score (CATS cohort) (596)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>35.7</td>
<td>31.9</td>
<td>19.1</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>89.6</td>
<td>91.9</td>
<td>98.2</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>6.7</td>
<td>22.1</td>
<td>42.9</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>98.5</td>
<td>94.9</td>
<td>94.4</td>
</tr>
</tbody>
</table>

The adapted score, like the original, was excellent in characterising patients at low risk for symptomatic VTE, but its ability to clearly identify those that would develop VTE was only slightly better than the original. There were, however, significant differences in the cohorts of patients investigated between the original Khorana study and the CATS study:

- Khorana analysed only those patients having chemotherapy, whereas CATS analysed patients who were untreated or underwent radiation treatment and/or surgery as well as chemotherapy.
- The CATS follow up time (21.4 months) was considerably longer than the Khorana follow up time (median 2.5 months)
- VTE rates were higher in the CATS study (7.4% v 2.1%)
- Brain (high grade glioma) tumours (very high risk), myeloma and kidney carcinoma (high risk) were included in the Ay modified score but not in the original Khorana score.

Both studies had obtained samples at baseline but did not collect blood at other timepoints.
1.59 Other validation studies for the Khorana score

Other studies have employed the Khorana score prospectively or retrospectively to assess its utility in other population cohorts.

1.59.1 The SAVE-ONCO study
Using the Khorana score in the analysis of the SAVE-ONCO study results, it was found that VTE incidence did rise with increasing Khorana score. In the placebo cohort 5.4% (15/279) of the patients who were stratified as high risk (≥3) were diagnosed with symptomatic VTE. The large majority of patients were intermediate risk (1-2 points) and 3.5% (35/1003) developed VTE. In the low risk (0 points) group 1.3% (4/301) developed VTE. These findings fit with a strong negative predictive value but also confirm the lack of sensitivity and low positive predictive value of this risk assessment model(597).

1.59.2 The PROTECHT study and score
The PROTECHT study investigators concluded that risk scores would be useful to tailor thromboprophylaxis, in cancer patients, to the high risk groups.

The Khorana score was adapted into the PROTECHT score by including the use of carboplatin, cisplatin and gemcitabine chemotherapy. A post hoc analysis of the PROTECHT data had found high rates of VTE in patients receiving these agents individually or as a platinum and gemcitabine doublet(598). If one of these agents was administered a score of one point was assigned to the patient but if a platinum agent was used in combination with gemcitabine then two points were assigned (see table 23). The investigators retrospectively analysed the data of 378 patients on the placebo arm of the PROTECHT study and compared the performances of the Khorana score to the adapted score. Fifteen (4%) VTE were diagnosed in this arm of the study during treatment. The patients were not screened for unsuspected VTE(599).

The PROTECHT score classified a higher proportion of patients in the high risk group than the Khorana score (124 (32.8%) v 45 (11.9%)) and also identified twice the number of patients who developed VTE as high risk (10 v 5). The scores showed good specificity and negative predictive value but poor sensitivity and positive predictive value, as the vast majority of patients
classified as high risk did not develop suspected VTE (Khorana 40/45 (89%) v PROTECHT 114/124 (92%) and only a small proportion of those in the low to intermediate risk group developed VTE (Khorana 10/336 (3%) v PROTECHT 5/254 (2%). This study was limited by its retrospective nature and small numbers of patients both with and without VTE. It will require prospective evaluation(599).

Table 23 The PROTECHT score- an adaptation of the Khorana score

<table>
<thead>
<tr>
<th>Cancer primary</th>
<th>Gastric, GOJ, pancreas</th>
<th>2 points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung, lymphoma, gynaecological, bladder, testicular</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>Other primaries</td>
<td>0 points</td>
</tr>
<tr>
<td>Hb &lt; 100g/L or use of ESA</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>WCC &gt; 11x10⁹/L</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>Platelet ≥ 350x10⁹/L</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>Body Mass Index ≥ 35kg/m²</td>
<td></td>
<td>1 point</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Platinum (cisplatin or carboplatin)</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>Platinum and gemcitabine</td>
<td>2 points</td>
</tr>
</tbody>
</table>

1.59.3 Other studies

1.59.3.1 Spanish pancreas cancer study
A retrospective review of 84 consecutive Spanish patients with locally advanced or metastatic pancreas adenocarcinoma were analysed to derive the incidence of suspected and unsuspected VTE and the utility of the Khorana score. Thirty
(35.7%) patients developed VTE with two-thirds experiencing VTE in the first six months after chemotherapy initiation. On Khorana score analysis 42.8% were intermediate risk and 57.2% were high risk. VTE events occurred in 33% of the intermediate category and 37.5% occurred in the high risk category. The Khorana score did not discriminate between the two groups but the study is limited by its retrospective nature and small sample size (600).

1.59.3.2 Philadelphia study
Kearney et al. retrospectively reviewed the medical records of 112 patients diagnosed with a range of solid tumours and lymphoma who had undergone chemotherapy treatment in the last two years. The score not only correlated with VTE risk but also with mortality in this cohort. A high Khorana score predicted VTE in 41.4% (12/29), intermediate score 15.9% (10/63) and low score 5% (1/20) (601).

1.59.3.3 Portal vein thrombosis (PVT) and pancreas cancer study
A Seattle group retrospectively reviewed the records of 108 patients with pancreas cancer of which 32 (30%) developed PVT. The high risk group did develop a higher proportion of PVTs (9/35 (26%)) than those of lower risk (10/73 (14%)) but this did not reach statistical significance (602).

1.60 Thrombin assays
A single global test of coagulation ability has been pursued for many years to enable the identification of patients with disrupted coagulation status, at risk of either thrombosis or haemorrhage. Precise and reproducible assays quantifying thrombin generation have been sought, but it has been challenging to measure thrombin levels in the blood due to its rapid episodic production and elimination. Assays developed to measure thrombin levels in vivo have been complex and time consuming, requiring significant laboratory technician time. To date, a standardised, precise, practical, efficient and affordable test that can be used in routine clinical practice has not been produced and validated as a predictive tool for VTE development in cancer patients.

Assays analysing thrombin generation were first described in the literature approximately 60 years ago but these assays were time consuming and labour
intensive involving manual evaluation of the produced thrombin’s ability to clot fibrinogen at different points in the coagulation process (603, 604). At one minute intervals, small aliquots of the clotting mixture were transferred from the main test tube to subsampling test tubes containing fibrinogen, and the time taken to develop visible clot was measured. This time was calculated to be inversely proportional to the concentration of thrombin in that particular subsample. Clot developing in the main test tube was moved to the side of the tube manually with the use of a small wooden stick to facilitate aliquot aspiration for subsampling (604, 605). Although imprecise, these were the first studies to graph the thrombin generation curve against time.

The Thrombin generation curve depicts 3 distinct phases:

- **Initiation phase** where thrombin is initially developed in small quantities after a lag phase where no detectable thrombin is produced.

- **Propagation phase** where the development of initial thrombin prompts an explosion of its own production many fold

- **Tail phase** where production drops to undetectable levels and endogenous anticoagulation processes neutralise the thrombin effects.

Thirty years later, Hemker et al. introduced a chromogenic substrate in the place of fibrinogen during the subsampling phase of the test, which was specific for thrombin and produced a colour when cleaved that could be detected using photomeric computer software. Prior to analysis each sample required defibrination to reduce turbidity as this would impair the detection of the colour produced during the coagulation process and consequently undercall the concentration of thrombin produced over time (606). A thrombin generation curve was again produced using the computer software which converted the units of colour detected into thrombin concentrations at different time points in the coagulation process.

The test was further refined using a slow-acting chromogenic substrate specific for thrombin to allow continuous measurement of the cleaved substrate and avoiding the need for subsampling. This fully automated test could only be
performed on defibrinated platelet free plasma samples, which significantly limited the global use of the test (607).

In 2000, a slow-acting fluorogenic substrate was developed in place of the chromogenic substrate, allowing analysis of plasma samples containing both platelets and fibrinogen/fibrin, as the detection of fluorescence was not impaired by the turbidity of the sample. This is one of two commercial assays that have been used over the last 14 years to investigate hyper- and hypocoagulability in numerous patient cohorts (608). This assay measures all of the above mentioned parameters of the thrombin generation curve but can also calculate the velocity index which is the highest rate of thrombin production during the thrombin burst or propagation phase.

This Thrombin Generation Assay (TGA) (Thrombinscope BV, Stago Australia/New Zealand) analyses plasma from venous blood collected in citrate blood tubes and can be used to look at many aspects of the coagulation system. Depending on what is being investigated platelet-rich or platelet-poor plasma can be used. The citrate prevents the coagulation process from initiating in the blood collection tube although cannot prevent contact activation which has led to research into the use corn trypsin inhibitor as a contact pathway inhibitor.

At analysis platelet free plasma is pipetted into microtitres. Each sample is assayed in triplicate to minimise the influence of anomalous results. Calcium chloride, synthetic phospholipid (PL) and Tissue Factor (TF) are added to the samples along with a fluorogenic agent known as Z-Gly-Gly-Arg-AMC.

As the coagulation cascade progresses thrombin is produced and the fluorogenic agent is cleaved by thrombin. The resulting fluorescence can be detected and quantified and is not affected by the turbidity of the sample due to clot development. It is, however, affected by so called inner filter effects such as quenching of the fluorescence signal by molecules that have already been converted and by the plasma colour which will differ between patients and plasma samples which need to be calibrated for. This is achieved using a control sample which contains a known amount of substrate converting activity (thrombin calibrator) and patient plasma without TF and PL to initiate coagulation. The calibrator is thrombin bound to alpha2-macroglobulin (A2M).
Thrombin bound in this form cannot be inhibited by plasma protease inhibitors, does not catalyse the clotting process but is able to cleave the fluorescent substrate. A2M is a large plasma protein produced mainly in the liver. It can also be produced in macrophages, fibroblasts and adrenocortical cells. This antiprotease is able to inactivate a large number of proteinases including thrombin and can also act as a carrier protein for many growth factors and cytokines. Neonates and children have relatively high levels of this protein in their blood but adults only have low levels which have a negligible effect on coagulation.

Calcium chloride (CaCl2) and the fluorogenic agent (FluCa) are the final components added to all wells at the same time to prompt commencement of the coagulation process and the fluorescence is recorded by a fluorometer. The fluorescence is a surrogate measurement of the thrombin concentration in the plasma under the assay conditions. In the calibrator well the fluorogenic agent is converted at a constant rate initially producing a linear tracing. The slope of this tracing gives the calibration factor needed to convert raw fluorescent units (RFU) into thrombin concentration units (nM). Towards the end of the experiment the linear curve bends due to the inner filter effects and consumption of fluorogenic agent. This deviation is analysed by computer software and used to correct the fluorescence measured in the measurement well. Spurious substrate conversion by the A2M-thrombin also generates fluorescence in the measurement well and is subtracted using the computer software. Once all this has been corrected for, the thrombin curve is produced (thrombinogram).

As seen in figure 24 the following parameters can be measured:

- The lag phase- calculates to the clotting time.
- Peak thrombin concentration.
- Time to peak thrombin concentration.
- Velocity Index- the steepest gradient of the propagation phase of the thrombin curve.
- Endogenous Thrombin Potential (area under the curve)- the total enzymatic work performed by the produced thrombin during the assay.

- Start tail/Tail phase- the time from peak thrombin level to return to baseline.

**Figure 24 The thrombin generation curve (thrombinogram)**

1.60.1 Areas of controversy with the TGA

Ongoing issues with this particular assay are related to the preanalytical phase, the amount of tissue factor, thromobomodulin and phosphatidylserine used, the use of corn trypsin inhibitor and the time it takes to prepare samples for analysis.

During venesection blood cells can haemolyse due to trauma. Haemolysis was a major factor that predicted for anomalous results with the TGA(609). This was more likely when blood was drawn from an intravenous catheter or butterfly system than when a conventional straight needle was used with Vacutainer technology(609).
Tissue Factor and phospholipid of varying concentrations can be used. Corn Trypsin Inhibitor (CTI), a specific inhibitor of factor XIIa, can prevent contact activation of the coagulation pathway if required. The addition of thrombomodulin or activated protein C (APC) can investigate the protein C pathway. Thrombin generation is influenced by age, sex, BMI, genetic factors and medications such as the oral contraceptive pill and anticoagulants.

Loeffen et al. have suggested simple guidelines to standardise the TGA preanalytical process:

- Use a conventional straight needle with vacutainer to minimise the risk of haemolysis.
- Use a Monovette tube and/or use CTI to minimise contact activation of the coagulation cascade.
- Use a 2.5-5 mL discard tube first when collecting blood samples.
- Obtain plasma from whole blood as soon as possible, snap freeze in liquid nitrogen and store at -80°C until analysis.
- Double centrifugation of samples- 2,000g for 5 mins then 10,000g for 10 mins.
- Once plasma is thawed, analyse immediately. If not possible, then plasma is most stable at room temperature.

These guidelines, however, only appear to impact on results of assays using no TF or a low concentration of TF (1pM). TGA results were not significantly altered by the variables described above when using 5pM of TF as an initiator in this study (609). Although it is important to try and standardise all reagents and methods for thrombin generation analysis, it is also important to acknowledge that a test destined for regular use in the clinic will have to be applicable to the “real world” where blood is obtained from patients using a number of methods. In the field of Oncology there is increasing use of CVADs such as PICC lines, Hickman lines and portacaths. These indwelling devices provide a convenient way for intravenous medications and chemotherapy to be administered and for
venous blood to be aspirated from cancer patients, some of whom may have challenging venous access or needle phobia. Although the blood drawing method did impact on sample haemolysis, in the study by Loeffen et al., other studies have not shown this to be a significant issue (610, 611).

It must also be noted that even though it is possible to add substrates such as TF, thrombomodulin and phospholipid to the assay it is not possible to assess the effects of blood flow or the endothelial lining in this test.

1.60.2 The TGA and predicting thrombosis in cancer patients
Ay et al. were the first to suggest that the TGA may predict for VTE development in cancer patients undergoing chemotherapy (584). They analysed venous blood collected in plasma vacuum citrate tubes using sterile, atraumatic puncture at baseline in patients recruited to the ongoing Cancer and Thrombosis Study (CATS). Batched aliquots of platelet poor plasma were analysed after storage at -80°C using a calibrated TGA. 1,033 patients were used in the analysis of which 77 (7.5%) had experienced VTE events over a median observation period of 517 days (584).

Cancer patients with VTE were found to have higher peak thrombin levels, shorter lag times, shorter time to peak thrombin and a higher velocity index compared with those without VTE. The ETP was not significantly different between the two groups. A peak thrombin >611 nM had a significantly higher six-month probability of VTE than those patients with lower peak levels. On multivariate analysis it was noted that the association of VTE with peak thrombin increased with every 100 nM increase of thrombin after adjustment for age, gender, surgery, chemotherapy and age. TGA could, therefore, be used as a predictive tool for VTE in cancer patients but requires further validation in other cancer populations with similar malignancies (584).

These patients only gave blood at entry to the study and did not supply blood routinely throughout the follow up process. Some patients also developed their VTE up to two years from baseline blood sampling. It is difficult to know what other factors may have contributed to VTE development in the intervening
period, and so prediction over a shorter timeframe may be more appropriate for this potential predictive tool.

It would appear advantageous to look at the global picture rather than focus on just one aspect of coagulation when it comes to cancer patients as there may be different underlying mechanisms that predispose individual patients to develop thrombosis or haemorrhage. Those with a thrombotic tendency may produce higher levels of thrombin and/or produce lower levels of endogenous anticoagulants, such as antithrombin, compared with their peers who have not experienced thromboembolic events. The opposite may be the case for cancer patients who experience haemorrhage.

1.61 Clinician opinion and VTE in cancer patients

There is ambivalence in clinicians’ attitudes with regard to the investigation and treatment of cancer associated VTE. The perception of large PEs being a “nice way to go” may not always be the case(612, 613). Patients with life-limiting illnesses may have goals they wish to achieve before they die, such as being at a child’s wedding or going on a holiday. A rapid death from VTE may not allow patients to achieve these goals and this may impact on family members’ grief. The VTE may not kill the patient but may severely disable them resulting in reduced quality of life, mobility, organ function and chronic symptoms that may prevent the patient from achieving and/or enjoying the achievement of their goals. Patient-centred care is what is strived for and so patients’ wishes and opinions must be sought as they may be quite different from those of the treating clinicians.

International guidelines have been published by a number of medical bodies to advise on the prevention and management of cancer associated VTE. Suboptimal management continues despite these readily accessible publications(459). Until recently, clinical trials inconsistently reported on thrombotic complications, as shown by a systematic review of pancreas cancer trials(614). Following the NCI-CTG PA.3 trial the 14 % VTE rate was not included in the initial publication but was subsequently published on request(615). This is most likely due to VTE not having been perceived as a drug-related adverse event(459).
1.62 Summary

Delayed management of both suspected and undiagnosed unsuspected VTE may impact on patient survival and quality of life both directly from venous obstruction by the thrombus and indirectly from tumour cell survival, growth and spread being supported by the unimpeded coagulation process. Anticoagulant treatment, in combination with chemotherapy, may potentially improve survival and response rates, as suggested in underpowered clinical trials investigating the treatment of small cell lung cancer(568, 570, 571). This provides intriguing but limited evidence suggesting that cancer progression can be affected by pathological changes in the haemostatic processes of the body which may be modified by targeted treatments once the pathophysiological mechanisms are fully understood in specific cancer types. Mortality attributed to cancer progression rather than clinically unsuspected or suspected VTE may be reduced with thromboprophylaxis in high risk patients and requires further research.

The Khorana score is the first published risk assessment model to focus on ambulant cancer outpatients receiving chemotherapy. These patients are important to target with thromboprophylaxis as they are usually of good performance status and VTE development may significantly reduce quality of life, performance status, fitness to receive antineoplastic treatments and survival. The original study cohorts used to develop and validate the Khorana score were not screened for unsuspected VTE, and so the precision of this score has not been fully validated. It is important to know if this score predicts for clinically unsuspected as well as clinically suspected VTE. Screening for VTE in clinical trials, however, is challenging with regards to cost and resource utilisation, and ethically raises concerns over longterm risk from CT radiation exposure versus the benefit from detection of VTE at an early stage. There is growing evidence, however, indicating that patients who develop clinically unsuspected VTEs experience poorer outcomes compared with patients who do not develop VTE and that those outcomes are similar to those of patients with suspected VTEs.

As bleeding risk is twice that of patients without cancer, a risk score will enable identification of patients at low risk for VTE who do not require
thromboprophylaxis and subsequently minimise iatrogenic bleeding complications and financial burden. However, the existing risk scores have low positive predictive value for VTE in patients classed intermediate or high risk, and have neglected unsuspected VTE. Further exploration of angiogenic and other factors, previously discussed in this literature review, and the TGA may yield useful biomarkers of risk.
Chapter 2
Research Study Outline
Chapter 2 Research Study Outline

Our knowledge of venous thromboembolic disease in cancer patients is predominantly based on retrospective data and extrapolated evidence from studies of medical patients, of whom only a proportion had been diagnosed with cancer. This study was designed to prospectively investigate the development of clinically suspected and unsuspected (as defined in the literature review section 1.3.3) VTE in patients with cancer, establish the prevalence of VTE at baseline assessment, and then the incidence during chemotherapy treatment. The design also enabled assessment of published risk assessment models for VTE prediction and investigation of potential novel markers of VTE risk and diagnosis in patients with cancer undertaking chemotherapy.

Study aims

1. Establish the prevalence/cumulative incidence of venous thromboembolism (VTE) in cancer patients undertaking chemotherapy at the Canterbury Regional Cancer and Haematology Service (CRCHS).

2. Identify potential clinicopathological biomarkers predictive or diagnostic for VTE development in cancer patients undertaking chemotherapy.

3. Identify differences in these potential biomarkers between cancer patients and volunteers without cancer at baseline assessment.

4. Assess the performance of the Khorana score, Khorana/Ay score (The Vienna model) and PROTECHT score as risk assessment models (RAMs) for predicting VTE development in cancer patients undertaking chemotherapy(24, 596, 599).

5. Investigate the utility of a calibrated, automated fluorogenic thrombin generation assay in predicting/diagnosing VTE development in cancer patients, at baseline and undertaking chemotherapy.

6. Investigate changes in thrombomodulin and antithrombin levels during chemotherapy and their association with VTE in cancer patients undertaking chemotherapy.
7. Investigate ratios of thrombin generation variables, thrombomodulin and or antithrombin in cancer patients undertaking chemotherapy and their association with VTE.

8. Investigate changes in angiopoietin-1, angiopoietin-2 and/or soluble Tie-2 receptor levels in cancer patients undertaking chemotherapy and their association with VTE.

9. Investigate ratios of angiopoietin-1, angiopoietin-2 and soluble Tie-2 receptor levels with each other in cancer patients undertaking chemotherapy and their association with VTE.

**Chapter 3 Materials and methods**

Outlines the process of recruitment, assessment and follow up.

**Chapter 4 Results**

**Sections 4.1-4.10 Recruitment and demographics**
This section outlines demographics and compares study findings between patients with cancer and volunteers without cancer.

**Sections 4.11-4.19 Baseline prevalence and cumulative incidence of VTE**
Describes VTE events at baseline and in follow up and analyses blood markers that may be associated with VTE risk and/or diagnosis. It also includes cumulative incidence and survival analyses.

**Sections 4.20-4.28 VTE events and associated changes in clinicopathological variables for cancer patients undertaking chemotherapy**
Describes chemotherapy regimens administered and focuses on VTE events on chemotherapy as well as further blood marker analysis for VTE prediction and/or diagnosis on chemotherapy.

**Sections 4.29-4.36 Risk Assessment Model analysis**
Analyses the utility of published risk assessment models in the diagnosis of VTE at baseline and in assessing VTE risk on chemotherapy.

**Sections 4.37-4.43 Thrombin Generation Assay analysis**
Focuses on results of the thrombin generation assay and ratios of its variables to antithrombin and thrombomodulin.

**Section 4.44-4.52 Angiopoietin-Tie-2 pathway analysis**
Focuses on the blood marker results of the Ang-Tie-2 pathway and ratios of the three markers assayed with each other.

**Chapter 5 Discussion and further work**
This summarises the main findings of the study and relates these to current knowledge.
Chapter 3
Materials and Methods
Chapter 3 Materials and Methods

Venous thromboembolism in cancer patients undertaking chemotherapy

3.1 Materials and methods

This prospective, observational study was approved by the New Zealand Upper South Island B Regional Ethics committee (URB/11/02/005) in accordance with the Declaration of Helsinki.

Consecutive adult cancer patients, assessed and treated at Christchurch Hospital and St. George’s Cancer Care Centre, Christchurch, New Zealand, were recruited after written informed consent was obtained. All patients were provided with an information sheet providing a brief overview of the background and requirements of the study prior to consent being obtained. The eligibility criteria for the study were as follows:

Inclusion criteria (must be “Yes”)

- Newly referred ambulant patients with a histological/cytological diagnosis of cancer OR
- Ambulant cancer patients with recurrent or progressive disease about to commence chemotherapy/chemoradiation.
- Males or females aged 18 years or older.
- Eligible for staging CT of the chest with contrast and ultrasound of the lower limbs OR
- Eligible for ultrasound of the lower limbs.
- Written informed consent obtained.

Exclusion criteria (must be “No”)

- Patients on anticoagulation (low molecular weight heparins, unfractionated heparins, direct and indirect factor Xa inhibitors and oral vitamin K
antagonists) or treated with anticoagulation in the last 4 weeks. Patients on aspirin, dipyridamole and/or clopidogrel were able to participate.

- Patients with a known clotting disorder or thrombophilia.
- Pregnant or lactating women.
- Previous chemotherapy treatment in the last three months.
- Previous radiation treatment in the last three months.
- Surgery in the last two weeks (excluding insertion of a central venous access device).
- Hypersensitivity to contrast media for the CT scan (PE prevalence study) cohort.
- Patients unable to comply with the protocol.

For participation in the study all inclusion criteria had to be fulfilled, but one exclusion criterion sufficed for exclusion.

### 3.2 Assessment and follow up

The target population were cancer patients due to commence chemotherapy. Consented patients were scheduled to attend up to three separate appointments from their usual clinical care during the following time points:

1. Baseline assessment prior to commencing chemotherapy.
2. 42 to 100 days from baseline assessment.
3. 12 weeks to six to nine months from baseline assessment at completion of chemotherapy treatment.

If patients presented to medical staff outside of scheduled appointments, with symptoms/signs concerning for VTE, a study visit assessment was performed at the time of the acute presentation. At each visit all patients underwent:

- an interview on their medical history, which included information about their cancer (see appendix 3).
- a clinical examination (see appendix 3).
- venesection for biomarker analysis.

Information was crosschecked or obtained from clinical, radiology, laboratory and/or pathology records in patients’ hospital files. Patients were assessed at any time if there was concern for a clinically suspected VTE during the follow up period. Participants were able to withdraw from involvement in the study at any point and were not followed up if they went on to receive treatment other than chemotherapy (radiation treatment, surgery, hormonal manipulation) following their initial assessment. If patients moved to another treatment centre outside of Christchurch, New Zealand, it was not possible to follow them up on study, because they would be unable to attend assessment in Christchurch or provide necessary blood samples.

### 3.2.1 Radiological assessment

Following clinical assessment all cancer patients underwent a Doppler compression ultrasound scan (CUS) of both lower limbs to screen for proximal lower limb deep vein thrombosis and/or superficial vein thrombosis. If patients developed symptoms or signs suggesting below knee or upper limb thrombosis, at any point during study participation, an ultrasound was performed in the relevant area to look for thrombus. Scans were to be performed within seven days of each clinical assessment and took place outside of usual working hours (0800 to 1600 hours), to avoid impact on hospital service provision, unless the clinical presentation required urgent investigation.

Only those cancer patients that required a computed tomography (CT) scan of their chest with contrast for routine cancer staging purposes were screened for pulmonary embolism (PE). This would minimise the radiation exposure to patients. These patients were consented to undergo a computed tomography pulmonary angiogram (CTPA) scan of the chest instead of the routine portal venous phase staging CT protocol (see section 1.27.1). This allowed radiologists to report on the presence of PE without impacting on the ability of the scan to detect the presence of intrathoracic malignancy or other abnormalities. The CT scanners and protocols used during this study are summarised in table 24.
Patients were excluded from CT imaging if they:

- Had previously experienced a hypersensitivity reaction to the intravenous contrast used during CT scans.
- Exhibited significant renal impairment (creatinine ≥150 µmol/l).
- Did not require a contrast CT scan of the chest for staging purposes as deemed by their treating clinician.
- Refused to undergo a CTPA examination.

Patients underwent CTPA scans at any point during the study if there was clinical concern for the presence of a PE, or further CT staging of the chest with contrast was required. CTPA imaging was not requested beyond the baseline assessment, in patients who did not require further cancer staging, to minimise radiation exposure. All CT scans of the abdomen and pelvis performed on cancer patients consented to this study were screened for venous thrombosis.

Table 24 Multi Detector Computed Tomography (MDCT) scanners and CTPA protocols

<table>
<thead>
<tr>
<th>CT</th>
<th>128- slice dual source</th>
<th>64-slice single source</th>
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<td>Imaging slice width (mm)</td>
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<tr>
<td>Rotation speed (s)</td>
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<td>Pitch</td>
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</tr>
<tr>
<td>Table speed (mm/ rotation)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Collimation</td>
<td>128x 0.6mm</td>
<td>64x 0.626mm</td>
</tr>
<tr>
<td>Imaging exposure</td>
<td>80-120kVp 200mAs</td>
<td>80-120kVp</td>
</tr>
<tr>
<td>Contrast</td>
<td>100-120ml @ 4-5ml/s</td>
<td>100-120ml @ 4-5ml/s</td>
</tr>
<tr>
<td>Delay of scan post contrast infusion (s)</td>
<td>22-24</td>
<td>22-24</td>
</tr>
</tbody>
</table>
Patients were not screened for upper extremity and cerebral vein/sinus thromboses unless they presented with clinical signs/symptoms suspicious for thrombosis at these sites.

### 3.2.2 Radiation exposure

Risks from radiation exposure during CT scanning were discussed with patients who were given the opportunity to ask questions at the consent process. Prior to ethical approval being given for this study, a radiation risk assessment report was provided by the Christchurch Hospital Medical Physics department in line with the New Zealand National Radiation Laboratory guidelines (Please see reports in Appendix 5).

### 3.2.3 Study endpoints

The endpoints for the study were:-

- Development of VTE (suspected or unsuspected) at any site, found on radiological imaging.
- Development of arterial thrombosis including myocardial infarction, transient ischaemic attack or stroke, confirmed by appropriate investigations.
- Death from any cause.
- End of observation period.
- Patient withdrawal.
- Loss to follow up.

### 3.2.4 Diagnosis of thrombotic events on study

Radiologists reported scan results at dedicated reporting workstations, which allow scrolling of CT image slices, visualisation of static images and, if required, short dynamic videos from ultrasound scans. All initial reports were to be completed within 24 hours of scans being performed, and the result reported and attached to the scan on the hospital radiology image viewing software (Inteleviewer version 4-2-1-P332 (64 Bit), Intelerad, Montreal, Canada).
Pulmonary embolism and venous thromboses were diagnosed on CT images when a filling defect was seen within a pulmonary vessel lumen on two consecutive image slices. The scans were reported by a consultant Radiologist, and subsequently read by a specialist chest CT radiologist. Both radiologists were blinded to the clinical presentation and to each other’s reports. Results were entered into a secure password-locked database using Google docs.

Ultrasound scans were performed by experienced sonographers at Christchurch Hospital, who reported an initial result which was then checked by the charge sonographer and a radiologist with a subspecialty interest in ultrasound imaging (Please see ultrasound protocols in Appendix 6). The charge sonographer and radiologist were blinded to the sonographer’s initial report and the clinical presentation.

### 3.2.5 Management of thrombotic events diagnosed on study

For those patients diagnosed with arterial thrombotic events, referral to the appropriate hospital service was made for decisions on treatment. For patients diagnosed with venous thromboembolism, treatment was administered as published by the current American College of Chest Physicians international clinical guidelines on the treatment of venous thromboembolism, unless there were clinical contraindications(20). Treatment was coordinated by study investigators in conjunction with the patient’s treating clinician and general practitioner, who were both informed of any thrombotic events during the study period by telephone and/or by letter. Patients were then referred back to their treating clinician and general practitioner for ongoing management of the thromboembolic event.

### 3.2.6 Volunteers without cancer

Volunteers without cancer were also recruited to the study to undergo one assessment only. This involved the medical interview, examination and blood tests, but radiological imaging was not performed unless there was clinical suspicion of VTE. This allowed comparison of cancer patient clinicopathological data with a local, contemporaneous, non-cancer population data set. Eligibility criteria were:
Inclusion criteria

- Females and males aged 18 years or over.
- Written informed consent.

Exclusion criteria

- Current diagnosis or past history of cancer, except non-melanoma skin cancer.
- History of VTE, or a current VTE being treated.
- Inherited blood clotting condition or thrombophilia.
- Received anticoagulant medication (low molecular weight or unfractionated heparin, direct and indirect factor Xa inhibitors or oral vitamin K antagonists) within the last four weeks. Patients on aspirin, dipyridamole and/or clopidogrel were eligible to participate.

3.3 Blood samples

Venous blood samples were collected from participants, at each assessment, using a 21-gauge butterfly needle and vacutainer system. If a central venous access device (CVAD) was in place, or a cannula was being inserted at the time of venesection, blood was obtained during cannulation or from the CVAD. The first five millilitres of blood obtained from CVADs were discarded as “dead space” in case the line had been heparin-locked.

Venous blood (10mL) was obtained using the Vacutainer system (BD Vacutainer, BD PL6 7BP UK), collected into plain EDTA and lithium heparin tubes, and sent to Canterbury Health Laboratories (CHL) for routine tests as described in tables 25, 26 and 27 below. A further 12.5 mL was collected in 0.109M citrate tubes for assessment of a novel thrombin generation assay (TGA) (Stago, New Zealand/Australia) and ELISAs not routinely available at CHL (see section 1.6). The tubes were allowed to sit for 30 minutes following venesection and centrifuged (Heraeus Instruments Megafuge 1.0R Thermofisher Scientific Albany, North Shore, New Zealand) at 4,000rpm (2,800g) for 10 minutes to obtain platelet free plasma. To confirm that platelet free plasma was...
obtained, the first five plasma samples obtained from patients were checked using a Coulter counter, which confirmed that the single centrifugation process was adequate. One millilitre of plasma was obtained for the TGA, in accordance with the manufacturers instructions, and the remaining plasma was spun again at 4,000rpm (2,800g) for 10 minutes before further one millilitre aliquots were pipetted into Greiner Bio-One GmbH Cryo.S tubes. Each tube was labelled with the study name, visit number, the study participant’s initials and unique research identification number. The cryotubes were then frozen at -80°C until required for batched analysis. This meant that all obtained bloods had to be processed through Canterbury Health Laboratories within one hour of venesection to maintain standardisation of the pre-analytical phase.

3.4 Routine blood tests performed at Canterbury Health Laboratories (CHL)

Routine haematology tests were performed on whole blood (no centrifugation performed) using a Sysmex XE-2100 (Kobe, Japan) for analysis (see table 25).

<table>
<thead>
<tr>
<th>Blood Test</th>
<th>Type of test</th>
<th>Normal range</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>Oxyhaemoglobin SLS detection</td>
<td>130-175 g/dL (M) 115-155 g/dL (F)</td>
<td>0.7</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>Ratio</td>
<td>0.4-0.52 (M) 0.35-0.46 (F)</td>
<td>1.0</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>Flow cytometry</td>
<td>4-11x10^9/L</td>
<td>1.8</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>Flow cytometry</td>
<td>1.9-7.5x10^9/L</td>
<td>2.9</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>Flow cytometry</td>
<td>1-4x10^9/L</td>
<td>3.0</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>Flow cytometry</td>
<td>0.2-1x10^9/L</td>
<td>11.1</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Direct current or flow cytometry</td>
<td>150-400x10^9/L</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Biochemistry and coagulation tests were centrifuged at 4,000rpm (2,800g) in a Heraeus Megafuge for 10 minutes prior to evaluation.

All biochemistry analyses (see table 26) were performed using an Abbott c16000 analyser (Abbott reagents) except for high sensitivity CRP (Siemens
BNII nephelometer, Siemens Reagent (Germany) and serum beta hCG (Abbott i2000SR analyser, Abbott reagents).

**Table 26 Routine biochemistry tests performed (CHL)**

<table>
<thead>
<tr>
<th>Blood Test</th>
<th>Type of test</th>
<th>Normal range</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>Indirect ion specific electrode</td>
<td>135-145 mmol/L</td>
<td>0.8</td>
</tr>
<tr>
<td>Potassium</td>
<td>Indirect ion specific electrode</td>
<td>3.5-5.2 mmol/L</td>
<td>1.5</td>
</tr>
<tr>
<td>Urea</td>
<td>Enzyme kinetic method</td>
<td>3.2-7.7 mmol/L</td>
<td>2.0</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Classical Jaffe reaction</td>
<td>50-110μmol/L (M) 45-90μmol/L (F)</td>
<td>2.0</td>
</tr>
<tr>
<td>Urate</td>
<td>Modified Trinder method</td>
<td>0.20-0.42 mmol/L (M) 0.15-0.36mmol/L (F)</td>
<td>1.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>Enzymatic Hexokinase method</td>
<td>Random 3.5-7.7mmol/L</td>
<td>2.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Molybdate reaction at 340nm</td>
<td>0.8-1.5 mmol/L</td>
<td>1.9</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Arsenazo dye complex at 572nm</td>
<td>0.6-1.2 mmol/L</td>
<td>2.6</td>
</tr>
<tr>
<td>Corrected Calcium</td>
<td>Arsenazo dye in acid solution</td>
<td>2.2-2.6 mmol/L</td>
<td>1.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>Bromocresol Green – Albumin complex at 628nm</td>
<td>35-50 g/L</td>
<td>1.6</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Formation of p-nitrophenol at 404nm</td>
<td>30-50 U/L</td>
<td>3.5</td>
</tr>
<tr>
<td>Alanine Transaminase</td>
<td>IFCC, Bergmeyer 1986 method</td>
<td>0-40 U/L (M) 0-30 U/L (F)</td>
<td>3.6</td>
</tr>
<tr>
<td>Aspartate Transaminase</td>
<td>IFCC, Bergmeyer 1977 method</td>
<td>10-50 U/L</td>
<td>2.0</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Colourmetric Jendrassik and Grof</td>
<td>2-20 μmol/L</td>
<td>2.3</td>
</tr>
<tr>
<td>Gamma GT</td>
<td>Enzymatic colourimetric method</td>
<td>10-50 U/L (M) 10-35 U/L (F)</td>
<td>1.7</td>
</tr>
<tr>
<td>High sensitivity C-reactive protein</td>
<td>Particle enhanced immuno-nephelometry</td>
<td>&lt;1 mg/L</td>
<td>5.9</td>
</tr>
<tr>
<td>Serum beta HCG</td>
<td>CMIA method</td>
<td>&lt; 5 non pregnant</td>
<td>6.9</td>
</tr>
</tbody>
</table>

F= Female, M= Male

All coagulation tests (see table 27) were performed on an ACL TOP 700 (Instrumentation Laboratory, Bedford, MA, USA).
Table 27 Routine coagulation tests performed (CHL)

<table>
<thead>
<tr>
<th>Blood Test</th>
<th>Manufacturer</th>
<th>Type of test</th>
<th>Normal range</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>HemosIL Recombioplastin 2G</td>
<td>Clotting</td>
<td>0.8-1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>APTT</td>
<td>TriniCLOT APTT HS</td>
<td>Clotting</td>
<td>23-35 secs</td>
<td>2.9</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>HemosIL Fibrinogen-C</td>
<td>Clotting</td>
<td>1.5-4.0g/L (adult)</td>
<td>1.5-6.0g/L (elderly)</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>HemosIL D-Dimer HS</td>
<td>Latex Immunoassay</td>
<td>&lt;250ng/mL</td>
<td>5.0</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>HemosIL Liquid Antithrombin</td>
<td>Chromogenic substrate</td>
<td>75-135%</td>
<td>4.0</td>
</tr>
</tbody>
</table>

3.5 Thrombin Generation Assay (TGA)

Thrombin generation was measured using a commercially available assay kit (Thrombinscope BV, Stago, Australia/ New Zealand) on a fully automated, computer-controlled microplate reader (Fluoroskan) and results were analysed using specifically designed Thrombinscope software. The assay was performed by trained staff in the coagulation laboratory of CHL. Venous blood was collected as described above in citrate tubes. The samples were centrifuged and platelet free plasma was obtained and aliquoted into one millilitre cryovials for storage at -80°C until batched analysis could take place.

At analysis 80uL aliquots of thawed (37°C for 10 minutes) platelet poor plasma from a patient were added to four wells in a 96-well microplate. This allowed samples to be measured in triplicate. The fourth well was used for the thrombin calibrator solution. This allowed samples from up to 24 patients to be analysed in each batch. At time 0 seconds, the sample was recalcified with 20 uL of FluCa solution (40:1 calcium chloride buffer and fluorogenic substrate) and 20 uL of reagent (5pM of Tissue Factor, 4pM phospholipid and deionised water) were added at 37°C. Thrombin generation could then take place which acted on the fluorescent-labelled substrate. The fluorescence emitted was then continuously measured over time and converted by Thrombinscope software to a thrombin concentration measured in nM. The average of the three readings would then be calculated to give an overall result for each patient sample which
Venous thromboembolism in cancer patients undertaking chemotherapy could be compared and standardised to the thrombin calibrator. Figure 25 below summarises how the microplate reader functions.

**Figure 25 A diagram outlining the microplate reader functions of the TGA**

Reproduced with permission from Stago, Australia/New Zealand

- Halogen lamp light source (excitation source) (1).

- Light passes through the excitation filter (390nM) that rotates depending on the particular wavelength that is selected (2).

- Fluorescence emitted from the sample, following the reaction with the FluCa (4), and the original light emitted from the excitation light is filtered by the emission filter (460nM) and corrected (6).

- This is measured by the photomultiplier tube and converted to units (7)

- The autocalibration process- the reference detector is used to compare the measured fluorescence value to the value in the reference chip, and corrects for drift in the photomultiplier tube and the lamp (3).
3.6 Enzyme-Linked Immunosorbent Assays (ELISA) performed in the university research laboratory

Sandwich enzyme-linked immunosorbent assays (ELISAs; R&D Systems) were used to quantitate plasma levels of soluble P-selectin (sP-selectin) (catalogue no SBBE6), thrombomodulin (catalogue no. DTHBD0), angiopoietin-1 (catalogue no. SANG10), angiopoietin-2 (catalogue no. SANG20) and soluble Tie-2 (sTie-2) (catalogue no. DTE200) in both cancer patients and volunteers without cancer. ELISAs were performed according to the manufacturer’s instructions by the author (A. Rahman) and a laboratory scientist, blinded to any clinical information on the patients at the time of analysis.

Standards and samples were assayed in duplicate and the ELISA was analysed on a microplate reader at 450 and 650 nm. Readings at 650nm were then subtracted from readings at 450nm to correct for optical imperfections in the plate that may lead to less accurate results. Table 28 below summarises and compares the intra- and inter-assay variability for each ELISA with that of the published CVs in the manuals produced by the manufacturers.

Table 28 Summary of the precision of each assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>CV (assay in manual)</th>
<th>CV (Christchurch study population)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-assay</td>
<td>Inter-assay</td>
</tr>
<tr>
<td>Soluble P-selectin (n)</td>
<td>4.9-5.6</td>
<td>7.9-9.9</td>
</tr>
<tr>
<td>(sP-selectin)</td>
<td>(10)</td>
<td>(18)</td>
</tr>
<tr>
<td>Thrombomodulin (n) (TM)</td>
<td>2.3-3.6</td>
<td>5.7-7.9</td>
</tr>
<tr>
<td>(TM)</td>
<td>(20)</td>
<td>(40)</td>
</tr>
<tr>
<td>Angiopoietin-1 (n) (Ang-1)</td>
<td>2.4-3.3</td>
<td>5.5-6.4</td>
</tr>
<tr>
<td>(Ang-1)</td>
<td>(20)</td>
<td>(40)</td>
</tr>
<tr>
<td>Angiopoietin-2 (n) (Ang-2)</td>
<td>4.2-6.9</td>
<td>7.4-10.4</td>
</tr>
<tr>
<td>(Ang-2)</td>
<td>(20)</td>
<td>(40)</td>
</tr>
<tr>
<td>Human soluble Tie-2 (n) (sTie-2)</td>
<td>4.3-5.3</td>
<td>5.2-8.5</td>
</tr>
<tr>
<td>(sTie-2)</td>
<td>(20)</td>
<td>(40)</td>
</tr>
</tbody>
</table>
3.7 Risk assessment models
Cancer patients recruited to this study and due to undertake chemotherapy were analysed, at baseline, using three published risk assessment models (RAMs) for VTE risk on chemotherapy as described in sections 1.58 and 159.2 (see tables 20, 21 and 23)(24, 596, 599).

3.8 Data collection
Clinical history, examination, laboratory and radiological data collected in this study were recorded, in paper form, on clinical assessment sheets (please see Appendix 3). A prospective, observational, password-locked database was also established using MOSAIQ™ software at the Oncology department of Christchurch Hospital in June 2011 to log information electronically.

3.8.1 Blood borne variables
Variables analysed in blood samples provided by study participants and considered on univariate and multivariate analyses are listed in the tables above (see tables 25-27).

3.8.2 Statistical analysis
At the end of the study period, data was exported to Microsoft Excel for analysis. Statistical analysis of the data was performed using SPSS version 22.

Protocol development occurred in consultation with a biostatistician who advised on the numbers of participants to be recruited. The same biostatistician oversaw analyses of the data collected.

The prevalence of VTE at baseline was calculated and the cumulative incidence of VTE over the following six to nine months was calculated using the Kaplan-Meier method. Clinicopathological data were summarised and compared between cancer patients and non cancer patients and cancer patients with and without baseline VTE using descriptive statistics, analysis of variance (ANOVA), Mann-Whitney U tests, chi-square and Fisher’s exact tests as appropriate, and receiver operator characteristic (ROC) curves were used to quantify the diagnostic characteristics of potential biomarkers. Comparisons were also made between cancer patients who developed VTE and those that did not during each timeframe using ANOVA, chi-square and Fisher’s exact tests as
appropriate, and ROC curves. Logistic regression analysis was also used in this context to define the independent roles of potential predictors in the development of VTE. Cumulative mortality was estimated using the Kaplan-Meier method and compared between groups over the follow-up period using log rank tests. Validation of the risk assessment scores were undertaken to evaluate the accuracy of the prediction of patient outcomes, and investigate the individual contribution of all the variables contributing to the scores. Derived TGA variables were compared between cancer and non cancer cohorts as well as cancer patients with and without VTE using ANOVA. The study aimed to recruit 300 cancer patients and 50 volunteers without cancer with the expected VTE rate on study to be approximately four percent (4%).
Chapter 4
Results
Chapter 4 Results

Recruitment and demographics

4.1 Participant recruitment

Between July 2011 and August 2013, 272 potential study participants (see figure 26) were screened for accrual. Six patients did not meet eligibility criteria due to:

- having received chemotherapy/radiotherapy treatment within the last three months (n=3).
- receiving anticoagulation within four weeks of potential baseline assessment (n=2).
- being unable to consent to the study due to impaired cognition (n=1).

Nine patients declined entry to the study due to the need for extra appointments (n=2) or that they felt unable to cope with anything more than their current treatment plan (n=7).

A total of 257 participants (207 cancer patients and 50 age- and gender-matched volunteers without cancer) were recruited to the study. Following written informed consent and baseline assessment, four protocol violations had occurred in the cancer cohort. These patients were initially thought to have newly diagnosed or recurrent cancer but this was subsequently disproved on further investigations (see footnote).¹

¹Protocol violations: Female patient presented with hypercalcaemia and a history of treated, early stage breast cancer. She was found to have primary hyperparathyroidism and no cancer recurrence. Female patient presented with a four week history of hoarse voice and a history of early stage breast cancer but was found to have a benign lesion on her vocal cords. Male patient presented with probable cutaneous lymphoma but was diagnosed with pseudolymphoma. Male patient presented with weight loss and rising CEA with a history of colon cancer but was found to have a normal CT scan, clinical examination and colonoscopy.
Venous thromboembolism in cancer patients undertaking chemotherapy
Two hundred and three cancer patients were subsequently available for analysis. Eleven (5.4%) patients withdrew consent during the study but were happy to allow analysis of collected data up to the date of their withdrawal. Twenty (9.9%) patients were lost to follow up, because they had moved away from the area, were receiving ongoing care outside of the Canterbury District Health Board (n=7 (3.4%)) or were unable to attend appointments at the timepoints required (n=13 (6.4%)). Twenty two (10.8%) patients underwent only one assessment and 16 (7.9%) underwent two assessments before receiving radiation treatment (n=21), hormonal manipulation (n=5), surgery (n=11) or investigations for a second pathology (n=1), off study protocol. Nine (4.4%) patients died within 100 days of their baseline assessment and were not assessed a second time and five (2.5%) died following their second assessment before they could be seen for a third time. As of the May 2014, 75 (36.9%) study patients had died. All of these patients were included in the final data analysis.

4.2 Demographics

Tables 29, 30 and 31 below summarise and compare the characteristics of the cancer and non cancer cohorts. A higher proportion of females than males were recruited to the study in both cohorts. Most patients were of New Zealand European ethnicity which is in keeping with the ethnic diversity currently seen in the South Island of New Zealand. The non cancer cohort was slightly younger than the cancer cohort but age ranges were similar. This finding was influenced predominantly by the male participants who were slightly older in the cancer cohort.
Table 29 Study participant demographics

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cancer patients</th>
<th>Volunteers without cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient numbers (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>203 (100)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>129 (63.5)</td>
<td>29 (58)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>74 (36.5)</td>
<td>21 (42)</td>
</tr>
<tr>
<td><strong>Median age (range)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59 years (26-90)</td>
<td>56 years (26-84)</td>
</tr>
<tr>
<td>Female</td>
<td>59 years (26-90)</td>
<td>58 years (29-84)</td>
</tr>
<tr>
<td>Male</td>
<td>63 years (32-82)</td>
<td>58 years (26-73)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>108 F*/ 56 M**</td>
<td>26 F/ 19 M</td>
</tr>
<tr>
<td>Maori</td>
<td>3 F/ 3 M</td>
<td>0 F/ 0 M</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>1 F/ 1 M</td>
<td>0 F/ 0 M</td>
</tr>
<tr>
<td>Asian</td>
<td>2 F/ 2 M</td>
<td>1 F/ 0 M</td>
</tr>
<tr>
<td>Other</td>
<td>15 F/ 12 M</td>
<td>2 F/ 2 M</td>
</tr>
<tr>
<td><strong>ECOG performance status (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>162 (79.8)</td>
<td>50 (100.0)</td>
</tr>
<tr>
<td>2-3</td>
<td>39 (19.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Karnofsky performance status (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90-100</td>
<td>156 (76.8)</td>
<td>49 (98.0)</td>
</tr>
<tr>
<td>70-80</td>
<td>42 (20.7)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>50-60</td>
<td>3 (1.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Previous VTE (%)</strong></td>
<td>9 (4.4)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td><strong>Family history of VTE (%)</strong></td>
<td></td>
<td>28 (13.8)</td>
</tr>
<tr>
<td><strong>Family history thrombophilia (%)</strong></td>
<td></td>
<td>3 (1.5)</td>
</tr>
</tbody>
</table>

*F= female, **M=male
Both cohorts contained participants with good performance status as measured by both the European Collaborative Oncology Group (ECOG) and Karnofsky performance scales (see appendix 4).

**Figure 27 Comparison of performance status scores between the cancer and non cancer cohorts**

4.3 Comorbidities

Congestive cardiac failure, peripheral vascular disease, stroke/transient ischaemic attack (TIA) and chronic obstructive pulmonary disease (COPD) were not present in the non-cancer population sampled but only small numbers of cancer patients had been diagnosed with these conditions. Comorbidities were otherwise well balanced between the two cohorts (see table 30 and figure 28).
### Table 30 Study participant comorbidities

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cancer patients n=203 (%)</th>
<th>Volunteers without cancer n=50 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>59 (29)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>42 (20.7)</td>
<td>15 (30)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>15 (7.4)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Ischaemic heart disease (IHD)</td>
<td>13 (6.4)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Congestive cardiac failure (CCF)</td>
<td>3 (1.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Peripheral vascular disease (PVD)</td>
<td>2 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>5 (2.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>9 (4.4)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>COPD</td>
<td>7 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Connective tissue disorder (CTD)</td>
<td>6 (3)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Varicose veins</td>
<td>64 (31.5)</td>
<td>14 (28.0)</td>
</tr>
</tbody>
</table>
4.4 Smoking status, alcohol consumption and medications

There were a higher proportion of current smokers and ex-smokers in the cancer cohort with nearly two-thirds of the non cancer cohort having never smoked. A higher proportion of participants identified themselves as a non-alcohol drinker in the cancer cohort. Similar proportions of participants were taking regular doses of aspirin, non steroidal anti inflammatory drugs (NSAIDs), statins and clopidogrel in both cohorts. The use of the oral contraceptive pill or hormone replacement therapy was, however, more prevalent in the female non cancer cohort (see table 31 and figure 29).
Table 31 Smoking history, alcohol intake and medications

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cancer patients n=203 (%)</th>
<th>Volunteers without cancer n=50 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>23 (11.3)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>94 (46.3)</td>
<td>15 (30.0)</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>84 (41.4)</td>
<td>32 (64.0)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male ≥ 15 standard drinks</td>
<td>4/74 (5.4)</td>
<td>2/21 (9.5)</td>
</tr>
<tr>
<td>Male &lt; 15 standard drinks</td>
<td>46/74 (62.2)</td>
<td>16/21 (76.2)</td>
</tr>
<tr>
<td>Male non-drinkers</td>
<td>24/74 (32.4)</td>
<td>3/21 (14.3)</td>
</tr>
<tr>
<td>Female ≥10 standard drinks</td>
<td>17/129 (13.2)</td>
<td>3/29 (10.3)</td>
</tr>
<tr>
<td>Female &lt; 10 standard drinks</td>
<td>61/129 (47.3)</td>
<td>20/29 (69.0)</td>
</tr>
<tr>
<td>Female non-drinkers</td>
<td>51/129 (39.5)</td>
<td>6/29 (20.7)</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>36 (17.7)</td>
<td>7 (14.0)</td>
</tr>
<tr>
<td>Other NSAID</td>
<td>12 (5.9)</td>
<td>5 (10.0)</td>
</tr>
<tr>
<td>Statin</td>
<td>31 (15.3)</td>
<td>10 (20.0)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>1 (0.5)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Oral contraceptive or hormone replacement*</td>
<td>4/129 (3.1)</td>
<td>5/29 (17.2)</td>
</tr>
</tbody>
</table>

*p=<0.05 indicating significant difference between groups.
Figure 29 Medication use in the cancer and non cancer cohorts

* *p<0.05

4.5 Cancer primaries

Patients with a broad range of cancers were recruited to the study with breast (26.1%) and colorectal (21.2%) being the most common primary cancers (see figure 30).

Figure 30 Cancer primaries of patients at baseline assessment (n=203)
4.5.1 Cancer stage

As shown in figure 31 below 123 (60.6%) patients presented with metastatic disease and 80 (39.4%) presented with local or locally advanced disease at baseline assessment.

Figure 31 Cancer primaries and stage (n=203)

4.6 Blood marker findings at baseline

Clinical examination and blood borne variables of the 203 cancer patients were compared with the 50 volunteers without cancer. Table 32 summarises the variables that were significantly different between the cohorts on univariate analysis using ANOVA.
Table 32 Variables found to be significantly different between cancer patients (n=203) and volunteers without cancer (n=50) at baseline assessment

<table>
<thead>
<tr>
<th>Continuous variables significantly higher/longer in cancer cohort on univariate analysis (ANOVA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complete blood count</strong></td>
<td>Platelet count**, leukocyte count*, neutrophil count**</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>Corrected calcium****, potassium*, ALP*, GGT*</td>
</tr>
<tr>
<td><strong>Coagulation</strong></td>
<td>APTT*, fibrinogen****, D-dimer*, INR*</td>
</tr>
<tr>
<td><strong>Thrombin Generation Assay</strong></td>
<td>Lag time**, ETP*, peak****, velocity index****</td>
</tr>
<tr>
<td><strong>Angiogenesis/Inflammation</strong></td>
<td>hs CRP**, Ang-1*, Ang-2**</td>
</tr>
<tr>
<td><strong>Parameter Ratios</strong></td>
<td>Ang-2:Tie2*, peak:AT**, velocity index:AT**</td>
</tr>
<tr>
<td><strong>Clinical examination variables</strong></td>
<td>Pulse rate****, respiratory rate*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Continuous variables significantly lower/shorter in cancer cohort on univariate analysis (ANOVA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complete blood count</strong></td>
<td>Haemoglobin****</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>Albumin****, sodium**</td>
</tr>
<tr>
<td><strong>Parameter ratios</strong></td>
<td>Start tail:TM*</td>
</tr>
<tr>
<td><strong>Clinical examination variables</strong></td>
<td>Lying and standing diastolic BP*</td>
</tr>
</tbody>
</table>

*p<=0.05, **p<=0.01, ***p<=0.001, ****p<0.0001
A number of variables were different between the cohorts. Haemoglobin (p<0.0001), albumin (p<0.0001) and sodium (p=0.008) concentrations were lower in the cancer cohort while the platelet (p=0.007), leukocyte (p=0.032) and neutrophil (p=0.001) counts, corrected calcium (p<0.0001), potassium (p=0.022), alkaline phosphatase (ALP) (p=0.013) and gamma-glutamyl transferase (GGT) (p=0.018) concentrations were significantly higher. On examination the pulse (p<0.0001) and respiratory rates (p=0.011) were higher in cancer patients but the lying (p=0.041) and standing (p=0.038) diastolic blood pressures were significantly lower.

The APTT (p=0.013), INR (p=0.038) and fibrinogen concentration (p<0.0001) were also higher in cancer patients. Antithrombin (p=0.820), thrombomodulin (p=0.132) and soluble P-selectin (p=0.333) concentrations were similar in both cohorts.

As oral contraceptive pill (OCP) use is associated with thrombotic risk and there were a higher proportion of patients on these drugs in the cohort without cancer, the univariate ANOVA was repeated having removed all participants taking the OCP. This revealed the same results as the initial analysis with no change in the variables that were significantly different nor similar between the cohorts.

4.6.1 D-dimer and hsCRP

At baseline assessment, D-dimer concentrations were available in 199 (98%) patients with cancer and hsCRP concentrations were available in 200 (98.5%). These were compared with results from the 50 volunteers without cancer and concentrations were found to be significantly higher in cancer patients (D-dimer p=0.013 and hsCRP p=0.006, Mann-Whitney U test) (see table 33).
Table 33 Participants with D-dimer and hsCRP concentrations above the upper limit of normal as stipulated by Canterbury Health Laboratories (CHL)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cancer (n=200)</th>
<th>Non cancer (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer ≥ 250 ng/mL (%)</td>
<td>108 (54.3)</td>
<td>4 (8.0)</td>
</tr>
<tr>
<td></td>
<td>(Range 21-10,375)</td>
<td>(Range 28-502)</td>
</tr>
<tr>
<td>hsCRP ≥ 3 mg/L (%)</td>
<td>118 (59.0)</td>
<td>8 (16.0)</td>
</tr>
<tr>
<td></td>
<td>(Range 0.15-163)</td>
<td>(Range 0.15-155)</td>
</tr>
</tbody>
</table>

A significantly higher proportion of patients with cancer, compared with volunteers without cancer, presented with D-dimer and hsCRP concentrations above the upper limit of normal (ULN) at baseline assessment. Figure 32 shows the distribution of D-dimer concentrations in the two cohorts.

Figure 32 Box and whisker plots comparing plasma D-dimer concentrations between participants with and without cancer at baseline assessment
The D-dimer concentration in the cancer cohort was significantly higher than that of the non cancer population (Mann-Whitney U-test, $p<0.001$) despite the outliers which skewed the mean.

### 4.7 Thrombin Generation Assay (TGA)

On direct comparisons of mean TGA variables in each cohort, the lag time ($p=0.001$) was longer and endogenous thrombin potential (ETP) ($p=0.016$), peak thrombin ($p<0.0001$) and velocity index ($p<0.0001$) were higher in cancer patients than volunteers without cancer. The time to peak thrombin ($p=0.383$) was similar between the cohorts.

### 4.8 Angiopoietin-Tie-2 pathway

Both angiopoietins 1 ($p=0.045$) and 2 ($p=0.001$) (Ang-1 and Ang-2) were higher in cancer patients but sTie-2 concentrations ($p=0.159$) were similar between the cohorts.

### 4.9 Ratios

Ratios of peak thrombin ($p=0.003$) and velocity index ($p=0.001$) with antithrombin (AT) concentrations were higher in cancer patients as was Ang-2 ratioed with sTie-2 ($p=0.022$). The start tail ratioed with thrombomodulin (TM) level was significantly lower in cancer patients ($p=0.024$).

### 4.10 Summary

The cancer and non cancer cohorts in this study are comparable and contemporaneous. The cancer cohort was slightly older than the non cancer cohort, being skewed by the male population. More females in the non cancer cohort were on the oral contraceptive pill or hormone replacement therapy than in the cancer cohort, but this did not impact on differences seen in laboratory variables identified on univariate analyses. A number of variables which play a part in angiogenic, inflammatory and coagulation processes were significantly different between the cohorts. These differences were seen, not only in routinely measured variables, but also in variables of the TGA and Ang-Tie-2 pathway.

Routinely measured components of the complete blood count and biochemistry profiles were different between the cohorts. Notably the mean haemoglobin was
lower and platelet and leukocyte counts were higher in cancer patients. The coagulation markers INR, APTT, fibrinogen and D-dimer were also higher in cancer patients although INR and APTT results remained within normal laboratory ranges in both cohorts. Fifty four percent of cancer patients presented with D-dimer concentrations and 59% with hsCRP concentrations above the upper limit of normal (ULN) at baseline assessment.

Higher concentrations of angiopoietin (Ang)-2 and the Ang-2:sTie-2 ratio have been previously recognised in cancer patients compared with healthy volunteers (442, 443, 446). This study also identified higher concentrations of circulating Ang-1 in cancer patients but similar concentrations of sTie-2 compared to volunteers without cancer, which are different to previous findings (442, 443, 446). After TGA analysis, the cancer patient cohort were found to have a longer mean lag time but higher velocity index resulting in similar times to peak thrombin in comparison to volunteers without cancer. Mean peak thrombin and ETP were higher in the cancer cohort and the start tails were similar between the groups.
Baseline prevalence and cumulative incidence of VTE

4.11 Venous thromboembolic events

Sixty two venous thromboembolic events occurred in 51 (25.1%) of the 203 cancer patients during the study period. Seventeen (8.4%) had already developed VTE at baseline assessment with a further 34 (16.7%) diagnosed in the follow up period. Thirty two (94.1%) of the 34 patients had received chemotherapy (see table 34 and figure 33). No death was clinically attributed to VTE and no post mortem examinations were required in study patients who had died, to objectively report the cause of death.

Table 34 Venous thromboembolic events diagnosed in recruited cancer patients

<table>
<thead>
<tr>
<th>Visit timeframe</th>
<th>Number of VTE events (%)</th>
<th>Baseline</th>
<th>Baseline to 100 days</th>
<th>Beyond 100 days</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All VTE (%)</td>
<td></td>
<td>17 (33.3)</td>
<td>16 (31.4)</td>
<td>18 (35.3)</td>
<td>51 (100)</td>
</tr>
<tr>
<td>PE</td>
<td></td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>18 (35.3)</td>
</tr>
<tr>
<td>DVT</td>
<td></td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>16 (31.4)</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>6 (11.8)</td>
</tr>
<tr>
<td>PE and DVT</td>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>PE and SVT</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>DVT and SVT</td>
<td></td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>4 (7.8)</td>
</tr>
</tbody>
</table>

Similar numbers of patients were diagnosed with VTE at baseline and the two prespecified follow up periods. Eleven (21%) patients had two simultaneously diagnosed VTE events.
Figure 33 Breakdown of venous thromboembolic events by timeframe

- DVT/SVT: Beyond 100 days: 1, Baseline to 100 days: 3, Baseline: 0
- PE/SVT: Beyond 100 days: 0, Baseline to 100 days: 1, Baseline: 1
- PE/DVT: Beyond 100 days: 1, Baseline to 100 days: 3, Baseline: 3
- SVT: Beyond 100 days: 0, Baseline to 100 days: 3, Baseline: 3
- DVT: Beyond 100 days: 4, Baseline to 100 days: 5, Baseline: 7
- PE: Beyond 100 days: 4, Baseline to 100 days: 6, Baseline: 8

No. patients with VTE
4.12 VTE and cancer primary

The highest rates of VTE occurred in cancers of the colon/rectum (30.2%), pancreas (30.8%) and stomach/gastro-oesophageal junction (GOJ) (66.7%). High rates were also seen in patients with breast cancer (22.6%) and lymphoma (26.3%). Relatively small numbers of patients, with cancer primaries other than breast or colorectal, were recruited (see figure 34).

Figure 34 Venous thromboembolic events associated with cancer primary
4.13 VTE and cancer morphology

Most patients were diagnosed with adenocarcinoma (including invasive ductal breast carcinoma (n=129)) with 29.5% developing VTE during the study. Twenty eight percent of patients with invasive ductal carcinoma of the breast and 33% of patients with other types of adenocarcinoma developed VTE. Although there were only small numbers of B cell lymphomas, 33.3% were diagnosed with VTE (see figure 35). Despite these high proportions of VTE events, none of the associations between VTE and cancer morphology reached statistical significance.

Figure 35 Venous thromboembolic events associated with tumour histology (n=203)
4.14 VTE and cancer stage

Thirty seven (72.5%) of the 51 patients diagnosed with VTE had previously been diagnosed with widespread metastatic disease. Of the 14 (27.5%) patients with local or locally advanced disease, two (3.9%) had locally advanced pancreas cancer which was not resectable. The remaining 12 (23.5%) patients were undergoing potentially curative neoadjuvant or adjuvant therapy for localised/locally advanced lymphoma, hepatobiliary, gastric/gastro-oesophageal, breast and colorectal carcinomas (see figure 36).

Figure 36 VTE associated with cancer primary and stage (n=203)
4.14.1 VTE events

At baseline assessment 17 (8.4%) of the 203 recruited cancer patients were diagnosed with 21 VTE events. Twelve (70.6%) had developed pulmonary emboli, of whom four (23.5%) were simultaneously diagnosed with two VTE events (three with DVT and one with SVT). Five (29.4%) patients had developed DVT alone (see table 35).

During follow up, a further 43 VTE events were diagnosed in 34 patients. Seven (20.6%) patients were simultaneously diagnosed with two VTE events. Sixteen (47.1%) patients developed VTE during the first 100 days and a further 18 (52.9%) developed VTE beyond 100 days from baseline assessment.

Of the 138 (68.0%) cancer patients who were not diagnosed with VTE at baseline, received at least one cycle of chemotherapy and were treated in Christchurch, 31 (22.5%) were diagnosed with VTE in follow up. One patient moved to another cancer centre prior to commencing chemotherapy and developing VTE. This patient was excluded from further analyses due to the lack of prospective follow up data apart from objective evidence of VTE diagnosis on radiological imaging. Two patients recruited to the study developed VTE without having undergone chemotherapy and so were also not included in further analyses. They were:

- A male with resectable cholangiocarcinoma who proceeded directly to potentially curative surgery and developed a portal vein thrombosis in the postoperative period.

- A male with metastatic adenocarcinoma of the colon who developed a left lower extremity DVT and left upper extremity SVT prior to commencement of chemotherapy.

4.14.1.1 Breakdown of VTE events and clinical suspicion

All VTE events diagnosed at baseline were clinically unsuspected. Beyond baseline, the majority of diagnosed DVTs (57.1%) and SVTs (63.6%) were clinically suspected, but the majority of PEs (61.5%) were clinically unsuspected. Only five (20%) of the 25 PEs diagnosed were clinically...
suspected and overall, only 20 (33.9%) of the 59 VTE events were clinically suspected VTE on study (see table 35 and figures 37 and 38).

Table 35 Clinically suspected and unsuspected VTE events at baseline and follow up in patients undertaking chemotherapy in Christchurch

<table>
<thead>
<tr>
<th>Clinically suspected</th>
<th>Baseline (n)</th>
<th>Baseline to 100 days (n)</th>
<th>Beyond 100 days (n)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>PE (n)</td>
<td>0</td>
<td>12</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>DVT (n)</td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>SVT (n)</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Total (n)</td>
<td>0</td>
<td>21</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 37 Breakdown of suspected and unsuspected VTE events by timeframes

[Bar chart showing the breakdown of VTE events by timeframes]
Figure 38 Breakdown of suspected and unsuspected VTE events by VTE type

<table>
<thead>
<tr>
<th>% VTE events</th>
<th>PE</th>
<th>DVT</th>
<th>SVT</th>
<th>All VTE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>14%</td>
<td>5%</td>
<td>39%</td>
</tr>
<tr>
<td>Suspected</td>
<td>5%</td>
<td>8%</td>
<td>7%</td>
<td>20%</td>
</tr>
<tr>
<td>Unsuspected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.14.2 Sites of thrombi in the pulmonary arterial tree at baseline assessment (n=203)

Of the 12 (5.9%) patients diagnosed with PE (all unsuspected) at baseline, four (33.3%) were found to have a single site of thrombus and eight (66.7%) had more than one site affected by thrombus. Five (41.7%) patients were diagnosed with thrombi in both lungs. The most proximal site of thrombi were the lobar arteries in five (41.7%) patients, the segmental arteries in six (50.0%) and the subsegmental arteries in only one (8.3%) patient (see figure 39 and table 36).

Figure 39 Diagram of the branches of the pulmonary arterial tree in cross section
Table 36 Most proximal site of pulmonary artery tree occupied by thrombus in patients diagnosed with PE at baseline assessment

<table>
<thead>
<tr>
<th>PE site</th>
<th>Baseline n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Lobar</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>Segmental</td>
<td>6 (50.0)</td>
</tr>
<tr>
<td>Subsegmental</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
</tr>
</tbody>
</table>

4.15 Blood marker comparisons

Baseline clinicopathological findings were compared between cancer patients with VTE, cancer patients without VTE and volunteers without cancer.

4.15.1 Cancer patients without VTE compared with volunteers without cancer

Cancer patients not diagnosed with VTE (n=185) were compared with volunteers without cancer (n=50). The findings were similar to the comparisons between all 203 recruited cancer patients and the 50 volunteers without cancer except that the International Normalised Ratio (INR) (p=0.068) was similar in both cohorts (see table 37).

4.15.2 Cancer patients with VTE compared with volunteers without cancer

Findings were different, however, when cancer patients with VTE at baseline (n=16 as blood samples were lost after venesection in one patient due to earthquake occurrence) were compared with volunteers without cancer (n=50). Alanine aminotransferase (ALT) (p=0.037), aspartate transaminase (AST) (p=0.009), soluble Tie2 (sTie-2) (p=0.012), INR (p=0.001), soluble P-selectin (sP-selectin) (p<0.0001) and thrombomodulin (TM) (p=0.004) were higher than in the non cancer cohort. Antithrombin (AT) (p=0.033) was significantly lower than the non cancer cohort. Corrected calcium (p=0.055) and angiopoietin-1 (Ang-1) (p=0.740) concentrations were similar to non cancer patients unlike results observed in cancer patients without VTE.
As was seen in cancer patients without VTE the mean Angiopoietin-2 (Ang-2) concentration (p<0.0001), Ang-2:sTie-2 (p=0.011), peak:AT (p<0.0001) and velocity index (VI):AT (p=0.001) ratios were higher than in volunteers without cancer but, additionally, the endogenous thrombin potential (ETP):AT (p=0.001), tpeak:AT (p=0.004) and tail:AT (p=0.014) ratios were also significantly higher (see table 37).

Table 37 Baseline variables that were significantly different between cancer patients with (n=16) and without VTE (n=185) and volunteers without cancer (n=50)

| Variables higher/longer in cancer patients with and without VTE v volunteers without cancer on univariate analysis (ANOVA) |
|---|---|---|
| Variable | Cancer patients without VTE (n=185) | Cancer patients with VTE (n=16) |
| Complete blood count | Platelet count*, leukocyte count*, neutrophil count** | Platelet count**, neutrophil count** |
| Biochemistry | ALP**, GGT*, corrected calcium****, potassium* | ALP**, ALT*, AST**, GGT**, potassium* |
| Coagulation | APTT*, fibrinogen****, D-dimer**** | APTT*, fibrinogen****, D-dimer****, INR**, sP-selectin****, thrombomodulin** |
| Thrombin Generation Assay | Lag time**, ETP*, peak****, VI**** | Lag time**, ETP**, peak**, VI* |
| Angiogenesis/Inflammation | hs CRP*, Ang-1*, Ang-2** | hs CRP**, Ang-2****, sTie-2* |
| Clinical examination | Pulse rate****, respiratory rate* | Pulse rate**, respiratory rate** |
Variables significantly lower/shorter in cancer patients with and without VTE v volunteers without cancer on univariate analysis (ANOVA)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cancer patients without VTE (n=185)</th>
<th>Cancer patients with VTE (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count</td>
<td>Haemoglobin****</td>
<td>Haemoglobin****</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Albumin**, sodium*</td>
<td>Albumin****, sodium**</td>
</tr>
<tr>
<td>Coagulation</td>
<td>-</td>
<td>Antithrombin*</td>
</tr>
<tr>
<td>Parameter ratios</td>
<td>Tail:TM*</td>
<td>-</td>
</tr>
<tr>
<td>Clinical examination</td>
<td>Lying and standing diastolic BP*</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

4.15.3 Cancer patients with VTE (n=16) compared with cancer patients without VTE (185) at baseline

Comparisons of baseline variables in the cancer cohorts with and without VTE were subsequently performed and summarised in tables 38 and 39 below. The haemoglobin level (p=0.002) was significantly lower in cancer patients with VTE but platelet (p=0.152), leucocyte (p=0.688) and neutrophil (p=0.983) counts were similar in both groups. Liver enzyme concentrations were higher in the VTE cohort except ALT (p=0.129). Antithrombin (AT) (p=0.011) results were significantly lower in the VTE cohort as were albumin (p=0.005) concentrations. The D-dimer (p<0.0001) concentrations were significantly higher as well as sP-selectin (p<0.0001), INR (p=0.033), Ang-2 (p=0.018), sTie-2 (p=0.001), hsCRP (p=0.023) and ratios of all TGA variables with antithrombin except for the lag time (p=0.075). The mean Ang-2:sTie-2 ratio (p=0.079) was similar between the groups.
Table 38 Variables that were significantly different between cancer patients with (n=16) and without (n=185) VTE at baseline assessment

<table>
<thead>
<tr>
<th>Continuous variables significantly higher/longer in cancer patients with VTE (n=16) on univariate analysis (ANOVA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemistry</td>
<td>ALP****, AST*, GGT**</td>
</tr>
<tr>
<td>Coagulation</td>
<td>D-dimer****, sP-selectin****, INR*</td>
</tr>
<tr>
<td>Angiogenesis/Inflammation</td>
<td>Ang-2*, sTie-2**, hsCRP*</td>
</tr>
<tr>
<td>Clinical examination</td>
<td>Circumference L arm below medial epicondyle*</td>
</tr>
</tbody>
</table>

Continuous variables significantly lower/shorter in cancer patients with VTE (n=16) on univariate analysis (ANOVA)

| Complete blood count | Haemoglobin** |
| Biochemistry | Albumin** |
| Coagulation | Antithrombin* |

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Table 39 Comparisons of baseline D-dimer and hsCRP levels in cohorts of cancer patients with and without VTE

<table>
<thead>
<tr>
<th>Variable</th>
<th>VTE and cancer (n=16)</th>
<th>No VTE and cancer (n=185)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer ≥ 250 ng/mL (%)</td>
<td>14 (87.5) (Range 108-10,375)</td>
<td>94 (50.8) (Range 21-3,183)</td>
</tr>
<tr>
<td>hsCRP ≥ 3 mg/L (%)</td>
<td>13 (81.3) (Range 0.54-135)</td>
<td>105 (56.8) (Range 0.15-163)</td>
</tr>
</tbody>
</table>

Figures 40 to 46 below summarise these variables in graphical form.
Figure 40 Mean (+/-SEM) baseline values of variables that were significantly different between cancer patients with and without VTE

<table>
<thead>
<tr>
<th>Variable (units)</th>
<th>VTE (n=16)</th>
<th>No VTE (n=185)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>26±2</td>
<td>19±1</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>31.5±2.6</td>
<td>14.8±1.2</td>
</tr>
<tr>
<td>sP-selectin (ng/mL)</td>
<td>58±3.4</td>
<td>24±2.0</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>37.6±1.7</td>
<td>40.6±0.8</td>
</tr>
</tbody>
</table>

*p<0.05  **p<0.01  ****p<0.0001

Figure 41 Mean (+/-SEM) International Normalised Ratios (INR) between cancer patients with and without VTE at baseline

Mean INR

VTE (n=16)  No VTE (n=185)

1.06±0.03  1±0.00

*p<0.05

Venous thromboembolism in cancer patients undertaking chemotherapy
Figure 42 Mean (+/-SEM) values of variables that were significantly different at baseline assessment between cancer patients with and without VTE

![Figure 42](image)

* p<0.05  ** p<0.01  **** p<0.0001

Figure 43 Mean (+/-SEM) values of variables that were significantly different at baseline assessment between cancer patients with and without VTE

![Figure 43](image)

* p<0.05  **** p<0.0001
Figure 44 Mean (+/-SEM) ttPeak:AT ratios in cancer patients with and without VTE

**p<0.01

Figure 45 Mean (+/-SEM) ETP:AT ratios in cancer patients with and without VTE at baseline

**p<0.01

Figure 46 Mean values of variable ratios that were different between cancer patients with and without VTE at baseline

*p<0.05  **p<0.01
4.15.4 Multivariate analysis

On multivariate analysis of the above variables using stepwise logistic regression analysis, D-dimer and GGT were independently associated with the presence of VTE at baseline assessment. When ECOG performance status was added to the analysis this was also found to be independently associated with VTE diagnosis. All other variables identified in the table above correlated closely with each other as well as with D-dimer and/or GGT. As can be seen from figure 47 although there is a significant difference in median D-dimer concentrations between the two groups (Mann-Whitney U-test, p<0.001), there is some overlap in the distributions.

**Figure 47** Box and whisker plots comparing plasma D-dimer concentrations between cancer patients with and without VTE at baseline assessment

![Box and whisker plots](image)

ROC curves were produced for D-dimer and GGT concentrations and ECOG performance status (see figures 48 and 49).
Figure 48 ROC curves of D-dimer and GGT levels associated with VTE diagnosis in cancer patients at baseline assessment

The area under the D-dimer ROC curve was 0.783 (se=0.066, p<0.0001). A D-dimer cut off of 280 ng/mL was associated with baseline VTE with a sensitivity of 81.3% and specificity of 66.8%. The ROC curve for GGT concentrations was not significant with an area of 0.534 (SE=0.082, p=0.656).
Figure 49 A ROC curve of ECOG performance status and association with VTE diagnosis in cancer patients at baseline assessment

For ECOG performance status the area under the ROC curve was 0.751 (SE=0.057, p=0.001). An ECOG performance status greater than 1 was associated with baseline VTE with a sensitivity of 93.8% and specificity of 43.8%.

4.16 VTE in chemotherapy patients

Of the 203 cancer patients recruited to the study 138 (68.0%) undertook chemotherapy. The CONSORT diagram below (figure 50) summarises outcomes.
Figure 50 CONSORT diagram for cancer patients receiving chemotherapy during the VTE study

Cancer patients consented and undertaking baseline assessment n=203

Cancer patients not receiving chemotherapy n=48
Cancer patients with VTE at baseline n=17

Chemotherapy patients undertaking baseline assessment n=138

Total losses baseline to second assessment n=13
Underwent definitive surgery n=1
Lost to follow up n=2
Withdrew consent n=4
Died n=6

Number of cancer patients attending second assessment n=121

Between baseline assessment and 100 days follow up n=107
Patients assessed and diagnosed with VTE n=10
Patients diagnosed with VTE but not assessed n=4
Patients without VTE n=93

Beyond 100 days from baseline assessment n=14
Patients assessed and diagnosed with VTE n=3
Patients without VTE n=11

Total losses beyond 100 days n=30
Received radiation/hormone treatment n=9
Underwent definitive surgery n=6
Lost to follow up n=9
Withdrew consent n=1
Died n=4
Mistaken diagnosis of VTE n=1

Number of cancer patients attending third assessment beyond 100 days follow up n=74
Patients assessed and diagnosed with VTE n=14
Patients diagnosed with VTE but not assessed n=3
Patients without VTE n=57
4.16.1 CT scans performed in cancer patients undertaking chemotherapy

As CT scans could only be performed when clinically indicated, to minimise patient radiation exposure, only 96 (69.6%) patients underwent a baseline CT. Thirty one (22.5%) patients underwent a CT scan at three visits and 28 (20.3%) patients did not undergo any CT scanning. Thirty nine (28.3%) patients underwent one CT and 40 (29.0%) underwent two CTs (see figure 51).

Figure 51 Time points when CTPAs were performed on cancer patients
Table 40 summarises the VTE events that occurred in patients undertaking chemotherapy beyond baseline assessment.

Table 40 VTE events in cancer patients undertaking chemotherapy

<table>
<thead>
<tr>
<th>Number of VTE events (%)</th>
<th>Baseline to 100 days</th>
<th>Beyond 100 days</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All VTE (%)</td>
<td>14 (45.2)</td>
<td>17 (54.8)</td>
<td>31 (100.0)</td>
</tr>
<tr>
<td>PE</td>
<td>4</td>
<td>6</td>
<td>10 (32.3)</td>
</tr>
<tr>
<td>DVT</td>
<td>2</td>
<td>6</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>SVT</td>
<td>3</td>
<td>3</td>
<td>6 (19.4)</td>
</tr>
<tr>
<td>PE and DVT</td>
<td>1</td>
<td>1</td>
<td>2 (6.5)</td>
</tr>
<tr>
<td>PE and SVT</td>
<td>1</td>
<td>0</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>DVT and SVT</td>
<td>3</td>
<td>1</td>
<td>4 (12.9)</td>
</tr>
</tbody>
</table>

4.16.2 Sites of thrombi in the pulmonary arterial tree in follow up assessments of cancer patients treated with chemotherapy (n=138)

Thirteen (9.6%) of the 138 patients were diagnosed with PE in follow up of which five (38.4%) were clinically suspected and eight (61.5%) were clinically unsuspected. Five (38.5%) patients were diagnosed with thrombus at a single site and eight (61.5%) were found to have thrombi at more than one site. In patients diagnosed with multiple thrombi six (46.2%) patients had developed thrombi in both lungs. All four patients with clinically suspected PEs had thrombi in both lungs. The most proximal site of thrombus in patients with clinically suspected PE were the lobar arteries in two (50.0%) and the segmental arteries in two (50.0%) patients. In patients with clinically unsuspected PE, the lobar arteries were involved in three (33.3%), the segmental arteries in four
(44.4%) and the subsegmental arteries in two (22.2%) patients (see table 41 and figure 52).

Table 41 Most proximal site of thrombus in the pulmonary arterial tree of chemotherapy patients diagnosed with pulmonary embolism

<table>
<thead>
<tr>
<th>PE site</th>
<th>Up to 100 days follow up n (%)</th>
<th>Beyond 100 days follow up n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Lobar</td>
<td>2 (33.3)</td>
<td>3 (42.9)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>Segmental</td>
<td>4 (66.7)</td>
<td>1 (14.3)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>Subsegmental</td>
<td>0 (0.0)</td>
<td>3 (42.9)</td>
<td>3 (23.1)</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 52 The most proximal branches of the pulmonary arterial tree containing thrombus in patients diagnosed with PE at baseline as well as on chemotherapy

![Figure 52](image)
4.16.3 Clot burden and PE

Almost two thirds of the patients diagnosed with PE were diagnosed with multiple thrombi and 44% of the patients were diagnosed with bilateral PEs (see table 42).

Table 42 Clot burden in patients diagnosed with PE at baseline and on chemotherapy

<table>
<thead>
<tr>
<th>Study timepoint</th>
<th>Number of patients with PE (n=25)</th>
<th>Number of patients with PE in both lungs at diagnosis N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n=12)</td>
<td>Single thrombus 4 (33.3)</td>
<td>8 (66.7) 5 (41.6)</td>
</tr>
<tr>
<td></td>
<td>Multiple thrombi 8 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Post baseline (n=13)</td>
<td>5 (38.5)</td>
<td>8 (61.5) 6 (46.2)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (36.0)</td>
<td>16 (64.0) 11 (44.0)</td>
</tr>
</tbody>
</table>

4.16.4 DVT and superficial vein thrombosis (SVT)

Thirty (15.0%) of the 200 cancer patients analysed on study developed DVT and/or SVT including nine (4.5%) at baseline assessment. Twenty one (15.2%) of the 138 patients who received chemotherapy developed DVT and/or SVT during follow up.

Overall, nine lower extremity DVTs, seven upper extremity DVTs and seven abdominopelvic DVTs were diagnosed in 22 (15.9%) patients. Seven upper extremity SVTs and five lower extremity SVTs were diagnosed in 12 (8.7%) patients. Two patients were diagnosed with both PE and DVT, four (3.0%) with both DVT and SVT, one (0.7%) with both PE and SVT, eight (5.9%) with DVT alone and six (4.4%) with SVT alone (see tables 43 and 44).

Upper extremity DVTs and/or SVTs were diagnosed in 10 (7.2%) patients of which eight (4.4%) were associated with a CVAD. A total of 45 PICC lines, one Hickman line and four portacaths were inserted in 50 patients calculating to a thrombotic complication rate of 16% on study. Two SVTs of the upper
extremity were not associated with a CVAD and occurred during the chemotherapy course.

Table 43 Sites of DVT diagnosed in patients at baseline and on chemotherapy

<table>
<thead>
<tr>
<th>DVT site</th>
<th>Baseline n (%)</th>
<th>Up to 100 days n (%)</th>
<th>Beyond 100 days n (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower extremity</td>
<td>6 (60.0)</td>
<td>2 (33.3)</td>
<td>1 (12.5)</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Upper extremity</td>
<td>0 (0.0)</td>
<td>4 (66.7)</td>
<td>3 (37.5)</td>
<td>7 (29.2)</td>
</tr>
<tr>
<td>Abdominopelvic</td>
<td>4 (40.0)</td>
<td>0 (0.0)</td>
<td>4 (50.0)</td>
<td>8 (33.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 44 Sites of SVT diagnosed in patients at baseline and on chemotherapy

<table>
<thead>
<tr>
<th>SVT site</th>
<th>Baseline n (%)</th>
<th>Up to 100 days follow up n (%)</th>
<th>Beyond 100 days follow up n (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower extremity</td>
<td>1 (100.0)</td>
<td>2 (28.6)</td>
<td>2 (50.0)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>Upper extremity</td>
<td>0 (0.0)</td>
<td>5 (71.4)</td>
<td>2 (50.0)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>
4.17 Cumulative incidence of VTE on chemotherapy

The 90-day, 183-day and 365-day cumulative incidences of VTE, for the 138 cancer patients undergoing chemotherapy, was calculated using Kaplan-Meier curves. Tables 45 to 48 below summarise these results for all VTE, PE, DVT and SVT events.

Table 45 Cumulative incidences of all VTE events in chemotherapy patients

<table>
<thead>
<tr>
<th></th>
<th>Cumulative Incidence (% +/-SEM) n=138</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>All VTE (n=31)</td>
<td>8.4 (2.7)</td>
</tr>
<tr>
<td>Suspected VTE (n=15)</td>
<td>6.2 (2.1)</td>
</tr>
<tr>
<td>Unsuspected VTE (n=16)</td>
<td>4.4 (1.9)</td>
</tr>
</tbody>
</table>

Table 46 Cumulative incidences of PE events in chemotherapy patients

<table>
<thead>
<tr>
<th>PE</th>
<th>Cumulative Incidence (% +/-SEM) n=138</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>All PE (n=13)</td>
<td>4.8 (1.9)</td>
</tr>
<tr>
<td>Suspected PE (n=5)</td>
<td>2.3 (1.3)</td>
</tr>
<tr>
<td>Unsuspected PE (n=8)</td>
<td>2.6 (1.5)</td>
</tr>
</tbody>
</table>
### Table 47 Cumulative incidences of DVT events in chemotherapy patients

<table>
<thead>
<tr>
<th></th>
<th>Cumulative Incidence (% +/- SEM) n=138</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>All DVT (n=14)</td>
<td>4.0 (1.8)</td>
</tr>
<tr>
<td>Suspected DVT (n=8)</td>
<td>3.1 (1.5)</td>
</tr>
<tr>
<td>Unsuspected DVT (n=6)</td>
<td>0.9 (0.9)</td>
</tr>
</tbody>
</table>

### Table 48 Cumulative incidences of SVT events in chemotherapy patients

<table>
<thead>
<tr>
<th></th>
<th>Cumulative Incidence (% +/- SEM) n=138</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>All SVT (n=11)</td>
<td>5.0 (2.0)</td>
</tr>
<tr>
<td>Suspected SVT (n=7)</td>
<td>3.2 (1.6)</td>
</tr>
<tr>
<td>Unsuspected SVT (n=4)</td>
<td>1.8 (1.3)</td>
</tr>
</tbody>
</table>
4.18 Cumulative mortality
Mortality from all causes was also calculated using Kaplan-Meier curves and direct comparison was made between groups.

4.18.1 Cumulative mortality following VTE diagnosis
Mortality was significantly higher in cancer patients diagnosed with VTE at baseline assessment compared with those without VTE (see table 49 and figures 53 and 54).

Table 49 Cumulative mortality data for recruited cancer patients

<table>
<thead>
<tr>
<th>VTE</th>
<th>Cumulative mortality (% +/-SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>No VTE- all cancer patients recruited</td>
<td></td>
</tr>
<tr>
<td>(n=152)</td>
<td>6.6 (2.0)</td>
</tr>
<tr>
<td>No VTE- all cancer patients on chemotherapy (n=107)</td>
<td>4.7 (2.0)</td>
</tr>
<tr>
<td>All baseline VTE (n=17)</td>
<td>35.3 (11.6)</td>
</tr>
<tr>
<td>Baseline PE (n=12)</td>
<td>41.7 (14.2)</td>
</tr>
<tr>
<td>Baseline DVT (n=8)</td>
<td>37.5 (17.1)</td>
</tr>
<tr>
<td>Baseline SVT (n=1)</td>
<td>N/E</td>
</tr>
<tr>
<td>All post baseline VTE patients undertaking chemotherapy (n=31)</td>
<td>6.5 (4.4)</td>
</tr>
</tbody>
</table>
Patients diagnosed with VTE at baseline assessment (n=17, p=0.027) and chemotherapy patients diagnosed with VTE beyond baseline assessment (n=31, p=0.034) demonstrated significantly poorer survival than chemotherapy patients who were not diagnosed with VTE. The hazard ratio for mortality for those diagnosed with VTE at baseline compared with chemotherapy patients that did not develop VTE was 2.25 (95% CI 1.07-4.70). No differences in cumulative mortality were observed between the two VTE cohorts (p=0.670). Median survival had not been reached in the no VTE cohort on chemotherapy (n=107).
at the time of analysis but median survival was 400 days in the baseline VTE cohort and 674 days in the cohort who developed VTE beyond baseline (see figure 55).

**Figure 55** Kaplan-Meier curve of cumulative survival in cancer patients with VTE at baseline (n=17) and post baseline (n=31) compared with cancer patients who did not develop VTE on chemotherapy (n=107)

4.18.2 Cumulative mortality in cancer patients undertaking chemotherapy and diagnosed with VTE beyond baseline assessment

When comparing chemotherapy patients who were diagnosed with VTE beyond baseline during the study and those that were not, cumulative mortality was significantly higher in the VTE group (see table 50 and figure 56).
Table 50 Cumulative mortality for chemotherapy patients with and without VTE during follow up beyond baseline

<table>
<thead>
<tr>
<th></th>
<th>Cumulative mortality (%+/SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day % (+/-SEM)</td>
</tr>
<tr>
<td>VTE (n=138)</td>
<td></td>
</tr>
<tr>
<td>No VTE on chemotherapy (107)</td>
<td>4.7 (2.0)</td>
</tr>
<tr>
<td>All VTE (n=31)</td>
<td>6.5 (4.4)</td>
</tr>
<tr>
<td>Suspected VTE (n=15)</td>
<td>13.3 (8.8)</td>
</tr>
<tr>
<td>Unsuspected VTE (n=16)</td>
<td>0.0 (N/E)</td>
</tr>
</tbody>
</table>

Figure 56 Cumulative mortality for chemotherapy patients with and without VTE

Suspected VTE (n=15) were associated with significantly poorer survival when compared with the no VTE cohort (n=107) (p=0.01). Survival was, however, similar between those patients diagnosed with unsuspected VTE and those who had no VTE (n=107) (p=0.417). Median survival in the suspected VTE cohort
was 456 days and 744 days in the unsuspected VTE cohort but this difference in survival was not significant (p=0.204) (see figure 57).

Figure 57 Kaplan Meier survival curves for patients with and without VTE with VTE patients stratified for clinical suspicion of VTE presence

4.18.3 Clinical variables in chemotherapy patients with suspected and unsuspected VTE

Fifteen (93.7%) of 16 patients with unsuspected VTE and eight (53.3%) of the 15 patients with suspected VTE had complete data collected for statistical analysis. The remaining eight (25.8%) were found to have missing data or were not assessed by the study investigator prior to anticoagulation being administered as they presented acutely to other hospital services. Two sets of blood samples were lost due to unforeseen circumstances relating to earthquakes that affected Christchurch between February 2011 and December 2012. The variables in table 51 and figures 58 to 62 were found to be higher or longer in patients with suspected VTE compared with patients with unsuspected VTE.
Table 51 Differences in variables between chemotherapy patients with suspected and unsuspected VTE at the time of diagnosis

<table>
<thead>
<tr>
<th>Variables significantly higher/longer in cancer patients with suspected VTE (n=8) compared with patients with unsuspected VTE (n=15) on univariate analysis (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation</td>
</tr>
<tr>
<td>D-dimer (p=0.008)</td>
</tr>
<tr>
<td>Thrombin Generation Assay</td>
</tr>
<tr>
<td>Lag time (p=0.011), ttPeak (p=0.013)</td>
</tr>
<tr>
<td>Parameter Ratios</td>
</tr>
<tr>
<td>ttPeak:TM (p=0.049), ttPeak:AT (p=0.021)</td>
</tr>
</tbody>
</table>

Figure 58 Comparison of mean D-dimer concentrations (+/-SEM) in chemotherapy patients diagnosed with suspected and unsuspected VTE beyond baseline
Figure 59 Comparison of mean lag times (+/-SEM) in chemotherapy patients diagnosed with unsuspected and suspected VTE beyond baseline

![Graph showing comparison of mean lag times with asterisk indicating p<0.05](image)

* *p<0.05

Figure 60 Comparison of mean time to peak thrombin concentrations (+/-SEM) in chemotherapy patients diagnosed with suspected and unsuspected VTE beyond baseline

![Graph showing comparison of mean time to peak thrombin concentrations with asterisk indicating p<0.05](image)

* *p<0.05
Figure 61 Comparison of mean time to peak thrombin to antithrombin concentration ratios (+/-SEM) in chemotherapy patients diagnosed with suspected and unsuspected VTE beyond baseline

![Diagram of Figure 61](image)

*p<0.05

Figure 62 Comparison of mean time to peak thrombin to antithrombin concentration ratios (+/-SEM) in chemotherapy patients diagnosed with suspected and unsuspected VTE beyond baseline

![Diagram of Figure 62](image)

*p<0.05
4.18.3.1 Multivariate analysis
Following stepwise logistic regression analysis, D-dimer was the only variable independently associated with suspected VTE presence compared with unsuspected VTE presence on chemotherapy treatment.

4.18.4 Pulmonary embolism (PE) in patients on chemotherapy
Pulmonary emboli (n=13), diagnosed in follow up beyond baseline, were associated with significantly poorer survival (median survival 697 days) in comparison with the no VTE cohort (n=107, p=0.02). Suspected PEs (n=5) were associated with poorer survival than both the no VTE cohort (n=107, p=<0.0001) and patients diagnosed with unsuspected PEs (n=8, p=0.002). Patients without VTE and patients with unsuspected PE had similar mortality rates (p=0.843) (see table 52 and figures 63 and 64).

Table 52 Cumulative mortality for chemotherapy patients with PE diagnosed in follow up beyond baseline

<table>
<thead>
<tr>
<th></th>
<th>Cumulative mortality (% +/-SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>No VTE on chemotherapy (n=107)</td>
<td>4.7 (2.0)</td>
</tr>
<tr>
<td>All PE (n=13)</td>
<td>15.4 (10.0)</td>
</tr>
<tr>
<td>Suspected PE (n=5)</td>
<td>40.0 (21.9)</td>
</tr>
<tr>
<td>Unsuspected PE (n=8)</td>
<td>0.0 (N/E)</td>
</tr>
</tbody>
</table>
Figure 63 Cumulative mortality in patients who developed PE on chemotherapy in follow up beyond baseline

![Cumulative mortality graph](image)

Figure 64 Kaplan Meier survival curves for patients with and without PE on chemotherapy stratified for clinical suspicion

![Survival curves graph](image)
4.18.4.1 Burden of thrombi

Patients diagnosed with more than one thrombus in their pulmonary arteries (n=16) exhibited higher mortality rates than patients without VTE (n=107, p=0.004). There were no significant differences in survival between patients diagnosed with a single thrombus (n=9) and patients without VTE (n=107, p=0.903) nor patients diagnosed with more than one thrombus (p=0.17) (see table 53).

Table 53 Cumulative mortality for cancer patients diagnosed with PE at baseline and follow up stratified by number of thrombi seen on CT scan

<table>
<thead>
<tr>
<th>Cumulative mortality (%+/-SEM)</th>
<th>90 day</th>
<th>183 day</th>
<th>365 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>No VTE on chemotherapy (107)</td>
<td>4.7 (2.0)</td>
<td>10.3 (2.9)</td>
<td>18.1 (3.8)</td>
</tr>
<tr>
<td>Single thrombus (n=9)</td>
<td>11.1 (10.5)</td>
<td>11.1 (10.5)</td>
<td>22.2 (13.9)</td>
</tr>
<tr>
<td>≥2 thrombi (n=16)</td>
<td>37.5 (12.1)</td>
<td>37.5 (12.1)</td>
<td>43.7 (12.4)</td>
</tr>
</tbody>
</table>

4.18.4.2 Lung involvement

In patients diagnosed with thrombus in both lungs (bilateral PE) (n=11) the mortality rate was higher than in patients with unilateral lung involvement (n=14, p=0.046) and in patients who did not develop VTE (n=107, p=0.001). There was no difference in survival between those with unilateral PE and those who were not diagnosed with VTE (p=0.712) (see table 54).

Table 54 Cumulative mortality for cancer patients diagnosed with PE at baseline and follow up stratified for lung involvement

<table>
<thead>
<tr>
<th>Cumulative mortality (%+/-SEM)</th>
<th>90 day</th>
<th>183 day</th>
<th>365 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>No VTE on chemotherapy (107)</td>
<td>4.7 (2.0)</td>
<td>10.3 (2.9)</td>
<td>18.1 (3.8)</td>
</tr>
<tr>
<td>Unilateral PE (n=14)</td>
<td>14.3 (9.4)</td>
<td>14.3 (9.4)</td>
<td>28.6 (12.1)</td>
</tr>
<tr>
<td>Bilateral PE (n=11)</td>
<td>45.5 (15.0)</td>
<td>45.5 (15.0)</td>
<td>45.5 (15.0)</td>
</tr>
</tbody>
</table>
4.18.4.3  Site of thrombi in pulmonary arterial tree
Thrombus present in the lobar arteries (n=10) was associated with poorer survival compared with patients without VTE (n=107, p=<0.0001) but not when compared with PE present in the segmental (n=12, p=0.072) or subsegmental (n=3, p=0.261) vessels. Patients with segmental (p=0.736) and subsegmental (p=0.891) PE exhibited similar survival to patients without VTE (see table 55 and figures 65 and 66).

Table 55 Cumulative mortality for cancer patients diagnosed with PE at baseline and follow up stratified for most proximal site of thrombus

<table>
<thead>
<tr>
<th>Site of thrombus</th>
<th>Cumulative mortality (% +/-SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>No VTE on chemotherapy</td>
<td>4.7 (2.0)</td>
</tr>
<tr>
<td>Lobar PE (n=10)</td>
<td>40.0 (15.5)</td>
</tr>
<tr>
<td>Segmental PE (n=12)</td>
<td>25.0 (12.5)</td>
</tr>
<tr>
<td>Subsegmental PE (n=3)</td>
<td>0.0 (N/E)</td>
</tr>
</tbody>
</table>

Figure 65 Cumulative mortality for cancer patients diagnosed with PE at baseline and follow up stratified for the most proximal site of thrombus development in the pulmonary arteries
Figure 66 Kaplan Meier survival curves for patients diagnosed with PE stratifying for most proximal site of thrombus
4.18.4.4 Unsuspected lobar PE diagnosis at baseline

Lobar pulmonary emboli were diagnosed in 10 patients on study of which eight were clinically unsuspected. Five of these unsuspected events were diagnosed at baseline assessment. A comparison of variables at baseline was undertaken between these five patients and chemotherapy patients without VTE (see table 56).

Table 56 Variables that were significantly different between cancer patients diagnosed with unsuspected lobar PE (n=5) and cancer patients without VTE (n=138) at baseline who commenced chemotherapy

<table>
<thead>
<tr>
<th>Variables significantly higher/longer at baseline in cancer patients with unsuspected lobar PE on univariate analysis (ANOVA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemistry</td>
<td>ALP****, AST****, ALT**, GGT*</td>
</tr>
<tr>
<td>Coagulation</td>
<td>D-dimer****, soluble P-selectin****, INR**, thrombomodulin*</td>
</tr>
<tr>
<td>Thrombin Generation Assay</td>
<td>Tail*</td>
</tr>
<tr>
<td>Angiogenesis/Inflammation</td>
<td>Ang-2****, sTie-2****, hsCRP****</td>
</tr>
<tr>
<td>Examination parameters</td>
<td>Pulse rate*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables significantly lower/shorter at baseline in cancer patients with unsuspected lobar PE on univariate analysis (ANOVA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemistry</td>
<td>Albumin**</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Antithrombin****</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.001

Antithrombin (AT) (p<0.0001) levels were lower in patients with baseline unsuspected lobar PE, as was seen when all baseline VTE patients were compared with chemotherapy patients without VTE. Mean thrombomodulin (TM) (p=0.021), INR (p=0.002), D-dimer (p<0.0001), sP-selectin (p<0.0001),
Ang-2 (p<0.0001), sTie-2 (p<0.0001) levels and the Ang-2:sTie-2 (p=0.012) and lag time:AT (p=0.004) ratios were all higher as was the pulse rate (p=0.032) on clinical examination. Ang-1 (p=0.344), Bilirubin (p=0.069) and haemoglobin (p=0.064) levels were not significantly different between the groups in this analysis.

### 4.18.4.5 Unsuspected lobar PE at baseline multivariate analysis

Using stepwise logistic regression multivariate analysis D-dimer and sTie-2 were found to be independently associated with the diagnosis of unsuspected lobar PE at baseline.

**Figure 67 ROC curves of D-dimer and soluble Tie-2 receptor levels for the diagnosis of unsuspected lobar PE at baseline**

The ROC curve shown in figure 67 for D-dimer was statistically significant with an area under the curve of 0.91 (SE 0.08, p=0.002) and the curve for sTie-2 was non significant with an area under the curve of 0.76 (SE 0.1, p=0.051). A D-dimer cut off of greater than 1600 ng/mL would be associated with unsuspected lobar PE presence with a sensitivity of 80% and specificity of 71%.
4.18.5 Deep vein thrombosis (DVT) on chemotherapy

Patients diagnosed with DVT (n=14) and SVT (n=11) had similar mortality rates to patients without VTE (n=107, p=0.169 and 0.848 respectively).

4.18.5.1 DVT site

DVT development in the abdomen (n=8, median survival 386 days, p=0.009) or lower limb (n=9, median survival 384 days, p=0.003) were associated with poorer survival compared to patients without VTE (n=107) (see table 57 and figures 68 to 70). No differences in survival were identified in comparisons between patients with upper limb, lower limb or abdominal DVT with survival rates in the upper limb DVT cohort also similar to patients without a VTE diagnosis. Two patients had both abdominal and lower limb DVTs.

Table 57 Cumulative mortality of chemotherapy patients diagnosed with DVT in follow up beyond baseline

<table>
<thead>
<tr>
<th></th>
<th>Cumulative mortality (%+/−SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>No VTE on chemotherapy (107)</td>
<td>4.7 (2.0)</td>
</tr>
<tr>
<td>All DVT (n=14)</td>
<td>7.1 (6.9)</td>
</tr>
<tr>
<td>Suspected DVT (n=8)</td>
<td>12.5 (11.7)</td>
</tr>
<tr>
<td>Unsuspected DVT (n=6)</td>
<td>0.0 (N/E)</td>
</tr>
<tr>
<td>Abdominal DVT events (n=8)</td>
<td>12.5 (11.7)</td>
</tr>
<tr>
<td>Lower limb DVT events (n=9)</td>
<td>44.4 (16.6)</td>
</tr>
<tr>
<td>Upper limb DVT events (n=7)</td>
<td>0.0 (N/E)</td>
</tr>
</tbody>
</table>
Figure 68 Cumulative mortality in chemotherapy patients diagnosed with DVT in follow up beyond baseline

Figure 69 Cumulative mortality in chemotherapy patients diagnosed with DVT in follow up beyond baseline stratified by site of DVT
Figure 70 Kaplan Meier survival curves for chemotherapy patients diagnosed with DVT in follow up beyond baseline stratified for clinical suspicion

![Cumulative Survival](image)

4.18.5.2 Superficial vein thrombosis (SVT)

No differences in survival were observed between cohorts of patients with suspected SVT (n=7), unsuspected SVT (n=4) and without VTE (n=107) (see table 58 and figures 71 and 72).

Table 58 Cumulative mortality for chemotherapy patients diagnosed with SVT in follow up beyond baseline

<table>
<thead>
<tr>
<th></th>
<th>Cumulative mortality (% +/- SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 day</td>
</tr>
<tr>
<td>No VTE on chemotherapy (107)</td>
<td></td>
</tr>
<tr>
<td>All SVT (n=11)</td>
<td>0.0 (NE)</td>
</tr>
<tr>
<td>Suspected SVT (n=7)</td>
<td>0.0 (NE)</td>
</tr>
<tr>
<td>Unsuspected SVT (n=4)</td>
<td>0.0 (NE)</td>
</tr>
</tbody>
</table>
Figure 71 Cumulative mortality for chemotherapy patients diagnosed with SVT in follow up beyond baseline

![Cumulative mortality chart]

Figure 72 Kaplan Meier for survival of chemotherapy patients diagnosed with SVT in follow up beyond baseline stratified for clinical suspicion

![Kaplan Meier chart]
4.19 Summary

A high proportion of cancer patients were diagnosed with VTE (25.1%), of which almost 60% of events were clinically unsuspected. Notably all VTE events at baseline (8.4%) were clinically unsuspected and most patients had developed multiple thrombi at the time of diagnosis. In patients diagnosed with PE, thrombi were commonly found in segmental and lobar arteries, regardless of the clinical suspicion for their presence, with the minority localised solely to the subsegmental arteries. Clinically unsuspected VTE were as common as suspected VTE in patients followed up on chemotherapy, with significant clot burden seen on radiological imaging despite the lack of prospectively identified symptoms and signs.

Cumulative incidences and mortality of VTE were high in patients with VTE demonstrating poorer survival in comparison to patients not diagnosed with VTE, irrespective of time of diagnosis during the study. Although patients diagnosed with VTE at baseline assessment exhibited poorer survival, patients diagnosed with unsuspected VTE in follow up did not when compared to patients without VTE. Suspected VTE patients, however, did demonstrate poorer survival compared to patients without VTE but not when compared to patients with unsuspected VTE. Patients with lobar PE exhibited poorer survival than patients without VTE but similar mortality rates when compared with patients with segmental and subsegmental PEs. Eight of the 10 lobar PEs were clinically unsuspected prior to diagnosis, including five at baseline assessment. In the five patients with unsuspected lobar PE at baseline a D-dimer cut-off of 1600 ng/mL or greater was associated with the diagnosis with a sensitivity of 80% and specificity of 71%.

Blood marker analysis revealed differences, at baseline assessment, between cancer patients with and without VTE. Liver function tests were more likely to be deranged and haemoglobin and antithrombin (AT) were lower in patients diagnosed with VTE although mean AT concentrations remained within the CHL normal range. Individual variables of the TGA were similar in both cohorts but ratios of all variables with AT (with the exception of the mean lag time) were higher in patients diagnosed with VTE. Ang-2 and sTie-2 were both
higher in VTE patients but the Ang-2:sTie-2 ratio was similar. D-dimer, hsCRP, sP-selectin and INR were also higher in VTE patients. Of interest, similar differences were found in patients diagnosed with unsuspected lobar PE, at baseline or in follow up, in comparison to all cancer patients recruited to the study, with the exception of the haemoglobin and ALP, AST and GGT. The Ang2:sTie-2 ratio was significantly higher in the PE cohort.
VTE events and associated changes in clinicopathological variables for cancer patients undertaking chemotherapy

Of the 203 cancer patients consented and assessed at baseline 138 patients without VTE commenced chemotherapy as described in the CONSORT diagram in section 4.16 (see figure 50).

4.20 Chemotherapy regimens received by cancer patients

One hundred and twenty one of the 138 chemotherapy patients were fully reviewed in follow up, on at least one occasion beyond baseline assessment, with questionnaire, examination, blood tests and appropriate radiological imaging. The most commonly administered chemotherapy regimens are summarised in table 59.

Table 59 Chemotherapy regimens commenced by the 121 patients fully assessed on at least 2 occasions during the study

<table>
<thead>
<tr>
<th>Chemotherapy regimens commenced</th>
<th>Number patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin/cyclophosphamide (AC)</td>
<td>21 (17.4%)</td>
</tr>
<tr>
<td>5-Fluorouracil (5-FU)/leucovorin/Oxaliplatin (FOLFOX)</td>
<td>15 (12.4)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>9 (7.4)</td>
</tr>
<tr>
<td>Docetaxel/trastuzumab</td>
<td>8 (6.6)</td>
</tr>
<tr>
<td>Rituximab/cyclophosphamide/vincristine/doxorubicin/prednisone (R-CHOP)</td>
<td>6 (5.0)</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>6 (5.0)</td>
</tr>
<tr>
<td>Epirubicin/cisplatin/5-FU (ECF)</td>
<td>6 (5.0)</td>
</tr>
<tr>
<td>Weekly cisplatin with radiation therapy</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>Carboplatin/gemcitabine</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>Other regimens</td>
<td>42 (34.7)</td>
</tr>
</tbody>
</table>
Seven patients (5.8%) received at least one dose of two different chemotherapy regimens prior to their second assessment on study. The regimen changes were as follows:

- One patient- gemcitabine and erlotinib to gemcitabine alone due to erlotinib related toxicity.

- Two patients- docetaxel/trastuzumab to 5-FU / epirubicin / cyclophosphamide (FEC) as per institution protocol for breast cancer treatment. One of these patients developed an upper extremity DVT which was not CVAD (PICC)-associated.

- One patient- doxorubicin/cyclophosphamide (AC) to doxorubicin alone due to severe cyclophosphamide reaction during first treatment.

- Two patients- AC to weekly paclitaxel or paclitaxel with 3-weekly trastuzumab as per institution protocol for breast cancer treatment.

- One patient- rituximab/ cyclophosphamide/ vincristine/ doxorubicin/ prednisone (R-CHOP) to gemcitabine/cisplatin/dexamethasone (GDP) for refractory diffuse large B cell lymphoma. This patient developed a CVAD (PICC)-associated upper extremity DVT.

Forty patients (33.1%) received a platinum agent (cisplatin, carboplatin or oxaliplatin), 12 (9.9%) patients received gemcitabine, 40 (33.1%) patients received an anthracycline (doxorubicin or epirubicin) and 42 (34.7%) patients received a fluoropyrimidine (5-fluorouracil or capecitabine) either alone or in combination. Six (5.0%) patients received platinum/anthracycline/ fluoropyrimidine triplet therapy and a further 18 (14.9%) patients received a fluoropyrimidine and platinum without anthracycline in their chemotherapy regimens. Three (2.5%) patients received an anthracycline and fluoropyrimidine without platinum in their treatment regimen and there were no patients who received a platinum and anthracycline without fluoropyrimidine.
4.21 VTE events on chemotherapy within 100 days of baseline assessment

Four of the six (66.7%) patients on fluoropyrimidine/platinum/anthracycline triplet therapy and five of the 18 (27.8%) patients receiving fluoropyrimidine and platinum (alone or in combination with other non-platinum chemotherapeutic agents) developed VTE within 100 days of baseline assessment. One patient of three (33.3%) who received an anthracycline and fluoropyrimidine and one of six patients (16.7%) receiving single agent gemcitabine-based chemotherapy also developed VTE within the 100 day timeframe (see table 60).

Table 60 Chemotherapy regimens associated with VTE during the 100 days following baseline assessment

<table>
<thead>
<tr>
<th>Chemotherapy regimen</th>
<th>Patients developing VTE n=14 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOLFOX n=15</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>ECF n=6</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>AC n=21</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Gemcitabine n=6</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>5-FU/epirubicin/cyclophosphamide (FEC) n=3</td>
<td>1# (33.3)</td>
</tr>
<tr>
<td>Capecitabine/oxaliplatin (CAPOX/XELOX) n=2</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>5-FU/leucovorin/irinotecan/oxaliplatin (FOLFIRINOX) n=1</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>Trastuzumab n=10</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Paclitaxel n=2</td>
<td>1 (50.0)</td>
</tr>
</tbody>
</table>

# Patient routinely changed from docetaxel and trastuzumab to FEC and diagnosed with VTE after commencing FEC.
Of the 17 patients diagnosed with VTE beyond 100 days, 11 had completed chemotherapy treatment and six were still receiving chemotherapy treatment. These patients are summarised in tables 61 and 62.

**Table 61 VTE events diagnosed in chemotherapy patients beyond 100 days from baseline assessment**

<table>
<thead>
<tr>
<th>Chemotherapy regimen received</th>
<th>Patients developing VTE n=17 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine n=6</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Capecitabine n=9</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Carboplatin/etoposide n=3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Carboplatin/paclitaxel n=3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Paclitaxel n=3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Gemcitabine/cisplatin/dexamethasone (GDP) n=1</td>
<td>1# (100.0)</td>
</tr>
<tr>
<td>Completed chemotherapy ≤30 days prior to VTE diagnosis (range 8-24 days)</td>
<td>6</td>
</tr>
<tr>
<td>Completed chemotherapy &gt;30 days prior to VTE diagnosis (range 32-147 days)</td>
<td>5</td>
</tr>
</tbody>
</table>

# Patient had initially received R-CHOP but changed to GDP due to disease progression and diagnosed with VTE after commencing GDP.

Of the 11 patients who had completed chemotherapy treatment only three had received their final dose of chemotherapy more than 40 days prior to diagnosis of VTE.
Table 62 Chemotherapy regimens received by patients who had completed chemotherapy prior to being diagnosed with VTE

<table>
<thead>
<tr>
<th>Chemotherapy regimen received</th>
<th>Number of patients (n=11)</th>
<th>Days between last dose of chemotherapy and VTE diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOLFOX</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>116</td>
</tr>
<tr>
<td>AC</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Docetaxel (followed AC)</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>1</td>
<td>147</td>
</tr>
<tr>
<td>Paclitaxel (followed AC)</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Carboplatin/ etoposide</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>1</td>
<td>19</td>
</tr>
</tbody>
</table>

Most VTE events were associated with fluoropyrimidine, platinum, anthracycline use or chemotherapy regimens containing one or more of these chemotherapeutic agents (see table 63). Due to the small numbers of patients receiving specific chemotherapy regimens it was not possible to statistically analyse the associations between individual chemotherapeutic agents or regimens and VTE occurrence.
Table 63 The number of VTE events associated with chemotherapeutic agents

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Number of VTE diagnoses associated with chemotherapy agent and time of VTE diagnosis</th>
<th>Total VTE events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤100 days</td>
<td>&gt;100 days, on chemotherapy</td>
</tr>
<tr>
<td>Fluoropyrimidine</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Platinum</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Anthracycline</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Gemcitabine (nucleoside analogue)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cyclophosphamide (alkylating agent)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Taxane</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Topoisomerase inhibitor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vinca alkaloid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Steroid</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rituximab</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
4.22 Intent of chemotherapy and presence of macroscopic cancer

One hundred and thirty eight patients commenced chemotherapy on study of which 65 (47.1%) had been diagnosed with local or locally advanced disease and 73 (52.9%) had metastatic disease. Sixty two (44.9%) patients commenced chemotherapy with curative intent and 76 (55.1%) received palliative chemotherapy. Of the 62 patients on potentially curative therapy 27 (43.5%) had evidence of macroscopic disease.

Of the 34 patients who developed VTE in the follow up period 31 had been commenced on chemotherapy in Christchurch. Twenty four (75.0%) of these patients were fully assessed at the time of VTE diagnosis and seven patients (25.0%) presented acutely with VTE to other medical services and were not assessed, as per study protocol, prior to the commencement of anticoagulation. Ten (16.1%) of the 62 patients treated with curative intent and 21 (27.6%) of the 76 patients treated with palliative intent developed VTE (see tables 64 and 65).
Four (40%) patients who developed VTE on curative therapy, had macroscopic cancer in situ and six (60%) did not. One PE, one leg DVT, two leg SVTs were diagnosed, as well as six with upper limb venous thrombosis, four associated with PICC lines. The PICC associated events involved the deep and superficial veins whereas the non PICC upper limb thromboses involved the superficial veins only. All patients received therapeutic dose low molecular weight heparin except the two patients with lower limb SVT. A repeat USS, performed five

<table>
<thead>
<tr>
<th>Patient study ID</th>
<th>Stage at cancer diagnosis</th>
<th>VTE</th>
<th>Time from baseline assessment to VTE diagnosis (days)</th>
<th>Macroscopic disease in situ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Locally advanced</td>
<td>PICC DVT</td>
<td>57</td>
<td>No</td>
</tr>
<tr>
<td>51</td>
<td>Locally advanced</td>
<td>Leg DVT</td>
<td>117</td>
<td>No</td>
</tr>
<tr>
<td>52</td>
<td>Locally advanced</td>
<td>PE</td>
<td>203</td>
<td>No</td>
</tr>
<tr>
<td>90</td>
<td>Locally advanced</td>
<td>Non PICC arm SVT</td>
<td>77</td>
<td>No</td>
</tr>
<tr>
<td>96</td>
<td>Locally advanced</td>
<td>Non PICC arm SVT</td>
<td>178</td>
<td>No</td>
</tr>
<tr>
<td>117</td>
<td>Locally advanced</td>
<td>Leg SVT</td>
<td>83</td>
<td>No</td>
</tr>
<tr>
<td>152</td>
<td>Locally advanced</td>
<td>PICC DVT</td>
<td>168</td>
<td>Yes</td>
</tr>
<tr>
<td>155</td>
<td>Locally advanced</td>
<td>PICC DVT/SVT</td>
<td>14</td>
<td>Yes</td>
</tr>
<tr>
<td>255</td>
<td>Metastatic</td>
<td>PICC DVT/SVT</td>
<td>131</td>
<td>Yes</td>
</tr>
<tr>
<td>257</td>
<td>Locally advanced</td>
<td>Leg SVT</td>
<td>104</td>
<td>Yes</td>
</tr>
</tbody>
</table>
days after the original investigation, showed that the thrombi had largely resolved in these patients and did not require further intervention.

Table 65 VTE events in chemotherapy patients treated with palliative intent

<table>
<thead>
<tr>
<th>Patient study ID</th>
<th>Stage at cancer diagnosis</th>
<th>VTE</th>
<th>Time from baseline assessment to VTE diagnosis (days)</th>
<th>Macroscopic disease in situ</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Locally advanced#</td>
<td>PICC DVT</td>
<td>276</td>
<td>Yes</td>
</tr>
<tr>
<td>22</td>
<td>Metastatic colon</td>
<td>PE</td>
<td>191</td>
<td>Yes</td>
</tr>
<tr>
<td>27</td>
<td>Locally advanced#</td>
<td>PE</td>
<td>290</td>
<td>Yes</td>
</tr>
<tr>
<td>46</td>
<td>Metastatic</td>
<td>PE</td>
<td>153</td>
<td>Yes</td>
</tr>
<tr>
<td>47</td>
<td>Metastatic</td>
<td>PE</td>
<td>79</td>
<td>Yes</td>
</tr>
<tr>
<td>67</td>
<td>Metastatic</td>
<td>PE</td>
<td>42</td>
<td>Yes</td>
</tr>
<tr>
<td>69</td>
<td>Metastatic</td>
<td>PICC DVT/SVT</td>
<td>51</td>
<td>Yes</td>
</tr>
<tr>
<td>84</td>
<td>Metastatic</td>
<td>leg SVT</td>
<td>107</td>
<td>Yes</td>
</tr>
<tr>
<td>97</td>
<td>Metastatic</td>
<td>PICC SVT</td>
<td>45</td>
<td>Yes</td>
</tr>
<tr>
<td>106</td>
<td>Metastatic</td>
<td>abdominal DVT/gonadal vein</td>
<td>183</td>
<td>Yes</td>
</tr>
<tr>
<td>110</td>
<td>Metastatic</td>
<td>PE/R Common iliac DVT</td>
<td>240</td>
<td>Yes</td>
</tr>
<tr>
<td>113</td>
<td>Metastatic</td>
<td>PVT</td>
<td>210</td>
<td>Yes</td>
</tr>
<tr>
<td>158</td>
<td>Metastatic</td>
<td>PE</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>163</td>
<td>Metastatic</td>
<td>PE</td>
<td>119</td>
<td>Yes</td>
</tr>
<tr>
<td>168</td>
<td>Metastatic</td>
<td>PVT</td>
<td>115</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Venous thromboembolism in cancer patients undertaking chemotherapy

<table>
<thead>
<tr>
<th>Patient study ID</th>
<th>Stage at cancer diagnosis</th>
<th>VTE</th>
<th>Time from baseline assessment to VTE diagnosis (days)</th>
<th>Macroscopic disease in situ</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>Metastatic</td>
<td>leg DVT</td>
<td>74</td>
<td>Yes</td>
</tr>
<tr>
<td>182</td>
<td>Metastatic</td>
<td>PE</td>
<td>53</td>
<td>Yes</td>
</tr>
<tr>
<td>187</td>
<td>Metastatic</td>
<td>PE</td>
<td>168</td>
<td>Yes</td>
</tr>
<tr>
<td>191</td>
<td>Metastatic</td>
<td>PE/leg DVT</td>
<td>44</td>
<td>Yes</td>
</tr>
<tr>
<td>202</td>
<td>Metastatic</td>
<td>PICC DVT</td>
<td>98</td>
<td>Yes</td>
</tr>
<tr>
<td>249</td>
<td>Metastatic</td>
<td>PE/leg SVT</td>
<td>63</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Twelve (15.8%) of the 76 patients on palliative chemotherapy were diagnosed with PE, four (5.3%) developed PICC associated DVT and/or SVT, four (5.3%) developed lower limb DVT or SVT and four (5.3%) developed intra-abdominal DVT, including two portal vein thromboses (PVTs). In contrast to patients treated with curative intent where one site of thrombosis was diagnosed, four (33.3%) of the 12 patients were diagnosed with thrombi at more than one site in the palliative cohort. All patients were treated with therapeutic dose low molecular weight heparin.

### 4.23 Clinicopathological variables in chemotherapy patients

Baseline and follow up variables were analysed and compared between the 138 cancer patients who undertook chemotherapy and cancer patients who developed VTE.

#### 4.23.1 Cancer patients with VTE (n=16) compared with cancer patients without VTE about to commence chemotherapy (n=138) at baseline assessment

The following table summarises the variables that were significantly different between the 16 cancer patients with VTE at baseline and those without VTE that commenced chemotherapy. Compared with previously described
baseline comparisons between all cancer patients without VTE and volunteers without cancer, a smaller proportion of variables were different between the cohorts. All liver function tests, including bilirubin (p=0.001), were different with mean values higher in the VTE cohort than in the no VTE cohort. D-dimer (p<0.0001), sP-selectin (p<0.0001) and hsCRP (p=0.016) were also higher in the VTE cohort and only haemoglobin (p=0.003) and albumin (p=0.006) were significantly lower. Of note, all individual TGA variables were similar between the two cohorts. Ang-2 (p=0.011) and sTie-2 (p=0.001) concentrations were higher in the VTE cohort as was the Ang-2:sTie-2 ratio (p=0.049). AT (p=0.02) was lower in the VTE cohort and ratios of all the variables of the TGA with AT, with the exception of the lag time, were higher (see table 66).
Table 66 Comparison of baseline variables between cancer patients with VTE and those due to commence chemotherapy without VTE

<table>
<thead>
<tr>
<th>Variables significantly higher/longer at baseline in cancer patients with VTE (n=16) compared with chemotherapy patients without VTE (n=138) on univariate analysis (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemistry</strong></td>
</tr>
<tr>
<td>ALP****, ALT**, AST****, bilirubin**, GGT****</td>
</tr>
<tr>
<td><strong>Coagulation</strong></td>
</tr>
<tr>
<td>D-dimer****, sP-selectin****</td>
</tr>
<tr>
<td><strong>Angiogenesis/Inflammation</strong></td>
</tr>
<tr>
<td>Ang-2*, sTie-2**, hsCRP*</td>
</tr>
<tr>
<td><strong>Parameter ratios</strong></td>
</tr>
<tr>
<td><strong>Clinical examination variables</strong></td>
</tr>
<tr>
<td>Circumference L arm below medial epicondyle*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables significantly lower/shorter at baseline in cancer patients with VTE (n=16) compared with chemotherapy patients without VTE (n=138) on univariate analysis (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complete blood count</strong></td>
</tr>
<tr>
<td>Haemoglobin**</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
</tr>
<tr>
<td>Albumin**</td>
</tr>
<tr>
<td><strong>Coagulation</strong></td>
</tr>
<tr>
<td>Antithrombin*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.0001
4.23.2 D-dimer and hsCRP

Over 80% of patients with cancer diagnosed with VTE at baseline had D-dimer and/or hsCRP levels above the ULN. Over 50% of patients with cancer not diagnosed with VTE, however, also had D-dimer and hsCRP levels above the ULN (see table 67).

Table 67 Comparisons of baseline D-dimer and hsCRP levels between cancer patients with VTE and those without VTE undertaking chemotherapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>VTE and cancer (n=16)</th>
<th>No VTE commencing chemotherapy (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer ≥ 250 ng/mL (%)</td>
<td>14 (87.5) (Range 108-10,375)</td>
<td>71 (51.4) (Range 21-3,183)</td>
</tr>
<tr>
<td>hsCRP ≥ 3 mg/L (%)</td>
<td>13 (81.3) (Range 0.54-135)</td>
<td>76 (55.1) (Range 0.15-163)</td>
</tr>
</tbody>
</table>

In chemotherapy patients not diagnosed with VTE at baseline assessment, a higher proportion of patients with widespread cancer presented with D-dimer and hsCRP levels above the ULN compared with patients with local or locally advanced cancer. D-dimer and hsCRP levels did not appear to correlate with the presence or absence of macroscopic cancer at the time of chemotherapy initiation although a higher proportion of patients with unresected macroscopic local/locally advanced cancer had raised hsCRP levels (see table 68).
Table 68 Comparisons of D-dimer and hsCRP levels in chemotherapy patients without VTE at baseline assessment stratifying for cancer stage and presence of macroscopic cancer (n=138)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Local/ locally advanced cancer</th>
<th>Widespread cancer (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macroscopic disease resected (n=31)</td>
<td>Macroscopic disease present (not resected) (n=34)</td>
</tr>
<tr>
<td>D-dimer ≥ 250 ng/mL (%)</td>
<td>13 (41.9) (Range 81-579)</td>
<td>9 (26.5) (Range 21-1,232)</td>
</tr>
<tr>
<td>hsCRP ≥ 3 mg/L (%)</td>
<td>9 (29.0) (Range 0.15-12.1)</td>
<td>13 (38.2) (Range 0.17-86.4)</td>
</tr>
</tbody>
</table>

### 4.23.3 Multivariate analysis

Using stepwise logistic regression multivariate analysis D-dimer and alanine transaminase (ALT) were independently associated with VTE diagnosis at baseline assessment. When ECOG performance status, hospital admission within four weeks of assessment and the presence of breathlessness were taken into consideration, ECOG status was also found to be independently associated with VTE development within 100 days on chemotherapy.

The ROC curves produced for both D-dimer and ECOG performance status were statistically significant with areas under the curve of 0.79 (SE 0.071, p<0.0001) and 0.76 (SE 0.06, p<0.001) respectively (see figures 73 and 74).
Figure 73 ROC curves of D-dimer levels and ECOG performance status associated with the diagnosis of VTE in cancer patients at baseline assessment.

An ECOG performance status of greater than 1 was associated with baseline VTE with a sensitivity of 93.3% and specificity of 46%. A D-dimer cut-off value of 270ng/ml was also associated with baseline VTE presence with a sensitivity of 86.7% and specificity of 53.3%.
Alanine transaminase (ALT) ROC curve analysis showed an area under the curve of 0.52 (SE 0.084, p=0.779) and was not statistically significant despite the multivariate analysis findings.

4.24 Beyond baseline assessments
One hundred and seven (77.5%) of the chemotherapy patients underwent a full second assessment within 100 days beyond baseline. Fourteen (10.1%) of the 138 patients developed a VTE during this period. Three (21.4%) of the patients who developed VTE presented acutely to other hospital departments and were not seen by the study team at the time of diagnosis. Complete clinical assessment data could not be gathered in these patients and so only available blood results prior to anticoagulation and relevant imaging confirming VTE diagnosis were subsequently used for analysis to minimise recall bias. Eleven (78.5%) patients were fully assessed at the time of VTE diagnosis. Ninety six
patients were assessed for a second time within 100 days and were not diagnosed with VTE.

### 4.24.1 Comparison of baseline variables between chemotherapy patients who developed VTE on chemotherapy within 100 days of baseline assessment and those that did not.

At baseline assessment, mean D-dimer concentrations ($p=0.0499$) were significantly higher and mean magnesium concentrations ($p=0.021$) were significantly lower in the patients who were diagnosed with VTE within 100 days of follow up compared with those who were not (see table 69 and figures 75 and 76). None of the other variables studied were significantly different.

**Table 69 Baseline variables that were different between chemotherapy patients that were diagnosed with VTE and those that were not within 100 days following baseline assessment**

<table>
<thead>
<tr>
<th>Variable at baseline assessment (Mean)</th>
<th>VTE cohort (n=11)</th>
<th>No VTE cohort (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (ng/mL)</td>
<td>558*</td>
<td>320</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.84*</td>
<td>0.90</td>
</tr>
</tbody>
</table>

*p<0.05
Figure 75 Comparison of mean (+/-SEM) baseline D-dimer concentrations between chemotherapy patients that develop VTE and those that did not within 100 days from baseline assessment (n=107)

![Bar chart showing comparison of mean D-dimer concentrations between VTE and No VTE groups.](image)

Although the mean magnesium concentration was significantly lower in VTE patients the median concentrations of magnesium were identical at 0.90 mmol/L in both groups. Most patients maintained magnesium concentrations within the normal range making it challenging to identify a discriminatory cut-off concentration that would be clinically meaningful.

Figure 76 Comparison of mean (+/- SEM) baseline magnesium concentrations between chemotherapy patients that develop VTE and those that did not within 100 days from baseline assessment (n=107)

![Bar chart showing comparison of mean magnesium concentrations between VTE and No VTE groups.](image)
A ROC curve was, therefore, only produced for D-dimer which had an area of 0.769 (SE 0.063, p=0.005). With a D-dimer cut-off of 210-215 ng/mL the sensitivity was 100 % and specificity was 48%. At a cut-off of 270ng/mL the sensitivity reduced to 80% but the specificity increased to 64% (see figure 77).

Figure 77 ROC curve of D-dimer concentrations for predicting VTE diagnosis on chemotherapy within 100 days of follow up from baseline assessment (n=107)
4.24.2 Suspected and unsuspected VTE prediction on chemotherapy and D-dimer levels at baseline

ROC curves were also produced to look at prediction of any suspected or unsuspected VTE events on chemotherapy, within and beyond 100 days of follow up (see figures 78 and 79).

Figure 78 A ROC curve of D-dimer concentrations and prediction of suspected VTE on chemotherapy within and beyond 100 days (n=107)

The ROC curve for suspected VTE had an area of 0.680 (SE 0.058, p= 0.024). A cut-off of 220 ng/mL provided a sensitivity of 93.3% and specificity of 47.6% in predicting the development of suspected VTE on chemotherapy (see figure 78).
The ROC curve for unsuspected VTE had an area under the curve of 0.611 (SE 0.070, p=0.152) (see figure 79).

4.24.3 Comparison of variables at second assessment between chemotherapy patients that developed VTE within 100 days from baseline and those that did not

When comparing variables between the 96 patients without VTE and the 11 fully assessed patients that did develop VTE at their second assessment, mean D-dimer (p<0.0001) and sP-selectin (p=0.004) were significantly higher and mean platelet count (p=0.026) and magnesium (p=0.012) were significantly lower in the VTE cohort on univariate analyses. Median magnesium concentrations were, however, again identical in the two groups at 0.9 mmol/L. All other variables were similar in the two cohorts (see table 70 and figures 80 to 83).
Table 70 Variables that were different between chemotherapy patients with and without VTE at second assessment

<table>
<thead>
<tr>
<th>Variable at second assessment (Mean)</th>
<th>VTE cohort (n=11) (Result obtained at time of VTE diagnosis)</th>
<th>No VTE cohort (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble P-selectin (ng/mL)</td>
<td>46.0**</td>
<td>22.7</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>1453****</td>
<td>344</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>165*</td>
<td>251</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.77*</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.0001

All patients diagnosed with VTE within 100 days on chemotherapy had D-dimer concentrations above the ULN. It was also noted that over 40% of patients not diagnosed with VTE also had raised D-dimer concentrations (see table 71).

Table 71 Comparison of D-dimer concentrations in chemotherapy patients at follow up within 100 days of baseline stratified by VTE presence

<table>
<thead>
<tr>
<th>Variable</th>
<th>VTE and cancer (n=11)</th>
<th>No VTE and cancer (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer ≥ 250 ng/mL (%)</td>
<td>11 (100) (Range 356-4,741)</td>
<td>42 (43.3) (Range 36-2,604)</td>
</tr>
</tbody>
</table>
Figure 80 Comparison of mean (+/-SEM) soluble P-selectin concentrations between chemotherapy patients with and without VTE at second assessment

**p<0.01

Figure 81 Comparison of mean (+/-SEM) D-dimer concentrations between chemotherapy patients with and without VTE at second assessment

****p<0.0001
4.24.4 Multivariate analysis of differences in variables between cancer patient diagnosed with VTE and those not diagnosed with VTE at second visit within 100 days

Using stepwise logistic regression multivariate analysis, D-dimer was the only variable in which changes in venous blood concentrations were independently associated with VTE diagnosis within 100 days of baseline assessment.
A ROC curve of D-dimer concentrations was produced with an area under the curve of 0.907 (SE=0.034, p=<0.0001). A D-dimer cut-off of 355 ng/mL for VTE diagnosis demonstrated a sensitivity of 100% and specificity of 75% (see figure 84).

**4.25 The impact of chemotherapy on clinicopathological variables**

Ninety six patients on chemotherapy attended a full second assessment within 100 days from baseline, and none were diagnosed with VTE. Summarised below in table 72 are the clinicopathological variables that significantly changed between assessments.
Table 72 Changes in clinicopathological variables in patients without VTE on chemotherapy within 100 days following baseline assessment

<table>
<thead>
<tr>
<th>Continuous variables significantly higher/longer to 100 day follow up on chemotherapy- univariate analysis (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiogenesis/Inflammation</td>
</tr>
<tr>
<td>Biochemistry</td>
</tr>
<tr>
<td>Coagulation</td>
</tr>
<tr>
<td>Clinical examination variables</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Continuous variables significantly lower/shorter within 100 day follow up on chemotherapy- univariate analysis (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Biochemistry</td>
</tr>
<tr>
<td>Coagulation</td>
</tr>
<tr>
<td>Angiogenesis/ inflammation</td>
</tr>
<tr>
<td>TGA parameters</td>
</tr>
<tr>
<td>Clinical examination variables</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.0001
4.25.1 Disease response to chemotherapy

When clinicopathological changes were correlated with cancer response to chemotherapy, only four variables were found to be significantly different between the responders and non-responders (see table 73).

Table 73 Relative changes in variables that were significantly different between responders and non-responders on chemotherapy at second visit within 100 days of baseline assessment

<table>
<thead>
<tr>
<th>Variable measured at both assessments</th>
<th>Response to chemotherapy/ stable disease (n=74) (Downstaged)</th>
<th>Disease progression on chemotherapy (n=22) (Upstaged)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count</td>
<td>⬇</td>
<td>⬆</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>⬇</td>
<td>⬆</td>
</tr>
<tr>
<td>Sodium</td>
<td>⬆</td>
<td>⬇</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>⬆</td>
<td>⬇</td>
</tr>
</tbody>
</table>

4.26 Comparison of changes in variables between assessments in cancer patients on chemotherapy diagnosed with and without VTE within 100 days of follow up

On comparison of the 11 patients diagnosed with VTE and the 96 patients without VTE who were fully assessed within 100 days, changes in the measured levels of the following variables were significantly different between the cohorts. Soluble P-selectin (p=0.001) and D-dimer (p<0.0001) were again significantly increased and the platelet count (p=0.003), APTT (p=0.012) and fibrinogen (p=0.038) concentrations were significantly reduced in the patients diagnosed with VTE compared to patients without VTE (see table 74 and figures 85 to 89).
Table 74 Comparison of variables between chemotherapy patients diagnosed with VTE at 100 days and those not diagnosed with VTE

<table>
<thead>
<tr>
<th>Variables significantly higher/longer within 100 day follow up on chemotherapy in patients diagnosed with VTE (n=11) v patients without VTE (n=96)- univariate analysis (ANOVA)</th>
<th>Coagulation</th>
<th>Soluble P-selectin**, D-dimer****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables significantly lower/shorter within 100 day follow up on chemotherapy in patients with VTE (n=11) v patients without VTE (n=96)- univariate analysis (ANOVA)</td>
<td>Complete blood count</td>
<td>Platelet count**</td>
</tr>
<tr>
<td></td>
<td>Coagulation</td>
<td>APTT*, fibrinogen*</td>
</tr>
<tr>
<td></td>
<td>Angiogenesis/ inflammation</td>
<td>Ang-2*</td>
</tr>
<tr>
<td></td>
<td>Parameter ratios</td>
<td>Ang-2:sTie-2*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.0001

Figure 85 Changes in mean (+/-SEM) soluble P-selectin concentrations on chemotherapy stratified by VTE status at 100 days

![Figure 85](image-url)
Figure 86 Changes in mean (+/-SEM) D-dimer concentrations on chemotherapy stratified by VTE status at 100 days

![Graph showing changes in mean D-dimer concentrations](image)

**p<0.0001

Figure 87 Changes in mean (+/-SEM) platelet count on chemotherapy stratified by VTE status at 100 days

![Graph showing changes in mean platelet count](image)

**p<0.01

Figure 88 Changes in mean (+/-SEM) APTT on chemotherapy stratified by VTE status at 100 days

![Graph showing changes in mean APTT](image)

*p<0.05
Figure 89 Changes in mean (+/−SEM) fibrinogen concentrations on chemotherapy stratified by VTE status at 100 days

4.26.1 Ang-sTie-2 pathway

Ang-2 concentrations (p=0.040) and the Ang-2:sTie-2 ratio (p=0.025) were significantly lower in patients with VTE than those without (see figures 90 and 91).

Figure 90 Changes in mean (+/−SEM) Ang-2 concentrations on chemotherapy stratified by VTE status at 100 days
Figure 91 Changes in the mean (+/-SEM) Ang-2:sTie-2 ratio on chemotherapy stratified by VTE status at 100 days.

*p<0.05
4.26.2 Multivariate analysis of changes in variables associated with VTE development in cancer patients within 100 days of baseline assessment

On stepwise logistic regression multivariate analysis an increase in D-dimer and decrease in the Ang-2:sTie-2 ratio during chemotherapy treatment were independently associated with the development of VTE within 100 days of baseline assessment.

Figure 92 ROC curve of changes in D-dimer concentration associated with VTE development on chemotherapy at 100 days (n=107)

The ROC curve for D-dimer was statistically significant with an area of 0.792 (SE 0.08, p=0.002). Any increase in D-dimer in follow up after baseline assessment was associated with VTE development. An increase of ≥6.5 ng/mL was associated with VTE development with a sensitivity of 90.9% and specificity of 54.7%. An increase of ≥130 ng/mL was associated with VTE development with a sensitivity of 72.7% and specificity of 76.8% (see figure 92).
Figure 93 ROC curve of changes in the Ang-2:sTie-2 ratio associated with VTE development on chemotherapy at 100 days (n=107)

The ROC curve for the Ang-2:sTie-2 ratio was statistically significant with an area of 0.68 (SE 0.078, p=0.046). A reduction in the ratio by ≥10 was associated with VTE development with a sensitivity of 81.8% and specificity of 41.1% (see figure 93).

4.27 Overall VTE in follow up beyond baseline

Only the mean baseline magnesium level was associated with VTE development within and beyond 100 days from baseline but, as described earlier, the median magnesium level was identical in both the VTE and no VTE cohorts, and levels were within the laboratory normal range limiting the clinical utility of this finding on statistical analysis.
4.28 Summary

Twenty two percent of the patients that undertook chemotherapy developed VTE, 16.1% of 62 treated with curative intent and 27.6% of 76 treated palliatively. The majority received a platinum agent, fluoropyrimidine, anthracycline or combination of these agents. No single chemotherapeutic agent was significantly associated with VTE development as most were given in combination with other agents. However, four of the six patients, receiving ECF chemotherapy for gastric/GOJ cancer, developed VTE. VTE was associated with a broad range of malignancies and chemotherapeutic regimens.

Six of the 10 patients diagnosed with VTE while undergoing curative chemotherapy were undertaking adjuvant treatment. For six patients the site of VTE was the upper limb, of which four were PICC-related and only one patient was diagnosed with PE. In contrast 12 (57.1%) of the 21 patients treated with palliative intent were diagnosed with PE.

Multiple blood borne variables differed between cohorts of cancer patients with VTE and the 138 chemotherapy patients without VTE at baseline. The differences closely mirrored those identified at baseline between patients with VTE and all cancer patients recruited to the study regardless of whether they started chemotherapy or not (n=185). In addition to those variables, the mean Ang-2:sTie2 ratio, bilirubin and ALT were higher in the VTE cohort. Multivariate and ROC curve analysis identified D-dimer and ECOG performance status as independently associated with baseline VTE. An ECOG performance status of >1 was associated with baseline VTE with a sensitivity 93% and specificity of 46%. A D-dimer cut-off value of 270 ng/mL was associated with baseline VTE with a sensitivity of 86.7% and specificity of 53.3%.

D-dimer and magnesium were the only variables at baseline that were associated with subsequent development of VTE on chemotherapy. A D-dimer cut-off of 210-215 ng/mL provided a sensitivity of 100% but a specificity of only 48%. A cut-off of 270 ng/mL reduced the sensitivity to 80% but increased the specificity to 64%. For patients who presented with VTE on chemotherapy within 100 days D-dimer was again the only variable to be associated with the
presence of VTE. ROC curve analysis showed that a D-dimer cut-off of 355ng/mL demonstrated a sensitivity of 100% and specificity of 75%. Using this curve, a value of 270 ng/mL or more would also have provided a sensitivity of 100% but the specificity would decrease to 60.4%.

Many of these variables assessed in the blood changed in patients on chemotherapy treatment who did not develop VTE within 100 days, including Ang-1, which decreased, and Ang-2 and sTie-2 which increased. Ratios of Ang-1:Ang2 and Ang-1:sTie-2 consequently decreased as did ratios of ETP, peak thrombin, VI and tail to AT and ETP to TM. This was because the AT concentration increased and the TGA variables decreased. Complete blood count components reduced on chemotherapy as did the albumin, magnesium and potassium but the D-dimer and sP-selectin did not change significantly although did show a smaller upwards trend.

For those patients who developed VTE within 100 days on chemotherapy significant increases in D-dimer and soluble P-selectin were seen, while decreases were seen in Ang-2, the Ang-2:sTie-2 ratio, platelet count, APTT and fibrinogen. All other variables had not changed significantly between baseline and the VTE diagnosis visit. On multivariate analysis only changes in D-dimer and the Ang2:sTie-2 ratio were associated with VTE diagnosis with any increase in D-dimer associated with the presence of VTE. An increase of 6.5ng/mL had a sensitivity of 90.9% and specificity of 54.7% whereas an increase of 130 ng/mL had a sensitivity of 72.7% and specificity of 76.8%. A decrease in the Ang-2:sTie-2 ratio of 10 or more was associated with VTE presence with a sensitivity of 81.8% and a specificity of 41.1%.
Risk assessment model performance

4.29 Aim
Assess the performance of the Khorana score, Khorana/Ay score (The Vienna model) and PROTECHT score as risk assessment models (RAMs) for predicting VTE development in cancer patients undertaking chemotherapy (24, 596, 599).

4.30 Introduction
At baseline assessment all recruited participants provided blood samples for blood marker analysis and risk assessment model analysis.

4.31 VTE prediction on chemotherapy at 100 days
The original Khorana score assessed VTE risk on chemotherapy at 2.4 months from baseline assessment. It was, therefore, decided that initial validation of the score, in this study, would occur at up to 100 days from baseline assessment with most chemotherapy patients being assessed between 1.5 and 3 months to coincide with clinical care assessments (n=138).

4.31.1 The Khorana score
The performance of the parameters published in the original Khorana score were assessed individually as well as collectively in this study.

4.31.1.1 Haemoglobin <10g/dL (<100g/L)
Tables 75 and 76 summarise the numbers of chemotherapy patients presenting with haemoglobin levels above and below this cut-off as well as how many went on to develop VTE within 100 days.

Table 75 Patients stratified by VTE and haemoglobin concentration <10g/dL

<table>
<thead>
<tr>
<th>VTE</th>
<th>Hb&lt;10g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>
Table 76 The performance of a haemoglobin level <10g/dL in predicting VTE development on chemotherapy within 100 days from testing

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>98.4</td>
<td></td>
</tr>
<tr>
<td>Positive predictive value (PPV) (%)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Negative predictive value (NPV) (%)</td>
<td>89.7</td>
<td></td>
</tr>
</tbody>
</table>

4.31.1.2 Leukocyte count >11x10⁹/L

Tables 77 and 78 summarise the numbers of patients presenting with leukocyte counts above and below this cut-off as well as how many went on to develop VTE within 100 days.

Table 77 Patients stratified by VTE and leukocyte count >11x10⁹/L

<table>
<thead>
<tr>
<th>Leukocyte count &gt;11x10⁹/L</th>
<th>VTE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

Table 78 The performance of a leukocyte count >11x10⁹/L in predicting VTE development on chemotherapy within 100 days from testing

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>91.9</td>
<td></td>
</tr>
<tr>
<td>Positive Predictive Value (PPV) (%)</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>Negative Predictive Value (NPV) (%)</td>
<td>90.5</td>
<td></td>
</tr>
</tbody>
</table>

4.31.1.3 Platelet count ≥350x10⁹/L

Tables 79 and 80 summarise the numbers of patients presenting with platelet counts above and below this cut-off as well as how many went on to develop VTE within 100 days.

Table 79 Patients stratified by VTE and platelet count ≥350x10⁹/L

<table>
<thead>
<tr>
<th>Platelet count ≥350x10⁹/L</th>
<th>VTE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>
Table 80 The performance of a platelet count $\geq 350 \times 10^9$/L in predicting VTE development on chemotherapy within 100 days from testing

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>35.7</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>82.3</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>18.5</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>91.9</td>
</tr>
</tbody>
</table>

4.31.1.4 Body Mass Index (BMI) $\geq 35$kg/m$^2$

Tables 81 and 82 summarise the numbers of patients presenting with BMIs above and below this cut-off as well as how many went on to develop VTE within 100 days.

Table 81 Patients stratified by VTE and BMI $\geq 35$kg/m$^2$

<table>
<thead>
<tr>
<th>BMI $\geq 35$kg/m$^2$</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTE Yes</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>VTE No</td>
<td>13</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>125</td>
</tr>
</tbody>
</table>

Table 82 The performance of a BMI $\geq 35$kg/m$^2$ in predicting VTE development on chemotherapy within 100 days from testing

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>89.5</td>
</tr>
<tr>
<td>Positive predictive value (PPV) (%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Negative predictive value (NPV) (%)</td>
<td>88.8</td>
</tr>
</tbody>
</table>

4.31.2 Khorana/Ay score

The addition of D-dimer and sP-selectin measurements improved the sensitivity of the original Khorana score(596). The assay used for D-dimer in this study is different from the assay used routinely at Canterbury Health Laboratories and so we calculated our own cut-off for this as described below.

4.31.2.1 D-dimer

The chosen cut-off for D-dimer was 210 ng/mL from the ROC curve (area 0.692, SE 0.063, p=0.019) for all patients with cancer undergoing chemotherapy (n=138) shown in figure 94 below:
Figure 94 ROC curve for D-dimer concentrations associated with the development of VTE on chemotherapy.

Tables 83 and 84 summarise the numbers of patients presenting with D-dimer results above and below this cut-off as well as how many went on to develop VTE within 100 days.

Table 83 Patients stratified by VTE and D-dimer concentration ≥210ng/mL

<table>
<thead>
<tr>
<th>VTE</th>
<th>D-dimer ≥210ng/mL</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>13</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>No</td>
<td>70</td>
<td>53</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>

Table 84 The performance of D-dimer ≥210ng/mL in predicting VTE development on chemotherapy within 100 days from testing

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>92.9</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>42.7</td>
</tr>
<tr>
<td>Positive predictive value (PPV) (%)</td>
<td>15.7</td>
</tr>
<tr>
<td>Negative predictive value (NPV) (%)</td>
<td>98.1</td>
</tr>
</tbody>
</table>
4.31.2.2 Soluble P-selectin ≥53.1ng/L

Tables 85 and 86 summarise the numbers of patients presenting with soluble P-selectin above and below this cut-off as well as how many went on to develop VTE within 100 days.

Table 85 Patients stratified by VTE and Soluble P-selectin concentration ≥53.1ng/mL

<table>
<thead>
<tr>
<th>VTE</th>
<th>Soluble P-selectin ≥53.1ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

Table 86 The performance of a soluble P-selectin ≥53.1ng/mL in predicting VTE development on chemotherapy within 100 days from testing

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>21.4</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>91.9</td>
</tr>
<tr>
<td>Positive predictive value (PPV) (%)</td>
<td>23.1</td>
</tr>
<tr>
<td>Negative predictive value (NPV) (%)</td>
<td>91.1</td>
</tr>
</tbody>
</table>

4.31.3 Canterbury population-adapted Khorana, Khorana/Ay and PROTECHT scores and variable performance

The performance of each variable and the collective risk assessment scores, using our own adapted cut-offs, were assessed. The following tables summarise the data used to calculate the sensitivities, specificities and positive and negative predictive values. In order to adapt the continuous variables to our different, predominantly New Zealand European population, two approaches were used:

1. The Canterbury Health Laboratory reference ranges for haemoglobin, leukocyte and platelet counts and the World Health Organisation (WHO)/New Zealand Ministry of Health (MOH) guidelines for the definition of obesity. Adaptations of each score, using these cut-offs, was appended with CHL during this study.

2. The use of percentiles and/or receiver operator characteristic (ROC) curves using study participant results (cancer patients) only. Adaptations of each score, using these cut-offs, were appended with CHCH PC during this study.
4.31.3.1 Haemoglobin
The 25th percentile cut-off for haemoglobin was 122g/L. Table 87 summarises the performance of this cut off in predicting VTE in chemotherapy patients within 100 days in comparison with the original Khorana and Canterbury Health Laboratories’ cut offs.

Table 87 The performance of haemoglobin level in predicting VTE development on chemotherapy within 100 days from baseline testing

<table>
<thead>
<tr>
<th>Gender</th>
<th>Khorana</th>
<th>CHL</th>
<th>CHCH PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>All</td>
<td>F/M cut offs</td>
<td>All</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>&lt;100g/L</td>
<td>115/130g/L</td>
<td>&lt;122g/L</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>0.0</td>
<td>21.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>98.4</td>
<td>79.8</td>
<td>75.8</td>
</tr>
<tr>
<td>Positive predictive value (PPV) (%)</td>
<td>0.0</td>
<td>10.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Negative predictive value (NPV) (%)</td>
<td>89.7</td>
<td>90.0</td>
<td>87.9</td>
</tr>
</tbody>
</table>

CHL=Canterbury Health Laboratories, CHCH PC=Christchurch percentiles, F=Female, M=Male

4.31.3.2 Leukocyte count
The 75th percentile cut-off for leukocyte count was 8.7x10⁹/L. Table 88 summarises the performance of this cut off in predicting VTE in chemotherapy patients within 100 days in comparison with the original Khorana and Canterbury Health Laboratories’ cut off.

Table 88 The performance of leukocyte count in predicting VTE development on chemotherapy within 100 days from baseline testing

<table>
<thead>
<tr>
<th>Leukocyte count</th>
<th>Khorana/ CHL</th>
<th>CHCH PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count</td>
<td>&gt;11x10⁹/L</td>
<td>8.7x10⁹/L</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>21.4</td>
<td>28.6</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>79.8</td>
<td>76.6</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>10.7</td>
<td>12.1</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>90.0</td>
<td>90.5</td>
</tr>
</tbody>
</table>

4.31.3.3 Platelet count
The 75th percentile cut-off for platelet count was 328x10⁹/L. Table 89 summarises the performance of this cut off in predicting VTE in chemotherapy
patients within 100 days in comparison with the original Khorana and Canterbury Health Laboratories’ cut offs.

Table 89 Platelet count performance in predicting VTE development on chemotherapy within 100 days from baseline testing

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>Khorana</th>
<th>CHL</th>
<th>CHCH PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>35.7</td>
<td>21.4</td>
<td>42.9</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>82.3</td>
<td>90.3</td>
<td>76.6</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>18.5</td>
<td>20.0</td>
<td>17.1</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>91.9</td>
<td>91.1</td>
<td>92.2</td>
</tr>
</tbody>
</table>

4.31.3.4 Body mass index (BMI)
The 75th percentile cut-off for BMI was 30.63 kg/m². Table 90 summarises the performance of this cut-off in predicting VTE in chemotherapy patients within 100 days in comparison with the original Khorana and WHO criteria/MOH guidelines for obesity cut-offs.

Table 90 The performance of BMI in predicting VTE development on chemotherapy within 100 days from baseline testing

<table>
<thead>
<tr>
<th>BMI</th>
<th>Khorana</th>
<th>CHCH PC</th>
<th>WHO/ NZ MOH guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.0kg/m²</td>
<td>0.0</td>
<td>28.6</td>
<td>42.9</td>
</tr>
<tr>
<td>30.63kg/m²</td>
<td>89.5</td>
<td>75.8</td>
<td>74.8</td>
</tr>
<tr>
<td>30.0kg/m²</td>
<td>0.0</td>
<td>11.8</td>
<td>16.2</td>
</tr>
<tr>
<td>35.0kg/m²</td>
<td>88.8</td>
<td>90.4</td>
<td>92.0</td>
</tr>
</tbody>
</table>

4.31.3.5 D-dimer
The chosen cut-off for D-dimer was 210 ng/mL from the ROC curve as described in section 4.31.2.1. The designated upper limit of normal for D-dimer at Canterbury Health Laboratories is 250 ng/mL.

The following data assesses the performances of these cut offs in predicting VTE in chemotherapy patients within 100 days in comparison with the original Khorana and Canterbury Health Laboratories’ cut offs (see table 91).
Table 91 The performance of D-dimer in predicting VTE development on chemotherapy within 100 days from testing

<table>
<thead>
<tr>
<th>D-dimer threshold</th>
<th>CHCH PC</th>
<th>CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥210ng/mL</td>
<td>92.9</td>
<td>78.6</td>
</tr>
<tr>
<td>≥250ng/mL</td>
<td>42.7</td>
<td>51.2</td>
</tr>
</tbody>
</table>

| Sensitivity (%)  | 92.9    | 78.6 |
| Specificity (%)  | 42.7    | 51.2 |
| PPV (%)          | 15.7    | 15.5 |
| NPV (%)          | 98.1    | 95.5 |

4.31.3.6 Soluble P-selectin
The chosen cut-off for soluble P-selectin was 33.5pg/mL from the ROC curve shown in figure 95 below. The area under the curve was 0.519 (SE 0.094). This was not statistically significant (p=0.815).

Figure 95 ROC curve for soluble P-selectin concentrations associated with the development of VTE on chemotherapy (n=138)

Table 92 summarises the performances of this cut-off and the published cut off of ≥53.1pg/mL used in the Khorana/Ay score in predicting VTE in chemotherapy patients within 100 days.
Table 92 The performance of soluble P-selectin in predicting VTE development on chemotherapy within 100 days from baseline testing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Khorana/Ay</th>
<th>ROC CHCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin cut-off</td>
<td>≥53.1pg/mL</td>
<td>≥33.5pg/mL</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>21.4</td>
<td>35.7</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>91.9</td>
<td>75.8</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>23.1</td>
<td>14.7</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>91.1</td>
<td>91.3</td>
</tr>
</tbody>
</table>

4.32 RAM performance for VTE diagnosed beyond baseline assessment but within 100 days of follow up n=14.

The two adaptations (CHL and CHCH PC) of each risk assessment model were produced and their performances compared. Table 93 below summarises the relevant values used for each score.

Table 93 Table of the original and adapted cut-offs for the continuous variables of the RAMs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Original score cut-offs</th>
<th>Adapted CHL cut-offs</th>
<th>Adapted CHCH PC cut-offs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td>&lt;100</td>
<td>&lt;115 (F)/130 (M)</td>
<td>&lt;122</td>
</tr>
<tr>
<td>Leukocyte count (x10⁹/L)</td>
<td>&gt;11.0</td>
<td>&gt;8.7</td>
<td></td>
</tr>
<tr>
<td>Platelet count (x10⁹/L)</td>
<td>≥350</td>
<td>≥400</td>
<td>≥328</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>≥35.0</td>
<td>≥30.0 (WHO guideline for obesity)</td>
<td>≥30.63</td>
</tr>
<tr>
<td>Soluble P-selectin (pg/mL)</td>
<td>≥53.1</td>
<td></td>
<td>≥33.5</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>≥144 (different assay from that used in Christchurch)</td>
<td>≥250</td>
<td>≥210</td>
</tr>
</tbody>
</table>

WHO=World Health Organisation

4.32.1 Khorana score and adapted Khorana Score performances

The relevant variables were put together into the original and adapted Khorana scores. As risk scores increased the likelihood of developing a VTE increased as
The Khorana CHL score appeared to identify a higher proportion of patients who developed VTE as high risk compared with the other two scores. Fifteen percent of those that developed VTE, however, were classified as low to intermediate risk and would not have received prophylactic anticoagulation had this guided decision making (see table 94 and figure 96).

Table 94 Original and adapted Khorana scores and VTE development within 100 days on chemotherapy

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Original Khorana Score</th>
<th>Khorana CHCH PC score</th>
<th>Khorana CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=138) (%)</td>
<td>No. with VTE ≤ 100 days (n=14) (%) VTE</td>
<td>No. Patients (n=138) (%)</td>
</tr>
<tr>
<td>0</td>
<td>74 (53.6)</td>
<td>5 (6.8)</td>
<td>38 (27.5)</td>
</tr>
<tr>
<td>1-2</td>
<td>51 (37.0)</td>
<td>7 (13.7)</td>
<td>77 (55.8)</td>
</tr>
<tr>
<td>≥3</td>
<td>13 (9.4)</td>
<td>2 (15.4)</td>
<td>23 (16.7)</td>
</tr>
</tbody>
</table>

Figure 96 Risk level and proportion of patients developing VTE within 100 days from baseline assessment on chemotherapy
4.32.2 The Khorana/Ay and adapted Khorana/Ay Score performances

The relevant variables were put together into the original and adapted Khorana/Ay scores. The original score cut-offs identified a higher proportion of patients who developed VTE as high risk. The D-dimer assay was, however, different to the one used by the CATS group (596). The adapted scores classified a higher number of patients as intermediate to high risk. Only one patient (7.1%) classed as low risk, by the CHL score, went on to develop VTE whereas five of the 14 (35.7%) patients with VTE were classed as low risk using the original score. No one in the low risk group developed VTE (22/138 (15.9%)) when using the percentile cut-off score. On the other hand, both adapted scores identified a larger number of patients as high risk and would have exposed more patients to prophylactic anticoagulation who would not have developed VTE had this score been used to guide thromboprophylaxis use (see table 95 and figure 97).

Table 95 Original and adapted Khorana/Ay scores and VTE development within 100 days on chemotherapy

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Original Khorana/Ay Score</th>
<th>Khorana/Ay CHCH PC Score</th>
<th>Khorana/Ay CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=138) (%)</td>
<td>No. with VTE ≤ 100 days (n=14) (%)</td>
<td>No. Patients (n=138) (%)</td>
</tr>
<tr>
<td>0</td>
<td>61 (44.2)</td>
<td>5 (8.2)</td>
<td>13 (9.4)</td>
</tr>
<tr>
<td>1-2</td>
<td>63 (45.7)</td>
<td>6 (9.5)</td>
<td>70 (50.7)</td>
</tr>
<tr>
<td>≥3</td>
<td>14 (10.1)</td>
<td>3 (21.4)</td>
<td>55 (39.9)</td>
</tr>
</tbody>
</table>
4.32.3 The PROTECHT and adapted PROTECHT Score performances

The relevant variables were put together into the original and adapted PROTECHT scores. As seen with the other scores the proportion of patients who developed VTE increased with increasing score. The absolute numbers of patients classified as high risk were small with each score. VTE developed at all risk levels and despite the CHL adapted PROTECHT score identifying a higher proportion of patients with VTE in the high risk group the number of patients developing VTE were evenly spread throughout the three risk stratifications with four in the low risk group and five each in the intermediate and high risk groups (see table 96 and figure 98).
Table 96 Original and adapted PROTECHT scores and VTE development within 100 days on chemotherapy

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Original PROTECHT Score</th>
<th>PROTECHT CHCH PC Score</th>
<th>PROTECHT CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=138) (%)</td>
<td>No. with VTE ≤ 100 days (n=14) (%)</td>
<td>No. Patients (n=138) (%)</td>
</tr>
<tr>
<td>0</td>
<td>72 (52.2)</td>
<td>5 (6.9)</td>
<td>38 (27.5)</td>
</tr>
<tr>
<td>1-2</td>
<td>50 (36.2)</td>
<td>6 (12.0)</td>
<td>74 (53.6)</td>
</tr>
<tr>
<td>≥3</td>
<td>16 (11.6)</td>
<td>3 (18.8)</td>
<td>26 (18.8)</td>
</tr>
</tbody>
</table>

Figure 98 Risk level and proportion of patients developing VTE within 100 days from baseline assessment on chemotherapy
4.33 Comparison of risk assessment model (RAM) performances

The following data summarises the performance of each RAM in patients presenting as high risk (score ≥3) at baseline assessment (see table 97).

Table 97 The performance of each RAM in predicting VTE on chemotherapy within 100 days in a high risk (score ≥3) patient

<table>
<thead>
<tr>
<th>Score ≥3</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV* (%)</th>
<th>NPV# (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Khorana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>14.3</td>
<td>91.1</td>
<td>15.4</td>
<td>90.4</td>
</tr>
<tr>
<td>CHCH PC</td>
<td>28.6</td>
<td>84.7</td>
<td>17.4</td>
<td>91.3</td>
</tr>
<tr>
<td>CHL</td>
<td>35.7</td>
<td>89.5</td>
<td>27.8</td>
<td>92.5</td>
</tr>
<tr>
<td><strong>Khorana/Ay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>21.4</td>
<td>91.1</td>
<td>21.4</td>
<td>91.1</td>
</tr>
<tr>
<td>CHCH PC</td>
<td>64.3</td>
<td>62.9</td>
<td>16.4</td>
<td>94</td>
</tr>
<tr>
<td>CHL</td>
<td>50</td>
<td>71</td>
<td>16.3</td>
<td>92.6</td>
</tr>
<tr>
<td><strong>PROTECHT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>21.4</td>
<td>89.5</td>
<td>18.8</td>
<td>91</td>
</tr>
<tr>
<td>CHCH PC</td>
<td>28.6</td>
<td>82.3</td>
<td>15.4</td>
<td>91.1</td>
</tr>
<tr>
<td>CHL</td>
<td>35.7</td>
<td>87.9</td>
<td>33.3</td>
<td>92.4</td>
</tr>
</tbody>
</table>

*PPV= positive predictive value, #NPV= negative predictive value

The CHL adaptation of the Khorana and PROTECHT scores appeared to improve the PPV of the model to 27.8% and 33.3% respectively without significant change in the NPV. All models described exhibited similar NPVs. At the high risk score of ≥3 these models would perform well in ruling out low risk patients from having to receive thromboprophylaxis but will not reliably predict those patients who will develop chemotherapy associated VTE within 100 days.

4.34 All VTEs diagnosed on study in chemotherapy patients in follow up beyond baseline (n=31)

The models exhibited slightly improved PPVs but poorer NPVs when used to predict for all 31 VTE events on study, including those developed beyond 100 days.
A higher proportion of patients who developed VTE were identified as high risk using the CHL cut-offs. However, only 13% of the cohort were identified as high risk and only 22.6% of the VTE events occurred in this group. Twelve patients developed VTE in each of the low and intermediate risk groups. These findings were similar in the other scores. The proportion of patients who developed VTE did not appreciably increase with increasing risk score (see table 98 and figure 99).

Table 98 Original and adapted Khorana scores and VTE development in follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Khorana Score</th>
<th>Khorana CHCH PC Score</th>
<th>Khorana CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=138) (%)</td>
<td>No. with VTE beyond baseline (n=31) (%)</td>
<td>No. Patients (n=138) (%)</td>
</tr>
<tr>
<td>0</td>
<td>74 (53.6)</td>
<td>15 (20.3)</td>
<td>38 (27.5)</td>
</tr>
<tr>
<td>1-2</td>
<td>51 (37.0)</td>
<td>13 (25.4)</td>
<td>77 (55.8)</td>
</tr>
<tr>
<td>≥3</td>
<td>13 (9.4)</td>
<td>3 (23.0)</td>
<td>23 (16.7)</td>
</tr>
</tbody>
</table>

Figure 99 Risk level and proportion of patients developing VTE beyond baseline assessment
The Khorana/Ay model was originally used to predict VTE to two years of follow up. The adapted scores were superior to the original cut-offs in identifying low risk patients who did not develop VTE and high risk patients who did develop VTE. Regardless of which score was used approximately half of the cohort were classified as intermediate risk but a higher proportion of patients were identified as high risk in the adapted scoring systems. The adapted scores would have classified 10% of the study cohort as low risk (see table 99 and figure 100).

**Table 99 Original and adapted Khorana/Ay scores and VTE development in follow up beyond baseline**

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Khorana/Ay Score</th>
<th>Khorana/Ay CHCH PC Score</th>
<th>Khorana/Ay CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=138) (%)</td>
<td>No. with VTE beyond baseline (n=31) (%)</td>
<td>No. with VTE beyond baseline (n=31) (%)</td>
</tr>
<tr>
<td>0</td>
<td>61 (44.2)</td>
<td>14 (23.0)</td>
<td>13 (9.4)</td>
</tr>
<tr>
<td>1-2</td>
<td>63 (45.7)</td>
<td>13 (20.6)</td>
<td>70 (50.7)</td>
</tr>
<tr>
<td>≥3</td>
<td>14 (10.1)</td>
<td>4 (28.6)</td>
<td>55 (39.9)</td>
</tr>
</tbody>
</table>

Venous thromboembolism in cancer patients undertaking chemotherapy
As was seen with the Khorana score analysis, the CHL adaptation of the PROTECHT score did identify a higher proportion of patients with VTE as high risk (see table 100 and figure 101).

**Table 100 Original and adapted PROTECHT scores and VTE development in follow up beyond baseline**

<table>
<thead>
<tr>
<th>Overall score</th>
<th>PROTECHT Score</th>
<th>PROTECHT CHCH PC Score</th>
<th>PROTECHT CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=138) (%)</td>
<td>No. with VTE beyond baseline (n=31) (%)</td>
<td>No. Patients (n=138) (%)</td>
</tr>
<tr>
<td>0</td>
<td>72 (52.2)</td>
<td>14 (19.4)</td>
<td>38 (27.5)</td>
</tr>
<tr>
<td>1-2</td>
<td>50 (36.2)</td>
<td>13 (26.0)</td>
<td>74 (53.6)</td>
</tr>
<tr>
<td>≥3</td>
<td>16 (11.6)</td>
<td>4 (25.0)</td>
<td>26 (18.8)</td>
</tr>
</tbody>
</table>
For prediction of VTE, regardless of timeframe, all risk scores showed a slight increase in PPV and reduction in NPV compared with the 100 day prediction. Using these models beyond 100 days is challenging due to the number of confounders that may impact over the longer period of time such as other treatments and comorbidities. They are, however, still reasonable scores for ruling out the need for thromboprophylaxis in low risk patients (see table 101).

Table 101 The performance of each RAM in predicting VTE in a high risk (score ≥3) patient beyond baseline assessment at any time point

<table>
<thead>
<tr>
<th></th>
<th>Score ≥3</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Khorana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>9.7</td>
<td>90.7</td>
<td>23.1</td>
<td>77.6</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>19.4</td>
<td>84.1</td>
<td>26.1</td>
<td>78.3</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>22.6</td>
<td>89.7</td>
<td>38.9</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><strong>Khorana/Ay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>12.9</td>
<td>90.7</td>
<td>28.6</td>
<td>78.2</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>51.6</td>
<td>63.6</td>
<td>29.1</td>
<td>81.9</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>35.5</td>
<td>70.1</td>
<td>25.6</td>
<td>78.9</td>
<td></td>
</tr>
<tr>
<td><strong>PROTECHT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>12.9</td>
<td>88.8</td>
<td>25</td>
<td>77.9</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>22.6</td>
<td>82.2</td>
<td>26.9</td>
<td>78.6</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>22.6</td>
<td>87.9</td>
<td>35</td>
<td>79.7</td>
<td></td>
</tr>
</tbody>
</table>
4.34.1 Comparisons between suspected and unsuspected VTE in follow up beyond baseline

When the VTE events were stratified for clinical suspicion of their presence, all score performances were superior in predicting suspected VTE. This was important as the scores were developed to predict clinically suspected VTE but not clinically unsuspected VTE (see tables 102 to 110 and figures 102 to 107).

Table 102 The original Khorana score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Original Khorana Score</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58 (54.7)</td>
<td>10 (62.5)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>1-2</td>
<td>38 (35.8)</td>
<td>6 (37.5)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>≥3</td>
<td>10 (9.4)</td>
<td>0 (0.0)</td>
<td>3 (20.0)</td>
</tr>
</tbody>
</table>

  - Sensitivity (%): 0.0 20.0
  - Specificity (%): 90.6 90.6
  - PPV (%): 0.0 23.1
  - NPV (%): 85.7 88.9

Table 103 The Khorana CHCH PC score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Khorana Score CHCH PC ranges</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30 (28.3)</td>
<td>6 (37.5)</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>1-2</td>
<td>59 (55.7)</td>
<td>9 (56.3)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>≥3</td>
<td>17 (16.0)</td>
<td>1 (6.3)</td>
<td>5 (33.3)</td>
</tr>
</tbody>
</table>

  - Sensitivity (%): 6.3 33.3
  - Specificity (%): 84.0 84.0
  - PPV (%): 5.6 22.7
  - NPV (%): 85.6 89.9
Table 104 The Khorana CHL score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Khorana Score using CHL ranges</th>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>42 (39.6)</td>
<td>9 (56.3)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>53 (50.0)</td>
<td>5 (31.3)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>11 (10.4)</td>
<td>2 (12.5)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sensitivity (%)</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Specificity (%)</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PPV (%)</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NPV (%)</td>
<td>87.2</td>
</tr>
<tr>
<td>Figure 102 Risk level and proportion of patients developing unsuspected VTE beyond baseline assessment</td>
<td>14.7 13.6</td>
<td>16.7 13.2</td>
<td>17.6 15.4</td>
<td>0 5.6 8.6</td>
</tr>
</tbody>
</table>
Figure 103 Risk level and proportion of patients developing suspected VTE beyond baseline assessment

Table 105 The original Khorana/Ay score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46 (43.4)</td>
<td>9 (56.3)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>1-2</td>
<td>50 (47.2)</td>
<td>7 (43.8)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>≥3</td>
<td>10 (9.4)</td>
<td>0 (0.0)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>0.0</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>90.6</td>
<td>90.6</td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td>0.0</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td>85.7</td>
<td>89.7</td>
<td></td>
</tr>
</tbody>
</table>
Table 106 The Khorana/Ay CHCH PC score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13 (12.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1-2</td>
<td>54 (50.9)</td>
<td>10 (62.5)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>≥3</td>
<td>39 (36.8)</td>
<td>6 (37.5)</td>
<td>10 (66.7)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 107 The Khorana/Ay CHL score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20 (18.9)</td>
<td>1 (6.25)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>1-2</td>
<td>54 (50.9)</td>
<td>12 (75.0)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>≥3</td>
<td>32 (30.2)</td>
<td>3 (18.8)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 104 Risk level and proportion of patients developing unsuspected VTE beyond baseline assessment

![Bar chart showing the proportion of patients with unsuspected VTE at low, intermediate, and high risk levels for different cut-off derivation methods.]

- **Low**, **Intermediate**, and **High** risk levels are represented.

**Patients with unsuspected VTE (%)**

- **Khorana/Ay original**:
  - Low: 16.4
  - Intermediate: 13.6
  - High: 0

- **Khorana/Ay CHCH PC**:
  - Low: 0
  - Intermediate: 5.6
  - High: 13.2

- **Khorana/Ay CHL**:
  - Low: 4.8
  - Intermediate: 8.6
  - High: 15.4

**Cut off derivation method**

Figure 105 Risk level and proportion of patients developing suspected VTE beyond baseline assessment

![Bar chart showing the proportion of patients with suspected VTE at low, intermediate, and high risk levels for different cut-off derivation methods.]

- **Low**, **Intermediate**, and **High** risk levels are represented.

**Patients with suspected VTE (%)**

- **Khorana/Ay original**:
  - Low: 9.8
  - Intermediate: 13
  - High: 28.6

- **Khorana/Ay CHCH PC**:
  - Low: 0
  - Intermediate: 8.5
  - High: 20.4

- **Khorana/Ay CHL**:
  - Low: 4.8
  - Intermediate: 10
  - High: 20

**Cut off derivation method**
Table 108 The original PROTECHT score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Original PROTECHT Score</th>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>57 (53.8)</td>
<td>9 (56.3)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>37 (34.9)</td>
<td>7 (43.8)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>12 (11.3)</td>
<td>0 (0.0)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td></td>
<td>0.0</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td></td>
<td>88.7</td>
<td>88.7</td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td></td>
<td>0.0</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td></td>
<td>85.5</td>
<td>89.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 109 The PROTECHT CHCH PC score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>PROTECHT Score CHCH PC ranges</th>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30 (28.3)</td>
<td>6 (37.5)</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>57 (53.8)</td>
<td>8 (50.0)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>19 (17.9)</td>
<td>2 (12.5)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td></td>
<td>12.5</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td></td>
<td>82.1</td>
<td>82.1</td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td></td>
<td>9.5</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td></td>
<td>86.1</td>
<td>86.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 110 The PROTECHT CHL score performance in predicting suspected and unsuspected VTE at any point during follow up beyond baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Patients with score (n=106) (%)</th>
<th>Unsuspected VTE beyond baseline (n=16) (%)</th>
<th>Suspected VTE beyond baseline (n=15) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>41 (38.7)</td>
<td>7 (43.8)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>1-2</td>
<td>52 (49.1)</td>
<td>7 (43.8)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>≥3</td>
<td>13 (12.3)</td>
<td>2 (12.5)</td>
<td>5 (33.3)</td>
</tr>
</tbody>
</table>

- Sensitivity (%) 12.5 33.3
- Specificity (%) 87.8 87.7
- PPV (%) 13.3 27.8
- NPV (%) 86.9 90.3

Figure 106 Risk level and proportion of patients developing unsuspected VTE beyond baseline assessment
The PPVs and NPVs were lower in predicting unsuspected VTE compared with suspected VTE regardless of the RAM used. As was seen earlier these scores are useful to rule out low risk patients but will not reliably identify patients at high risk. Overall the adapted CHCH PC Khorana/Ay model appeared to be the most discriminatory in identifying patients at low risk for suspected or unsuspected VTE on chemotherapy, since no one developed VTE in the low risk group. The NPVs for this model range from 81.9-94.0% in this study and were consistently higher than the other models. This model would rule out 9.4% of the population from receiving thromboprophylaxis if all intermediate and high risk patients were to be offered VTE prevention (see tables 111 and 112).
Table 111 Comparison of predictive score performances in predicting post baseline unsuspected VTE

<table>
<thead>
<tr>
<th></th>
<th>Score ≥3</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Khorana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0</td>
<td>90.6</td>
<td>0</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>6.3</td>
<td>84</td>
<td>5.6</td>
<td>85.6</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>12.5</td>
<td>89.6</td>
<td>15.4</td>
<td>87.2</td>
<td></td>
</tr>
<tr>
<td><strong>Khorana/Ay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0</td>
<td>90.6</td>
<td>0</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>37.5</td>
<td>63.2</td>
<td>13.3</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>18.8</td>
<td>69.8</td>
<td>8.6</td>
<td>85.1</td>
<td></td>
</tr>
<tr>
<td><strong>PROTECHT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0</td>
<td>88.7</td>
<td>0</td>
<td>85.5</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>12.5</td>
<td>82.1</td>
<td>9.5</td>
<td>86.1</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>12.5</td>
<td>87.8</td>
<td>13.3</td>
<td>86.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 112 Comparison of predictive score performances in predicting post baseline suspected VTE

<table>
<thead>
<tr>
<th></th>
<th>Score ≥3</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Khorana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>20</td>
<td>90.6</td>
<td>23.1</td>
<td>88.9</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>33.3</td>
<td>84</td>
<td>22.7</td>
<td>89.9</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>33.3</td>
<td>89.6</td>
<td>31.3</td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td><strong>Khorana/Ay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>26.7</td>
<td>90.6</td>
<td>28.6</td>
<td>89.7</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>66.7</td>
<td>63.2</td>
<td>20.4</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>53.3</td>
<td>69.8</td>
<td>20</td>
<td>91.4</td>
<td></td>
</tr>
<tr>
<td><strong>PROTECHT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>26.7</td>
<td>88.7</td>
<td>25</td>
<td>89.5</td>
<td></td>
</tr>
<tr>
<td>CHCH PC</td>
<td>33.3</td>
<td>87.7</td>
<td>27.8</td>
<td>90.3</td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td>33.3</td>
<td>82.1</td>
<td>20.8</td>
<td>86.1</td>
<td></td>
</tr>
</tbody>
</table>
4.35 Baseline VTE and RAM scores

The models were also assessed in all 203 cancer patients assessed at baseline for performance in identifying those that had already developed VTE at baseline. All 17 patients with VTE at baseline assessment were clinically unsuspected. The precision of the scores were similar to their predictive performance to 100 days. Note, however, that the scores were derived for VTE prediction in patients undertaking chemotherapy (see tables 113 to 116 and figures 108 to 110).

4.35.1.1 Khorana score

Table 113 Original and adapted Khorana scores and VTE development at baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Khorana Score</th>
<th>Khorana CHCH PC score</th>
<th>Khorana CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients</td>
<td>Baseline VTE (%)</td>
<td>No. Patients</td>
</tr>
<tr>
<td></td>
<td>(n=203) (%)</td>
<td>(n=17) (%)</td>
<td>(n=203) (%)</td>
</tr>
<tr>
<td>0</td>
<td>99 (48.8)</td>
<td>7 (7.1)</td>
<td>53 (26.1)</td>
</tr>
<tr>
<td>1-2</td>
<td>86 (42.6)</td>
<td>8 (9.3)</td>
<td>113 (55.7)</td>
</tr>
<tr>
<td>≥3</td>
<td>18 (8.9)</td>
<td>2 (11.1)</td>
<td>37 (18.2)</td>
</tr>
</tbody>
</table>

Figure 108 Risk level and proportion of all cancer patients developing VTE at baseline assessment
4.35.1.2 Khorana/Ay score

Table 114 Original and adapted Khorana/Ay scores and VTE development at baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Khorana/Ay Score</th>
<th>Baseline CHCH PC Score</th>
<th>Khorana/Ay CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=203) (%)</td>
<td>Baseline VTE (n=17) (%)</td>
<td>VTE (%)</td>
</tr>
<tr>
<td>0</td>
<td>81 (39.9)</td>
<td>4 (4.9)</td>
<td>20 (9.9)</td>
</tr>
<tr>
<td>1-2</td>
<td>101 (49.8)</td>
<td>10 (9.9)</td>
<td>97 (47.8)</td>
</tr>
<tr>
<td>≥3</td>
<td>21 (10.3)</td>
<td>3 (14.3)</td>
<td>86 (42.4)</td>
</tr>
</tbody>
</table>

Figure 109 Risk level and proportion of all cancer patients developing VTE at baseline assessment
4.35.1.3 PROTECHT score

Table 115 Original and adapted PROTECHT scores and VTE development at baseline

<table>
<thead>
<tr>
<th>Overall score</th>
<th>PROTECHT Score</th>
<th>PROTECHT CHCH PC Score</th>
<th>PROTECHT CHL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Patients (n=203) %</td>
<td>Baseline VTE (n=17) % VTE</td>
<td>No. Patients (n=203) %</td>
</tr>
<tr>
<td>0</td>
<td>97 (47.8)</td>
<td>7 (7.2)</td>
<td>53 (26.1)</td>
</tr>
<tr>
<td>1-2</td>
<td>84 (41.4)</td>
<td>7 (8.3)</td>
<td>110 (54.2)</td>
</tr>
<tr>
<td>≥3</td>
<td>22 (10.8)</td>
<td>3 (13.6)</td>
<td>40 (19.7)</td>
</tr>
</tbody>
</table>

Figure 110 Risk level and proportion of all cancer patients developing VTE at baseline assessment

![Risk level and proportion of all cancer patients developing VTE at baseline assessment](image)
Table 116 Performances of the RAMs in identifying cancer patients diagnosed with VTE at baseline assessment

<table>
<thead>
<tr>
<th>RAM</th>
<th>Score ≥3</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khorana</td>
<td>Original</td>
<td>11.8</td>
<td>91.4</td>
<td>11.1</td>
<td>91.9</td>
</tr>
<tr>
<td></td>
<td>CHCH PC</td>
<td>41.2</td>
<td>83.9</td>
<td>18.9</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>CHL</td>
<td>23.5</td>
<td>88.2</td>
<td>15.4</td>
<td>92.7</td>
</tr>
<tr>
<td>Khorana/Ay</td>
<td>Original</td>
<td>17.6</td>
<td>90.3</td>
<td>14.3</td>
<td>92.3</td>
</tr>
<tr>
<td></td>
<td>CHCH PC</td>
<td>70.6</td>
<td>60.2</td>
<td>14</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>CHL</td>
<td>58.8</td>
<td>67.7</td>
<td>14.3</td>
<td>94.7</td>
</tr>
<tr>
<td>PROTECHT</td>
<td>Original</td>
<td>17.6</td>
<td>89.8</td>
<td>13.6</td>
<td>92.3</td>
</tr>
<tr>
<td></td>
<td>CHCH PC</td>
<td>41.2</td>
<td>82.3</td>
<td>17.5</td>
<td>93.9</td>
</tr>
<tr>
<td></td>
<td>CHL</td>
<td>29.4</td>
<td>87.1</td>
<td>17.2</td>
<td>93.1</td>
</tr>
</tbody>
</table>

PPVs were low but NPVs were high indicating that patients with a score less than three using these models would have a lower likelihood of having a VTE at the time of assessment. Once again the adapted CHCH PC and CHL Khorana/Ay models were reliable in identifying patients without VTE as low risk with only one of the 17 patients with VTE being classified as low risk. Only 10% and 15% of the patient cohort in the percentile model and CHL model respectively, were classified as low risk, however.

4.36 Summary

The existing risk assessment models and adapted versions developed during this study are observed to have similar validity in predicting VTE risk and indicating those with a higher risk of VTE at the time of the patient assessment. Increasing scores usually correlated with increasing proportions of patients developing suspected VTE, but not clinically unsuspected VTE. Had these models been used to guide thromboprophylaxis use in those patients deemed high risk (score ≥3), up to 10% of the lower risk patients would still develop VTE within 100 days and approximately 75-80% of the higher risk patients would not develop VTE but may experience complications from anticoagulation. Adapted Khorana/Ay models, incorporating D-dimer and soluble P-selectin assays with the Khorana variables, appear to be the most reliable tools for identifying patients at low risk of VTE but would still classify 85-90% as intermediate to high risk. These patients would all need consideration for primary
thromboprophylaxis and perhaps screening for VTE up front. Importantly, D-dimer has been consistently associated with VTE development in this study. However, the baseline soluble P-selectin level was not predictive of VTE development at 100 days (non significant ROC curve) and was not independently associated with VTE diagnosis at baseline. It was also not independently associated with VTE at the time of diagnosis on chemotherapy within 100 days after multivariate analysis. This is because it correlated strongly with D-dimer and other markers. Further research into predictive biomarkers will be important to aid identification of the patients at high risk for VTE on chemotherapy and minimise the use of screening for VTE and thromboprophylaxis to those that require it.
4.37 Aims
Investigate the utility of a calibrated, automated fluorogenic thrombin generation assay in predicting/diagnosing VTE development in cancer patients, at baseline and undertaking chemotherapy.

Investigate changes in thrombomodulin and antithrombin levels during chemotherapy and their association with VTE in cancer patients undertaking chemotherapy.

Investigate ratios of thrombin generation variables, thrombomodulin and or antithrombin in cancer patients undertaking chemotherapy and their association with VTE.

4.38 Introduction
At each patient assessment blood was collected as previously described and stored for batched analysis. Antithrombin (AT) concentrations were measured using an assay routinely used at CHL. Thrombomodulin (TM) was measured using a previously described commercially available assay in the Mackenzie Cancer Research Laboratory at the University of Otago Christchurch. The TGA was performed in the coagulation laboratory at CHL and results for lag time, velocity index (VI), time to peak thrombin (ttPeak), peak thrombin, endogenous thrombin potential (ETP) and start of the thrombin tail (tail or start tail) were recorded (see section 1.60, figure 24).

4.39 Thrombin curves in patients with cancer compared with volunteers without cancer at baseline assessment
At baseline, results for cancer patients with and without VTE and volunteers without cancer were analysed. One hundred and ninety nine cancer patients and 50 volunteers without cancer had available results. The mean lag time was longer in the cancer patient cohort but the time to peak thrombin (ttPeak) and start tail (tail) were similar. Peak thrombin (peak), velocity index (VI) and endogenous thrombin potential (ETP) were all greater in cancer patients (see Table 117).
Table 117 Mean TGA variables results in cancer patients compared with volunteers without cancer

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Cancer patients (n=199)</th>
<th>Volunteers without cancer (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.32** (0.05)</td>
<td>3.22</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>149**** (3.0)</td>
<td>148</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.74 (0.07)</td>
<td>5.63</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>346**** (5.0)</td>
<td>341</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM/min)</td>
<td>1671* (24)</td>
<td>1636</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>21.5 (0.2)</td>
<td>21.1</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.0001

Mean thrombin curves for the two cohorts are shown below in figure 111.
Figure 111 A-C Mean thrombin generation curves for patients with cancer and patients without cancer at baseline assessment (see footnote)²

A  The complete thrombin curve.

B  The lag time.

² The thrombin curves depicted in the TGA results section are plotted using mean thrombin values recorded at specific time points during the assay and provide a visual comparison of thrombin generation between examined cohorts. They do not accurately depict the means of each specific variable (e.g. peak) listed in the tables.
C The propagation phase and peak.

![Graph showing mean thrombin concentration over time for cancer and non-cancer patients]

Antithrombin (AT) and thrombomodulin (TM) concentrations were similar between the two cohorts but peak thrombin and velocity index in ratio with AT were higher and the start tail in ratio with TM was lower in cancer patients compared with volunteers without cancer (see figures 112 and 113).

**Figure 112 Comparisons of TGA variables in ratio with thrombomodulin (TM) in participants with and without cancer**

![Bar chart showing mean ratio for tPeak:TM and Tail:TM]

ns-not significant  *P<0.05
**Figure 113** Comparisons of TGA variables in ratio with antithrombin (AT) in participants with and without cancer

The cancer cohort diagnosed with VTE at baseline assessment (n=16) demonstrated a significantly longer mean lag time and higher mean ETP, peak thrombin and velocity index than volunteers without cancer (see table 118 and figure 114). In cancer patients without VTE, these variables were also significantly greater than the non cancer cohort. The ttPeak and tail were similar in the cohorts.

**4.39.1 Cancer patients with (n=16) VTE and without VTE (n=183) v volunteers without cancer (n=50) at baseline assessment**

The cancer cohort diagnosed with VTE at baseline assessment (n=16) demonstrated a significantly longer mean lag time and higher mean ETP, peak thrombin and velocity index than volunteers without cancer (see table 118 and figure 114). In cancer patients without VTE, these variables were also significantly greater than the non cancer cohort. The ttPeak and tail were similar in the cohorts.
<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Cancer patients with VTE (n=16)</th>
<th>Volunteers without cancer (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.56 (0.3)**</td>
<td>3.31</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>153 (10.0)**</td>
<td>147</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.97 (0.37)</td>
<td>5.72</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>357 (14.0)**</td>
<td>360</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1789 (73)**</td>
<td>1841</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>22.3 (0.8)</td>
<td>21.9</td>
</tr>
</tbody>
</table>

**p<0.01
Figure 114 Mean TGA curves comparing cancer patients with VTE at baseline assessment (n=16) and volunteers without cancer (n=50)

A. The complete thrombin curves.

- Cancer baseline VTE (n=16)
- Non cancer (n=50)

B. The lag time.

- Cancer baseline VTE (n=16)
- Non cancer (n=50)
C The propagation phase and peak.

AT was significantly lower in cancer patients with VTE at baseline compared with volunteers without cancer although the concentrations remained within the CHL normal range. TM concentrations were significantly higher in cancer patients with VTE than volunteers without cancer (see figures 115 and 116).

Figure 115 Comparison of mean (+/-SEM) antithrombin (AT) concentrations between cancer patients with VTE and volunteers without cancer.

*p<0.05
Figure 116 Comparison of mean (+/-SEM) thrombomodulin (TM) concentrations between cancer patients with VTE and volunteers without cancer

When individual TGA variables were separately ratioed with AT and TM concentrations ETP:AT, peak:AT, tPeak:AT, VI:AT and tail:AT were all significantly higher in cancer patients with VTE than in volunteers without cancer. Variables in ratio with TM were similar between the two cohorts (see figures 117 to 119).

Figure 117 Comparison of the mean (+/-SEM) ETP:AT ratio with AT concentrations between cancer patients with VTE and volunteers without cancer
Figure 118 Comparison of the mean (+/-SEM) VI:AT and peak:AT ratio with AT concentrations between cancer patients with VTE and volunteers without cancer

Figure 119 Comparison of the mean (+/-SEM) ttPeak:AT and tail:AT ratios with AT concentrations between cancer patients with VTE and volunteers without cancer

The cancer cohort without VTE at baseline assessment (n=183) also demonstrated a significantly longer mean lag time and higher mean ETP, peak thrombin and velocity index than volunteers without cancer (see table 119 and figure 120). The ttPeak and tail were again similar in the cohorts.
Table 119 Mean TGA variables in cancer patients without VTE at baseline assessment (n=183) compared with volunteers without cancer

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Cancer patients without VTE baseline (n=183)</th>
<th>Volunteers without cancer (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.30 (0.05)**</td>
<td>3.22</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>149 (3.0)****</td>
<td>148</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.72 (0.06)</td>
<td>5.63</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>345 (5.0)****</td>
<td>341</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1661 (25)*</td>
<td>1623</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>21.4 (0.17)</td>
<td>21.1</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ****p<0.0001
Figure 120 Mean thrombin curves in cancer patients without VTE at baseline assessment compared with volunteers without cancer

A  The complete thrombin generation curve

<table>
<thead>
<tr>
<th></th>
<th>Cancer no VTE (n=183)</th>
<th>Non cancer (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean thrombin concentration (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (minutes)</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

B  The lag time

<table>
<thead>
<tr>
<th></th>
<th>Cancer no VTE (n=183)</th>
<th>Non cancer (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean thrombin concentration (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (minutes)</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
C  The propagation phase and peak.

Mean AT and TM concentrations were similar between the cohorts of cancer patients without VTE and volunteers without cancer. The peak:AT and VI:AT ratios were, however, significantly higher in cancer patients without VTE at baseline assessment compared with volunteers without cancer. The tail:TM ratio was significantly lower. All other TGA variable ratios were similar in both cohorts (see figures 121 and 122).
Figure 121 Comparison of mean (+/-SEM) tail:TM ratios between cancer patients without VTE at baseline and volunteers without cancer

* *p<0.05

Figure 122 Comparisons of the mean (+/-SEM) velocity index and peak thrombin in ratio with AT between cancer patients without VTE and volunteers without cancer at baseline

** *p<0.01

4.39.2 Cancer patients with VTE (n=16) compared with cancer patients without VTE (n=183) at baseline assessment

There were no significant differences in individual TGA variables between cancer patients diagnosed with VTE compared with cancer patients without VTE at baseline assessment (see table 120 and figure 123).
Table 120 TGA variables in cancer patients with \((n=16)\) and without \((n=183)\) VTE at baseline assessment

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Cancer patients without VTE baseline ((n=183))</th>
<th>Cancer patients with VTE baseline ((n=16))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.30 (0.05)</td>
<td>3.22</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>149 (3.0)</td>
<td>148</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.72 (0.06)</td>
<td>5.63</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>345 (5.0)</td>
<td>341</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1661 (25)</td>
<td>1623</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>21.4 (0.17)</td>
<td>21.1</td>
</tr>
</tbody>
</table>
Figure 123 Mean thrombin generation curves comparing cancer patients with and without VTE at baseline assessment

A  The complete thrombin generation curve

B  The lag time
C The propagation phase and peak

AT concentrations were significantly lower in cancer patients with VTE than cancer patients without VTE (p=0.011) although mean AT concentrations were maintained within the CHL normal range. Mean TM concentrations were similar in the two cohorts (see figure 124).

**Figure 124 Comparison of mean (+/-SEM) antithrombin (AT) levels between cancer patients with and without VTE at baseline**

\[\text{Mean thrombin concentration (nM)}\]

\[\text{Time (minutes)}\]

Cancer no baseline VTE (n=183)  Cancer baseline VTE (n=16)

\[\text{Cohort} \]

\[\text{Mean (%)} \]

Cancer no VTE n=184  Cancer VTE baseline n=16

108.6  97.2

*p<0.05

Venous thromboembolism in cancer patients undertaking chemotherapy
Consequently the ETP:AT (p=0.001), peak:AT (p=0.001), ttPeak:AT (p=0.001), VI:AT (P=0.018) and tail:AT (p=0.001) were all significantly higher in cancer patients with VTE compared with cancer patients without VTE as was also seen in non cancer cohort comparisons (see figures 125 to 127).

**Figure 125** Comparison of the ETP:AT ratio between cancer patients with and without VTE at baseline

**Figure 126** Comparisons of the VI:AT and peak:AT ratios between cancer patients with and without VTE at baseline
4.40 VTE events at baseline assessment and within 100 days

Forty eight cancer patients were diagnosed with VTE on study. Seventeen patients were diagnosed with VTE at baseline assessment prior to commencing treatment. Of the 138 patients without VTE at baseline who commenced chemotherapy, 31 developed VTE in follow up. Fourteen were diagnosed between baseline assessment and 100 days of follow up and the remaining 17 were diagnosed beyond 100 days of follow up.

Peak thrombin and ETP were lower at VTE diagnosis in chemotherapy patients diagnosed within 100 days of baseline assessment compared with patients diagnosed with VTE at baseline assessment (see table 121 and figure 128).
Table 121 TGA variables in cancer patients with VTE at baseline assessment compared with cancer patients with VTE on chemotherapy within 100 days

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>VTE baseline (n=16)</th>
<th>VTE within 100 days (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.56 (0.3)</td>
<td>3.31</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>153 (10.0)</td>
<td>147</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.97 (0.37)</td>
<td>5.72</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>357* (14.0)</td>
<td>360</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1789** (73)</td>
<td>1841</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>22.3 (0.8)</td>
<td>21.9</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Figure 128 Mean thrombin curves in cancer patients with VTE at baseline assessment and those diagnosed on chemotherapy within 100 days

A  The complete thrombin curve

B  The lag time
C  The propagation phase

![Graph showing the propagation phase]

D  The peak

![Graph showing the peak]

The mean AT concentration was lower in cancer patients diagnosed with VTE at baseline \((p=0.024)\) compared with chemotherapy patients diagnosed within 100 days of follow up (see figure 129). TM concentrations were again similar in
both cohorts (data not shown). ETP (p=0.016), VI (p=0.018) and peak (p=0.007) ratios with AT were all significantly lower in chemotherapy patients diagnosed with VTE within 100 days (see figures 130 and 131).

**Figure 129** Comparison of mean (+/-SEM) antithrombin concentrations between cancer patients diagnosed with VTE at baseline and those diagnosed within 100 days of follow up on chemotherapy

![Figure 129](image1)

**Figure 130** Comparison of the mean (+/-SEM) ETP:AT ratio between cancer patients diagnosed with VTE at baseline and those diagnosed within 100 days of follow up on chemotherapy

![Figure 130](image2)
4.41 VTE diagnosed after baseline assessment

At follow up within 100 days, 96 chemotherapy patients without VTE and 11 with VTE had available TGA data for comparison. There were no significant differences in individual TGA variables, AT, TM or variable ratios between the two groups (see table 122 and figure 132).
Table 122 TGA variables in cancer patients on chemotherapy with and without VTE in follow up within 100 days

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Cancer patients without VTE at second visit within 100 days (n=96)</th>
<th>Cancer patients with VTE at diagnosis within 100 days (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.35 (0.05)</td>
<td>3.23</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>138 (3.0)</td>
<td>133</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.80 (0.06)</td>
<td>5.57</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>325 (5.0)</td>
<td>322</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1559 (29)</td>
<td>1546</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>21.1 (0.17)</td>
<td>20.7</td>
</tr>
</tbody>
</table>
Figure 132 Mean TGA curves of cancer patients on chemotherapy with and without VTE within 100 days of follow up

A  The complete thrombin curve

B  The lag time
C  The propagation phase

![Graph showing mean thrombin concentration over time for patients with and without VTE.]

D  The peak

![Graph showing mean thrombin concentration over time for patients with and without VTE.]

Venous thromboembolism in cancer patients undertaking chemotherapy
4.41.1 Comparison of thrombin curves at baseline assessment and at VTE diagnosis in cancer patients who developed VTE within 100 days from baseline assessment

In the 11 chemotherapy patients diagnosed with VTE within 100 days of follow up the velocity index, peak thrombin and ETP were significantly lower at VTE diagnosis than at baseline assessment. Other variables were similar (see table 123 and figure 133).

Table 123 Changes in mean TGA variables between baseline and VTE diagnosis in chemotherapy patients who developed VTE within 100 days beyond baseline

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>TGA changes in chemotherapy patients who developed VTE within 100 days of baseline assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n=11)</td>
</tr>
<tr>
<td></td>
<td>Mean (SEM)</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.32 (0.16)</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>154* (8.0)</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.69 (0.19)</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>352* (15.0)</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1721* (86)</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>21.3 (0.5)</td>
</tr>
</tbody>
</table>

*p<0.05
Figure 133 Mean TGA curves at baseline assessment and at VTE diagnosis in chemotherapy patients who developed VTE within 100 days beyond baseline

**A**  The complete thrombin curve

**B**  The lag time
C The propagation phase and peak

AT concentrations (p=0.029) were higher at the time of VTE diagnosis compared with baseline levels in chemotherapy patients diagnosed with VTE within 100 days of follow up (see figure 134). TM concentrations were similar in the two cohorts (data not shown).
The mean peak thrombin and ETP levels in ratio with both AT (p=0.01, p=0.007 respectively) and TM (p=0.04, p=0.024 respectively) were lower at VTE diagnosis on chemotherapy than they had been at baseline assessment. This was also the case for the VI:AT (p=0.016) and tail:AT (p=0.03) ratios (see figures 135 to 138).
Figure 135 Comparison of the mean (+/-SEM) peak:TM and ETP:TM ratios between baseline visit and visit 2 in chemotherapy patients diagnosed with VTE at visit 2 within 100 days

![Bar chart showing comparison of mean peak:TM and ETP:TM ratios between baseline and visit 2 with significance levels indicated by asterisks.]

* p<0.05

Figure 136 Comparison of the mean (+/-SEM) VI:AT and peak:AT ratios between baseline visit and visit 2 in chemotherapy patients diagnosed with VTE at visit 2 within 100 days

![Bar chart showing comparison of mean VI:AT and peak:AT ratios between baseline and visit 2 with significance levels indicated by asterisks.]

* p<0.05
Figure 137 Comparison of the mean (+/-SEM) ETP:AT ratio between baseline visit and visit 2 in chemotherapy patients diagnosed with VTE at visit 2 within 100 days

![Bar chart showing comparison of mean ETP:AT ratio between baseline and visit 2 for VTE patients.](image)

**p<0.01

Figure 138 Comparison of mean (+/-SEM) tail:AT ratio between baseline visit and visit 2 in chemotherapy patients diagnosed with VTE at visit 2 within 100 days

![Bar chart showing comparison of mean tail:AT ratio between baseline and visit 2 for VTE patients.](image)

*p<0.05
4.41.2 Thrombin generation curves in cancer patients with clinically suspected VTE compared with cancer patients with clinically unsuspected VTE

Of the 31 chemotherapy patients diagnosed with VTE in follow up, 15 were clinically suspected and 16 were clinically unsuspected. Eight patients with suspected VTE and 16 patients with unsuspected VTE had available data for analysis.

Patients with suspected VTE were found to have a lower mean ETP, peak thrombin and velocity index and longer ttPeak when compared to patients diagnosed with VTE at baseline (see table 124 and figure 139).

**Table 124 TGA variables in cancer patients with VTE at baseline compared with patients diagnosed with suspected VTE on chemotherapy**

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Baseline VTE n=16</th>
<th>Suspected VTE on chemotherapy n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.56 (0.3)</td>
<td>3.31</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>153* (10.0)</td>
<td>147</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.97* (0.37)</td>
<td>5.72</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>357** (14.0)</td>
<td>360</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1789** (73)</td>
<td>1841</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>22.3 (0.8)</td>
<td>21.9</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Chemotherapy patients diagnosed with unsuspected VTE, in follow up, were found to have a significantly lower mean ETP but all other TGA variables were similar in comparison to patients diagnosed with VTE at baseline assessment (see table 125 and figure 139).

**Table 125 TGA variables in cancer patients with VTE at baseline compared with patients diagnosed with unsuspected VTE on chemotherapy**

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Baseline VTE n=16</th>
<th>Unsuspected VTE on chemotherapy n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.56 (0.3)</td>
<td>3.31</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>153 (10.0)</td>
<td>147</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.97 (0.37)</td>
<td>5.72</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>357 (14.0)</td>
<td>360</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1789** (73)</td>
<td>1841</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>22.3 (0.8)</td>
<td>21.9</td>
</tr>
</tbody>
</table>

**p<0.01**
Figure 139 Mean thrombin generation curves for cancer patients with baseline VTE and suspected and unsuspected VTE in follow up on chemotherapy

A  The complete thrombin curve

B  The lag time
C The propagation phase and peak

4.41.3 Suspected compared with unsuspected VTE

The mean lag time and ttPeak were significantly longer in patients diagnosed with suspected VTE than patients with unsuspected VTE on chemotherapy. All other TGA variables were similar between the cohorts (see table 126 and figure 140). TGA variable ratios with AT and TM were also similar in the two cohorts.
Table 126 TGA variables in cancer patients diagnosed with suspected VTE compared with patients diagnosed with unsuspected VTE on chemotherapy

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Suspected VTE on chemotherapy n=8</th>
<th>Unsuspected VTE on chemotherapy n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>4.31* (0.59)</td>
<td>4.06</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>106 (21)</td>
<td>109</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>7.35** (0.96)</td>
<td>6.49</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>270 (42)</td>
<td>282</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1345 (152)</td>
<td>1371</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>23.2 (2.0)</td>
<td>21.6</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Figure 140 Mean (+/-SEM) thrombin generation curves of cancer patients with suspected and unsuspected VTE in follow up on chemotherapy

A  The complete thrombin curve

B  The lag time
C The propagation phase and peak

![Graph showing mean thrombin concentration over time for suspected and unsuspected VTE on chemotherapy.]

4.41.4 Baseline VTE compared with suspected VTE

The mean lag time:TM ratio (p=0.011) was higher and the ETP:AT (p=0.009), VI:AT (p=0.003) and peak:AT (p=0.002) ratios were lower in chemotherapy patients diagnosed with suspected VTE in follow up compared with patients diagnosed with VTE at baseline assessment (see figures 141 to 143).
Figure 141 Comparison of the mean (+/-SEM) lag time:TM ratio between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up

![Comparison of mean lag time:TM ratio between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up.](image)

*p<0.05

Figure 142 Comparison of the mean (+/-SEM) ETP:AT ratio between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up

![Comparison of mean ETP:AT ratio between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up.](image)

**p<0.01
**Figure 143** Comparison of the mean (+/-SEM) VI:AT and peak:AT ratios between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up

**4.41.5 Baseline VTE compared with unsuspected VTE**
Chemotherapy patients diagnosed with unsuspected VTE in follow up had higher mean AT concentrations (P=0.023) than patients diagnosed with VTE at baseline assessment (see figure 144).

**Figure 144** Comparison of mean (+/-SEM) antithrombin concentrations between cancer patients with baseline VTE and chemotherapy patients that developed unsuspected VTE in follow up

352  Venous thromboembolism in cancer patients undertaking chemotherapy
Consequently, ttPeak:AT (p=0.02), Peak:AT (p=0.01), VI:AT (p=0.049) and ETP:AT (p=0.007) ratios were lower in unsuspected VTE patients (see figures 145 to 147).

**Figure 145** Comparison of the mean (+/-SEM) ttpeak:AT ratio between cancer patients with baseline VTE and chemotherapy patients that developed unsuspected VTE in follow up

![Comparison of the mean (+/-SEM) ttpeak:AT ratio between cancer patients with baseline VTE and chemotherapy patients that developed unsuspected VTE in follow up](image1)

* *p<0.05

**Figure 146** Comparison of the mean (+/-SEM) ETP:AT ratio between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up

![Comparison of the mean (+/-SEM) ETP:AT ratio between cancer patients with baseline VTE and chemotherapy patients that developed suspected VTE in follow up](image2)

**p<0.01
Figure 147 Comparison of the mean (+/-SEM) VI:AT and peak:AT ratios between cancer patients with baseline VTE and chemotherapy patients that developed unsuspected VTE in follow up

*p<0.05
4.42 TGA changes on chemotherapy in patients not diagnosed with VTE

Ninety six chemotherapy patients were reviewed for a second time within 100 days of their baseline assessment and were not diagnosed with VTE in that time. Between the two assessments the mean VI, peak and ETP had reduced although the lag time, ttPeak and tail were similar (see table 127 and figure 148).

Table 127 Comparison of TGA data at baseline and second assessment in chemotherapy patients who did not develop VTE within 100 days

<table>
<thead>
<tr>
<th>TGA variable</th>
<th>Baseline assessment (n=96)</th>
<th>Second assessment within 100 days (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Lag time (minutes)</td>
<td>3.20 (0.06)</td>
<td>3.18</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>147* (3.0)</td>
<td>144</td>
</tr>
<tr>
<td>Time to peak (minutes)</td>
<td>5.64 (0.06)</td>
<td>5.56</td>
</tr>
<tr>
<td>Peak thrombin (nM)</td>
<td>342* (5.0)</td>
<td>340</td>
</tr>
<tr>
<td>Endogenous thrombin potential (nM.min)</td>
<td>1663* (30)</td>
<td>1642</td>
</tr>
<tr>
<td>Start tail (minutes)</td>
<td>21.4 (0.17)</td>
<td>21.1</td>
</tr>
</tbody>
</table>

*p<0.05
**Figure 148** Mean (+/-SEM) thrombin generation curves of the baseline and second visit within 100 days for patients not diagnosed with VTE

**A** The complete thrombin curve

![Complete Thrombin Curve](image)

**B** The lag time

![Lag Time](image)
C The propagation phase and peak

The mean AT concentration (p=0.012) was found to be higher at second visit although, as was observed in earlier analyses, the AT concentrations remained within the CHL normal range (see figure 14). TM concentrations were similar at the two visits (data not shown).
**Figure 149** Comparison of mean (+/-SEM) antithrombin concentrations at baseline and second visit in chemotherapy patients who were not diagnosed with VTE within 100 days

Ratios of ETP with both AT (p=0.001) and TM (p=0.04) were found to be lower at second visit as were VI:AT (p=0.002), peak:AT (p=0.001) and tail:AT (p=0.009) ratios (see figures 150 to 153).

**Figure 150** Comparison of the mean (+/-SEM) ETP:TM ratio at baseline and second visit in chemotherapy patients who were not diagnosed with VTE within 100 days
Figure 151 Comparison of the mean (+/-SEM) ETP:AT ratio at baseline and second visit in chemotherapy patients who were not diagnosed with VTE within 100 days

![Graph showing comparison of mean ETP:AT ratios](image)

**p<0.01

Figure 152 Comparison of the mean (+/-SEM) VI:AT and peak:AT ratios at baseline and second visit in chemotherapy patients who were not diagnosed with VTE within 100 days

![Graph showing comparison of mean VI:AT and peak:AT ratios](image)

**p<0.01
4.43 Summary

This study has identified differences in TGA variables between cohorts of cancer patients and volunteers without cancer. The mean lag times were longer in the cancer cohorts compared with the cohort without cancer at baseline assessment, regardless of the presence of VTE. The mean velocity index, peak thrombin concentration and ETP were also higher in the cancer patient cohorts. No differences were seen, however, in TGA variables when cancer patients with VTE were compared with those without VTE at baseline assessment.

Mean AT and TM concentrations were similar between cancer patients without VTE at baseline and volunteers without cancer. In cancer patients with VTE at baseline, mean AT was significantly lower and mean TM was significantly higher than volunteers without cancer. The mean AT concentration was also significantly lower than that of cancer patients without VTE but TM concentrations were similar.

Ratios of the mean ETP, peak thrombin, ttPeak, VI and tail to AT were higher in cancer patients with VTE compared with those without VTE and volunteers without cancer at baseline assessment.
A reduction in the mean velocity index, peak thrombin and ETP were seen with no change in the lag time, ttPeak and tail in chemotherapy patients who developed VTE within 100 days. These changes were, however, also seen on chemotherapy in patients who did not develop VTE at 100 days follow up. The mean AT also increased in chemotherapy patients regardless of VTE development but TM levels remained similar.

Ratios of ETP, peak thrombin, VI and tail to AT, however, reduced in chemotherapy patients who developed VTE within 100 days as did ratios of ETP and peak thrombin to TM. These changes were different to those observed in chemotherapy patients who had not been diagnosed with VTE at 100 days. In this cohort the ETP:AT reduced but the ETP:TM and peak thrombin:AT, VI:AT and tail:AT ratios had increased.

When VTE events diagnosed in chemotherapy patients, during follow up, were stratified for clinical suspicion the suspected VTE cohort were found to have a significantly longer mean lag time, ttPeak and higher AT concentration. All other variables and ratios were similar. Compared with patients diagnosed with VTE at baseline (all unsuspected), patients with suspected VTE had a lower mean VI, peak and ETP but a longer mean ttPeak and similar mean tail, AT and TM. Mean ETP, peak and VI ratioed with AT were also lower but the mean lag time:TM ratio was higher. Chemotherapy patients diagnosed with unsuspected VTE in follow up had similar mean concentrations of individual TGA variables except the ETP, which was decreased compared to patients with baseline VTE. As was seen in the suspected VTE cohort, mean ETP:AT, peak:AT and VI:AT were reduced but in addition the unsuspected VTE cohort also had a reduced mean ttPeak:AT ratio and higher AT in comparison to the baseline VTE cohort.
4.44 Introduction
Enzyme-linked immunosorbent assays (ELISAs) for Ang-1, Ang-2 and sTie-2 were performed at the Mackenzie Cancer Research Laboratory at the University of Otago Christchurch. Assays were performed on platelet-free plasma prepared as described in the methods section and stored at -80°C for batched analysis. Mean Ang-1, Ang-2 and sTie-2 concentrations were ratioed with each other and analysed to look for any differences in patients diagnosed with VTE compared with those who did not.

At baseline assessment, results for cancer patients with and without VTE in comparison to volunteers without cancer have been analysed at baseline, and also in follow-up, including patients who received chemotherapy. Each analysis is presented in tabular form, including statistical significance, and then displayed as bar graphs.

4.45 Angiopoietin and sTie-2 concentrations in cancer patients compared with volunteers without cancer at baseline assessment
Mean Ang-1 and Ang-2 concentrations were higher in cancer patients who underwent a baseline assessment compared with volunteers without cancer. Soluble Tie-2 concentrations were similar in the two cohorts (see table 128 and figures 154 and 155).
### Table 128 Mean (+/-SEM) angiopoietin and sTie-2 concentrations in cancer patients and volunteers without cancer at baseline assessment

<table>
<thead>
<tr>
<th></th>
<th>Cancer (n=200)</th>
<th>Volunteers without cancer (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>2602* (164)</td>
<td>2143</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>3284** (144)</td>
<td>2670</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>19.5 (0.6)</td>
<td>18.0</td>
</tr>
<tr>
<td>Ang-1: Ang2</td>
<td>0.98 (0.08)</td>
<td>0.70</td>
</tr>
<tr>
<td>Ang-1: sTie-2</td>
<td>152 (12)</td>
<td>119</td>
</tr>
<tr>
<td>Ang-2: sTie-2</td>
<td>176* (7)</td>
<td>158</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Figure 154 Mean (+/-SEM) angiopoietin-1 and -2 concentrations in cancer patients and volunteers without cancer

![Graph showing mean concentrations of Ang-1 and Ang-2 in cancer (n=199) and non-cancer (n=49) cohorts.](image)

*p<0.05  **p<0.01

Figure 155 Mean (+/-SEM) sTie-2 concentrations in cancer patients and volunteers without cancer

![Graph showing mean concentrations of sTie-2 in cancer (n=199) and non-cancer (n=49) cohorts.](image)

Ang-2 in ratio with sTie-2 was also higher in cancer patients (see figure 156). Ang-1:Ang-2 and Ang-1:sTie-2 ratios were similar in the two cohorts.
4.45.1 Cancer patients with VTE compared with volunteers without cancer

Plasma Ang-2 and sTie-2 concentrations were found to be significantly higher in cancer patients with VTE compared with volunteers without cancer. Ang-1 concentrations were similar in the two cohorts (see table 129 and figures 157 and 158). The Ang-2:sTie-2 ratio was also higher in the cancer patient cohort (see figure 159).
Table 129 Mean (+/-SEM) angiopoietin and sTie-2 concentrations in cancer patients with VTE and volunteers without cancer at baseline assessment

<table>
<thead>
<tr>
<th></th>
<th>Cancer with VTE at baseline (n=16)</th>
<th>Volunteers without cancer (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>2048 (397)</td>
<td>1813</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>4435**** (627)</td>
<td>3659</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>25.9* (4.5)</td>
<td>18.7</td>
</tr>
<tr>
<td>Ang-1: Ang2</td>
<td>0.56 (0.14)</td>
<td>0.42</td>
</tr>
<tr>
<td>Ang-1: sTie-2</td>
<td>107 (28)</td>
<td>64</td>
</tr>
<tr>
<td>Ang-2: sTie-2</td>
<td>219* (41)</td>
<td>156</td>
</tr>
</tbody>
</table>

*p<0.05, ****p<0.0001
Figure 157 Mean (+/-SEM) angiopoietin-1 and -2 concentrations in cancer patients with VTE and volunteers without cancer at baseline assessment

![Bar chart showing mean angiopoietin-1 and -2 concentrations](chart_157)

****p<0.0001

Figure 158 Mean (+/-SEM) sTie-2 concentrations in cancer patients with VTE and volunteers without cancer at baseline assessment

![Bar chart showing mean sTie-2 concentrations](chart_158)

* p<0.05

Figure 159 Mean (+/-SEM) Ang-2:sTie-2 ratios in cancer patients with VTE and volunteers without cancer at baseline assessment

![Bar chart showing mean Ang-2:sTie-2 ratios](chart_159)

*p<0.05
4.45.2 Cancer patients without VTE compared with volunteers without cancer at baseline assessment

Ang-1 and Ang-2 concentrations were higher in cancer patients without VTE at baseline assessment. sTie-2 concentrations were similar between the cohorts (see table 130 and figures 160 and 161).

The Ang-2:sTie-2 ratio was higher in cancer patients without VTE compared with volunteers without cancer (see figure 162).

**Table 130 Mean (+/-SEM) angiopoietin and sTie-2 concentrations in cancer patients without VTE and volunteers without cancer at baseline assessment**

<table>
<thead>
<tr>
<th></th>
<th>Cancer without VTE at baseline (n=184)</th>
<th>Volunteers without cancer (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>2650* (174)</td>
<td>2170</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>3183** (145)</td>
<td>2640</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>19.0 (0.5)</td>
<td>18.0</td>
</tr>
<tr>
<td>Ang-1:Ang2</td>
<td>1.01 (0.08)</td>
<td>0.73</td>
</tr>
<tr>
<td>Ang-1:sTie-2</td>
<td>156 (12)</td>
<td>127</td>
</tr>
<tr>
<td>Ang-2:sTie-2</td>
<td>173* (7)</td>
<td>158</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Figure 160 Mean (+/-SEM) angiopoietin-1 and -2 concentrations in cancer patients without VTE and volunteers without cancer at baseline assessment

*Figure 161 Mean (+/-SEM) sTie-2 concentrations in cancer patients without VTE and volunteers without cancer at baseline assessment

*Figure 162 Mean (+/-SEM) Ang-2:sTie-2 ratios in cancer patients without VTE and volunteers without cancer at baseline assessment

*p<0.05  **p<0.01

Venous thromboembolism in cancer patients undertaking chemotherapy
4.46 Cancer patients with VTE (n=16) compared with cancer patients without VTE (n=184)

Plasma Ang-2 and sTie-2 concentrations were both higher in cancer patients with VTE compared to those without at baseline assessment. Ang-1 concentrations were similar in both cohorts (see table 131 and figures 163 and 164).

Table 131 Mean (+/SEM) angiopoietin and sTie-2 concentrations in cancer patients with and without VTE at baseline assessment

<table>
<thead>
<tr>
<th></th>
<th>Cancer with VTE at baseline (n=16)</th>
<th>Cancer without VTE at baseline (n=184)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>2048 (397)</td>
<td>1813</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>4435* (627)</td>
<td>3659</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>25.9** (4.5)</td>
<td>18.7</td>
</tr>
<tr>
<td>Ang-1: Ang2</td>
<td>0.56 (0.14)</td>
<td>0.42</td>
</tr>
<tr>
<td>Ang-1: sTie-2</td>
<td>107 (28)</td>
<td>64</td>
</tr>
<tr>
<td>Ang-2: sTie-2</td>
<td>219 (41)</td>
<td>156</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Figure 163 Mean (+/-SEM) angiopoietin concentrations in cancer patients with and without VTE at baseline

* p<0.05

Figure 164 Mean (+/-SEM) sTie-2 concentrations in cancer patients with and without VTE at baseline

** p<0.01

The mean and median Ang-2:sTie-2 ratios was similar between the two cohorts as were other variable ratios (see figure 165).

Figure 165 Mean (+/-SEM) Ang-2:sTie-2 ratios in cancer patients with and without VTE at baseline
**4.47 Cancer patients diagnosed with VTE compared to patient without VTE within 100 days of follow up**

At follow up visits taking place up to and including 100 days from baseline assessment, 107 chemotherapy patients underwent a full assessment including blood tests. Eleven patients (10.3%) had developed VTE, had undergone a full study assessment and provided blood samples. Concentrations of Ang-1, Ang-2 and sTie-2 were similar between those that developed VTE and those that did not on chemotherapy (see table 132 and figures 166 and 167).

**Table 132 Mean (+/-SEM) angiopoietin and sTie-2 concentrations in chemotherapy patients with and without VTE at 100 days of follow up from baseline assessment**

<table>
<thead>
<tr>
<th></th>
<th>Cancer with VTE within 100 days at diagnosis (n=11)</th>
<th>Cancer without VTE second visit within 100 days (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM) Median Range</td>
<td>Mean (SEM) Median Range</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>1413 (633) 1204 50-3060</td>
<td>2016 (221) 1559 50-12215</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>2740 (277) 2672 1719-4046</td>
<td>3074 (94) 2686 1101-10360</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>20.2 (1.6) 19.4 12.2-30.0</td>
<td>20.8 (0.6) 19.6 11.3-65.9</td>
</tr>
<tr>
<td>Ang-1: Ang2</td>
<td>0.56 (0.19) 0.47 0.01-1.44</td>
<td>0.76 (0.11) 0.62 0.01-5.33</td>
</tr>
<tr>
<td>Ang-1: sTie-2</td>
<td>77 (25) 63 2-212</td>
<td>106 (16) 68 2-898</td>
</tr>
<tr>
<td>Ang-2: sTie-2</td>
<td>139 (17) 132 37-86</td>
<td>159 (5) 144 33-619</td>
</tr>
</tbody>
</table>
Figure 166 Mean (+/-SEM) angiopoietin-1 and -2 concentrations in chemotherapy patients with and without VTE at 100 days of follow up from baseline assessment

Figure 167 Mean (+/-SEM) sTie-2 concentrations in chemotherapy patients with and without VTE at 100 days of follow up from baseline assessment
4.48 Changes on chemotherapy in patients who did not develop VTE within 100 days

For the 96 patients who commenced chemotherapy, were reviewed for a second time within 100 days from baseline assessment and were not diagnosed with VTE, the mean Ang-1 concentration was significantly reduced at second visit. Ang-2 concentrations increased and sTie-2 concentrations remained similar between the cohorts (see table 133 and figures 168 and 169).

Table 133 Mean (+/-SEM) angiopoietin and sTie-2 concentrations at baseline and second visit in chemotherapy patients not diagnosed with VTE at 100 days of follow up

<table>
<thead>
<tr>
<th></th>
<th>Cancer no VTE baseline (n=96)</th>
<th>Cancer no VTE visit 2 (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>2855** (224)</td>
<td>2336</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>2804* (95)</td>
<td>2461</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>17.9** (0.6)</td>
<td>17.4</td>
</tr>
<tr>
<td>Ang-1:Ang2</td>
<td>1.21** (0.11)</td>
<td>0.84</td>
</tr>
<tr>
<td>Ang-1:sTie-2</td>
<td>173** (16)</td>
<td>131</td>
</tr>
<tr>
<td>Ang-2:sTie-2</td>
<td>163 (5)</td>
<td>145</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Figure 168 Mean (±SEM) angiopoietin-1 and -2 concentrations at baseline and second visit in chemotherapy patients not diagnosed with VTE at 100 days of follow up

![Bar chart showing mean angiopoietin-1 and -2 concentrations, with Cohort and p-values indicated.]

*p<0.05  **p<0.01

Figure 169 Mean (±SEM) sTie-2 concentrations at baseline and second visit in chemotherapy patients not diagnosed with VTE at 100 days of follow up

![Bar chart showing mean sTie-2 concentrations, with Cohort and p-values indicated.]

**p<0.01

Mean Ang-1:sTie-2 and Ang-1:Ang-2 ratios were lower in follow up on chemotherapy than at baseline assessment in patients who were not diagnosed with VTE within 100 days (see figures 170 and 171).
Figure 170 Mean (+/SEM) Ang-1:Ang-2 ratios at baseline and second visit in chemotherapy patients not diagnosed with VTE at 100 days of follow up

**p<0.01

Figure 171 Mean (+/SEM) Ang-1:sTie-2 ratios at baseline and second visit in chemotherapy patients not diagnosed with VTE at 100 days of follow up

**p<0.01
4.49 Changes on chemotherapy in cancer patients who developed VTE within 100 days

For the 11 patients who were fully assessed at baseline and at a second visit within 100 days and were diagnosed with VTE at that second visit, mean Ang-1 concentrations were significantly lower at second visit. Ang-2 and sTie-2 concentrations were similar (see table 134 and figures 172 and 173).

Table 134 Mean (+/-SEM) angiopoietin and sTie-2 concentrations at baseline and second visit in chemotherapy patients diagnosed with VTE at 100 days of follow up

<table>
<thead>
<tr>
<th></th>
<th>VTE within 100 days baseline visit (n=11)</th>
<th>VTE visit 2 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>3146* (633)</td>
<td>2438</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>3330 (277)</td>
<td>2872</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>17.4 (1.6)</td>
<td>17.6</td>
</tr>
<tr>
<td>Ang-1:Ang2</td>
<td>1.05 (0.19)</td>
<td>0.85</td>
</tr>
<tr>
<td>Ang-1:sTie-2</td>
<td>183* (25)</td>
<td>139</td>
</tr>
<tr>
<td>Ang-2:sTie-2</td>
<td>193* (17)</td>
<td>170</td>
</tr>
</tbody>
</table>

*p<0.05
Figure 172 Mean (+/-SEM) angiopoietin-1 and -2 concentrations at baseline and second visit in chemotherapy patients diagnosed with VTE at 100 days of follow up

![Graph showing mean angiopoietin-1 and -2 concentrations at baseline and second visit in chemotherapy patients diagnosed with VTE at 100 days of follow up.](image)

Figure 173 Mean (+/-SEM) sTie-2 concentrations at baseline and second visit in chemotherapy patients diagnosed with VTE at 100 days of follow up

![Graph showing mean sTie-2 concentrations at baseline and second visit in chemotherapy patients diagnosed with VTE at 100 days of follow up.](image)

Mean Ang-1:sTie-2 and Ang-2:sTie-2 ratios were significantly lower in chemotherapy patients at the time of VTE diagnosis when compared to baseline assessment (see figure 174).
As reported in results section 4.26.1, a comparison of changes in variables was undertaken in chemotherapy patients who were diagnosed with VTE within 100 days and those who were not. In this analysis, the mean Ang-2 concentration and Ang-2:sTie-2 ratio had shown a significant reduction in patients diagnosed with VTE compared with baseline assessment. The mean plasma Ang-2 concentration had, in fact, increased in patients without VTE, and Ang-2:sTie-2 levels were similar between visits.

**4.50 Suspected and unsuspected VTE on chemotherapy**

Thirty one patients were diagnosed with VTE in follow up on chemotherapy. Fourteen patients were diagnosed within 100 days, and 17 beyond 100 days of follow up from baseline assessment. Of these, 16 were clinically unsuspected and 15 were clinically suspected with full prospective data available in 24 to analyse differences in angiopoietins and sTie-2 at the time of diagnosis. No significant differences in individual variables or ratios were found between the two cohorts (see table 135 and figures 175 and 176).
Table 135 Mean (±SEM) angiopoietin and sTie-2 concentrations in chemotherapy patients diagnosed with suspected and unsuspected VTE in follow up beyond baseline

<table>
<thead>
<tr>
<th></th>
<th>Unsuspected VTE (n=16)</th>
<th>Suspected VTE (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>1683 (343)</td>
<td>1626</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>3978 (642)</td>
<td>3230</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>22.8 (1.2)</td>
<td>22.4</td>
</tr>
<tr>
<td>Ang-1:Ang2</td>
<td>0.46 (0.09)</td>
<td>0.45</td>
</tr>
<tr>
<td>Ang-1:sTie-2</td>
<td>73 (15)</td>
<td>64</td>
</tr>
<tr>
<td>Ang-2:sTie-2</td>
<td>174 (23)</td>
<td>159</td>
</tr>
</tbody>
</table>
Figure 175 Mean (+/-SEM) angiopoietin-1 and -2 concentrations in cancer patients diagnosed with baseline VTE and chemotherapy patients diagnosed with suspected VTE in follow up

Figure 176 Mean (+/-SEM) sTie-2 concentrations in cancer patients diagnosed with baseline VTE and chemotherapy patients diagnosed with suspected VTE in follow up
4.51 Cancer patients with baseline VTE compared with cancer patients with VTE within 100 days on chemotherapy

Ang-2 concentrations were significantly lower in chemotherapy patients diagnosed with VTE in follow up within 100 days compared with those that were diagnosed with VTE at baseline assessment, prior to starting chemotherapy. Ang-1 and sTie-2 concentrations were not significantly different between the two cohorts (see table 136 and figures 177 and 178). Variable ratios studied were all similar between the groups.

Table 136 Mean (+/SEM) angiopoietin and sTie-2 concentrations in cancer patients diagnosed with baseline VTE and chemotherapy patients diagnosed with VTE in follow up within 100 days

<table>
<thead>
<tr>
<th></th>
<th>Cancer with VTE at baseline visit (n=16)</th>
<th>VTE between baseline and 100 days (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Median</td>
</tr>
<tr>
<td>Ang-1 (pg/mL)</td>
<td>2048 (397)</td>
<td>1813</td>
</tr>
<tr>
<td>Ang-2 (pg/mL)</td>
<td>4435* (627)</td>
<td>3659</td>
</tr>
<tr>
<td>sTie-2 (ng/mL)</td>
<td>25.9 (4.5)</td>
<td>18.7</td>
</tr>
<tr>
<td>Ang-1: Ang2</td>
<td>0.56 (0.14)</td>
<td>0.42</td>
</tr>
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<td>107 (28)</td>
<td>64</td>
</tr>
<tr>
<td>Ang-2: sTie-2</td>
<td>219 (41)</td>
<td>156</td>
</tr>
</tbody>
</table>

*p<0.05
Figure 177 Mean (+/-SEM) angiopoietin-1 and -2 concentrations in cancer patients diagnosed with baseline VTE and chemotherapy patients diagnosed with VTE in follow up within 100 days

![Angiopoietin-1 and -2 Concentrations](image)

*p<0.05

Figure 178 Mean (+/-SEM) sTie-2 concentrations in cancer patients diagnosed with baseline VTE and chemotherapy patients diagnosed with VTE in follow up within 100 days

![sTie-2 Concentrations](image)
4.52 Summary

Changes in the Angiopoietin-Tie-2 pathway associated with cancer have been well documented, but little has been published on the pathway’s association with venous thromboembolic disease in cancer patients. This study found higher mean levels of Ang-1, Ang-2 and the Ang-2:sTie-2 ratio in cancer patients compared to volunteers without cancer. When cancer patients were differentiated into those with and without VTE at baseline assessment prior to commencing chemotherapy, both cohorts had higher mean plasma Ang-2 concentrations and Ang-2:sTie-2 ratios than volunteers without cancer. Cancer patients with VTE also had higher sTie-2 concentrations, whereas cancer patients without VTE had higher Ang-1 concentrations.

On direct comparison of cancer patients with and without VTE at baseline assessment, both mean plasma Ang-2 and sTie-2 concentrations were higher in those with VTE, but the Ang-2:sTie-2 ratio was similar between the two cohorts.

Mean concentrations of Ang-1 and the mean Ang-1:Ang-2 and Ang-1:sTie-2 ratios decreased on chemotherapy in a cohort of patients assessed on two occasions within 100 days and not diagnosed with VTE. Mean Ang-2 concentrations were found to have increased. In chemotherapy patients who developed VTE within 100 days and were assessed in follow up at the time of their VTE diagnosis, the mean Ang-1 concentration and Ang-1:sTie-2 ratio had again decreased. The mean Ang-2:sTie-2 ratio had, however, also significantly decreased in VTE patients, in contrast to no significant change in patients without VTE in follow up. Mean Ang-2 and sTie-2 concentrations were similar at both assessments. Ang-2 concentrations were, however, significantly lower in chemotherapy patients diagnosed with VTE within 100 days of follow up compared with patients diagnosed with VTE at baseline assessment.

No differences were seen in the pathway variables or their ratios when patients were stratified by clinical suspicion at the time of diagnosis.
Chapter 5
Discussion and further work
Chapter 5 Discussion and further work

This prospective longitudinal study is the first of its kind to follow a cohort of cancer outpatients with good performance status through chemotherapy treatment (n=138), and screen for SVT, DVT and PE development and associated blood markers at more than one time point. Over 80% of the 48 cancer patients found to have VTE were fully assessed at the time of VTE diagnosis. This minimised recall bias and enabled validation of the precision of published VTE risk assessment models. The high proportion of fully assessed patients also allowed investigation of potential novel blood borne markers that may predict or aid in the early diagnosis of VTE in cancer patients undertaking chemotherapy. Comparisons could also be made with a cohort of volunteers (n=50) without cancer to provide some indication of our local population’s ‘normal’ range for novel biomarkers investigated during this study.

5.1 Study participants

The cohorts with and without cancer were recruited contemporaneously and were similar for comorbidity and performance status. Nearly two-thirds of both cohorts were female. The age ranges were similar in each group, although the median age in the cancer cohort was slightly higher (59 years v 56 years), influenced primarily by the age of the male cancer participants. Consistent with the demographics of the Canterbury population, most cancer patients (53.2%) recruited were New Zealand European limiting generalisability of study conclusions to other ethnic groups (http://www.stats.govt.nz/Census.aspx). Both cohorts were well balanced for the regular use of aspirin and simvastatin which have been associated with VTE risk reduction in patients with cancer(617). The non cancer cohort contained a significantly higher proportion of women (17.2% v 3.1%) who took an oral contraceptive pill, but this did not impact on differences seen in blood marker comparisons between the cohorts.

The 203 recruited cancer patients had been diagnosed with a number of different cancer primaries, of which breast and colorectal cancer were most highly represented (47.3%). Cancer was widespread in 72.5% of patients, and local/locally advanced in 23.5%. Adenocarcinoma (63.5%) was the most common morphological diagnosis. The patients in this study would, therefore,
be a good representation of patients that regularly present to oncology clinics throughout the developed world.

### 5.2 VTE and baseline VTE prevalence

As has been published previously in predominantly retrospective epidemiological studies, VTE risk is high in cancer patients. Over 25% of the cancer patients recruited to this study were diagnosed with VTE. Significantly, the prevalence of VTE at baseline assessment, prior to commencing chemotherapy, was 8.4%. All of these VTE events were clinically unsuspected, including 12 patients with PE. This is only the second prospective study to document a high prevalence of unsuspected VTE at baseline assessment (259). These findings are important to consider in the design of future primary thromboprophylaxis studies of cancer patients undertaking chemotherapy. It may be appropriate to make VTE screening mandatory for all potential study recruits, in order to accurately enrol patients eligible for thromboprophylaxis, rather than patients who already have VTE and require therapeutic anticoagulation (20). Had the SAVE-ONCO study screened for VTE at study entry, up to 270 patients may have been identified who had clinically unsuspected VTE. These patients may not have been adequately managed with an unproven anticoagulant at prophylactic doses (553). In the current study, similar numbers of patients appeared to be diagnosed with VTE during each of the three assessment periods of baseline, baseline to 100 days follow up, and beyond 100 days follow up. Epidemiological studies have reported that VTE incidence appears to be highest in the first six months following cancer diagnosis and is associated with chemotherapy treatment (181, 187). Results from this study suggest that perhaps some of these early diagnoses may relate to missed unsuspected VTE, developed prior to chemotherapy administration, that have become clinically overt during the chemotherapy course.

### 5.3 VTE and chemotherapy

As well as the high baseline VTE prevalence, over 22% of the patient cohort that received chemotherapy (31 of 138) developed VTE during follow up on this study. Twenty of these (64.5%) were diagnosed with VTE while still actively receiving chemotherapy and 11 patients (35.4%) were diagnosed with
VTE after completion of the course. Seventy-two percent (72%) of the post chemotherapy VTE events (n=11) were diagnosed within five weeks of receiving the final dose of chemotherapy. The remaining 28% occurred beyond eight weeks from chemotherapy completion and could not be associated with the chemotherapy treatment. More than 20% of the patients with VTE developed thrombus at two different sites. The proportion of patients developing VTE in this study (22%) is considerably higher than those diagnosed by Khorana et al. (2.2% and 2.1%, respectively, in the derivation and validation cohorts for the Khorana RAM), in part, due to this study screening for clinically unsuspected as well as suspected VTE(24).

Most patients received a platinum agent, fluoropyrimidine, anthracycline or a combination of these agents. Relative frequencies were too small to enable robust statistical comparisons and thereby identify specific regimens or individual drugs that may be associated with VTE. Despite low statistical power the following findings warrant mention. Four out of 6 (66%) patients on ECF chemotherapy for the treatment of gastric/GOJ adenocarcinoma developed VTE. Thirty eight out of 129 (29.5%) of patients with adenocarcinoma, encompassing patients with gastric, GOJ, pancreas, colon and breast cancers, developed VTE. Five of 15 (33.3%) patients diagnosed with B-cell lymphomas were also diagnosed with VTE.

Ten of 62 (16.1%) patients undertaking potentially curative chemotherapy developed VTE, with 60% of these patients (6 of 10) undertaking adjuvant treatment (i.e. no evidence of macroscopic cancer present at the time of treatment). Six patients developed upper extremity thrombosis and only one patient was diagnosed with PE (~1.6%), which contrasts the patients treated with palliative intent of whom 12 of 76 patients (15.8%) were diagnosed with PE.

### 5.4 VTE site and clinical suspicion

Thirty three of the 48 patients (68.8%) with venous thrombosis were diagnosed with clinically unsuspected VTE. The diagnosis was made at baseline assessment in 17 patients, and during chemotherapy treatment in 16 patients.
Only 15 of the 138 (10.9%) patients on chemotherapy were diagnosed with clinically suspected VTE.

Twenty five (52.1%) patients were diagnosed with PE and 30 (62.5%) with DVT and/or SVT. In those patients diagnosed with PE, 64% were found to have developed more than one thrombus and 44% had bilateral lung involvement. Only four patients (16%) diagnosed with PE had thrombus located solely in subsegmental pulmonary arteries. Eighty four percent (84%) were found to have thrombus in more proximal vessels with 40% developing thrombus in lobar arteries. Eighty percent (80%) of the patients with thrombus present in lobar arteries were diagnosed with clinically unsuspected PE. Patients with DVT/SVT were diagnosed at sites in the abdomen as well as in the upper and lower limb, with 80% of the upper limb thrombotic events associated with PICC lines used to administer chemotherapy.

5.5 Cumulative incidence and mortality

Cumulative incidences of VTE on chemotherapy (n=31) at 90, 183 and 365 days were high (8.4%, 27.6% and 69.9%, respectively) in keeping with previous studies(460, 618, 619). Compared with chemotherapy patients who were not diagnosed with VTE, mortality at 90 days was high in patients diagnosed with VTE at baseline assessment and in patients diagnosed with VTE on chemotherapy treatment, despite therapeutic anticoagulation (41.7% v 6.6% respectively) with a hazard ratio for death in patients with VTE at baseline of 2.25 (95% CI 1.07-4.70). Of note, Gary et al. also found a high rate of unsuspected lower limb VTE (18%) at baseline assessment which was associated with poorer survival at nine months despite anticoagulation (HR for death 2.4; 95% CI 1.2-5.3)(259). These findings suggest that the diagnosis of VTE may be a marker of more aggressive tumour biology and poorer prognosis.

On further analysis of chemotherapy patients diagnosed with VTE beyond baseline, suspected but not unsuspected VTE was associated with poorer survival. PE patients diagnosed with suspected or unsuspected lobar pulmonary artery thrombus, bilateral lung involvement or more than one thrombus in one lung were also found to have higher mortality rates than patients without VTE.
on chemotherapy. The development of SVT and subsegmental PE did not impact on survival outcome although patient numbers were small.

This study did not investigate the natural history of unsuspected VTE, since most diagnosed VTEs were treated. However, it is reasonable to hypothesise that patients developing multiple or more proximal thrombi in larger vessels are at higher risk for developing clinically overt thrombosis, and resultant morbidity and mortality, compared with patients with no VTE or with a single thrombus in small peripheral vessels at initiation of chemotherapy. These findings support previously published research, and add weight to the argument that targeted thromboprophylaxis in patients without VTE at the commencement of chemotherapy treatment must be further investigated, using newer anticoagulants as well as older, more established treatments(620).

5.6 VTE risk assessment models (RAMs)
The RAMs utilised by this study were proficient in identifying patients at low risk of developing VTE on chemotherapy (high specificities and NPVs) but were limited in clearly identifying those patients that were at high risk(24, 596, 599). The models performed better when predicting VTE development within 100 days compared with VTE beyond 100 days from baseline assessment. All three models and their adaptations show that with increasing risk level there is an increase in the proportion of patients that develop VTE. The sensitivities and PPVs of the models are, however, low and classify a large number of patients at intermediate to high risk. Many of these patients would potentially receive thromboprophylaxis, never develop VTE and be at increased risk of haemorrhagic complications secondary to anticoagulants. However, the SAVE-ONCO study did not show a significant increase in major bleeding events in cancer outpatients who were undergoing chemotherapy and receiving prophylactic anticoagulants (1.2%) compared with placebo (1.2% v 1.0%, respectively, HR, 1.05; 95% CI, 0.55-1.99), though clinically relevant non major bleeding events were higher in the semuloparin cohort (1.6% v 0.9%, respectively, HR, 1.86, 95% CI, 0.98-3.68)(553). In contrast, the recently completed MAGELLAN study did report a slightly higher rate of major or clinically relevant bleeding at 3.6% (bleeding rates - rivaroxaban 5.4% and
enoxaparin 1.7%), but these were hospitalised cancer patients(542). Historically, the increased bleeding risk for cancer patients on anticoagulants has been attributed to therapeutic doses used to treat established thrombosis, and so it may not be appropriate to extrapolate these findings to patients on prophylactic doses(23).

As well as using the RAMs for VTE prediction on chemotherapy, their utility was addressed in identifying patients with VTE at baseline assessment. Although all these patients had clinically unsuspected VTE, the models attained similar sensitivities, specificities, PPVs and NPVs to those calculated for prediction of VTE to 100 days, with the exception of the adaptations of the Khorana/Ay model(596). Both adaptations had higher sensitivities but lower specificities, although the PPVs and NPVs were similar to other models.

The Khorana/Ay model appeared to perform best and was improved slightly by using local population-adapted cut-offs for the risk score parameters. This model was developed from the original Khorana model with the addition of D-dimer and soluble P-selectin measurements by the CATS researchers(24, 596). They are currently following cancer patients for up to two years to see if they develop VTE on various cancer treatment regimens. It may be more practical to predict VTE within a shorter timeframe, such as three to six months, to minimise the influence of changing confounding factors (e.g. other comorbidities and treatments). Shorter timeframes may also improve our understanding of the impact of specific cancer treatments on VTE risk, as over time, many different treatments (e.g. chemotherapy, radiation, surgery) may be offered to cancer patients, limiting our ability to understand the relative risks posed by any individual treatment. The original Khorana score was developed and validated using a population of cancer patients undertaking chemotherapy over a follow up period of 2.4 months(24). All data were collected prospectively and focused on symptomatic/clinically suspected VTE development. Our results unsurprisingly show that these scores are more reliable in ambulant cancer patients with clinically suspected VTE compared with unsuspected VTE.

Using the Christchurch percentile (CHCH PC) adapted Khorana/Ay RAM developed in this study, no patient classified as low risk developed suspected or
unsuspected VTE in follow up during or after completion of chemotherapy, and only one (5.9%) low risk patient had VTE out of 17 diagnosed at baseline. No other RAM was this consistent at the low risk level. Although this is helpful in ruling out thromboprophylaxis for this low risk group, only ~10% of the chemotherapy population were classified as low risk in this study. Of the remaining intermediate to high risk patients, 75-80% would not have developed VTE but would potentially have received thromboprophylaxis. Further research into diagnostic and predictive VTE biomarkers is required to tailor the administration of thromboprophylaxis to those genuinely at high risk for VTE development. This is important because screening cancer patients for PE and DVT may not be cost-effective nor be realistic in overburdened/under-resourced health systems.

In contrast to previously published studies, D-dimer was the only individual RAM variable to be independently associated with VTE diagnosis or prediction in this study(24, 596). Soluble P-selectin (sP-selectin), leukocyte count, platelet count and haemoglobin level were identified on univariate analyses but were found to correlate strongly with D-dimer and/or each other on multivariate analysis and may indicate a proinflammatory as well as procoagulant state. It may be that sP-selectin evaluation, which is not routinely available in medical laboratories, is not necessary as a component of the Khorana/Ay score, because D-dimer appeared to impact most strongly on prediction and diagnostic analyses and is correlated with sP-selectin. Removal of sP-selectin from the score would simplify the model and reduce its cost. This finding contrasts with that of other studies and therefore requires further prospective investigation in cancer patients(583, 596, 621, 622).

5.7 Blood markers of VTE risk/diagnosis

Differences in blood markers were identified in comparisons of samples obtained from cancer and non cancer patients at baseline assessment, and in chemotherapy patients during follow up. Some of these differences were seen in variables (components of the angiopoietin-Tie-2 pathway and TGA) not previously investigated in published studies on VTE in chemotherapy patients. Components of the routinely checked complete blood count and biochemistry
profiles can be deranged in patients with cancer on and off chemotherapy. Baseline mean platelet, leukocyte and neutrophil counts were significantly higher in cancer patients than non cancer volunteers, and the haemoglobin concentration was lower. Liver function tests were more likely to be deranged in cancer patients as was the corrected calcium concentration. Of note, from a coagulation perspective, the APTT was longer, the INR was higher, and fibrinogen and D-dimer concentrations were greater in cancer patients. This may suggest compensated consumption of coagulation factors and components in cancer patients. However, in most cases, APTT, INR and fibrinogen concentrations remained within CHL sanctioned normal assay ranges making the clinical interpretation of these findings challenging. Increased fibrinogen concentrations have been associated with inflammation but whether it causes thrombosis is a source of debate(623). In vitro research suggests that fibrinogen may increase the likelihood of thrombosis by increasing blood viscosity and fibrin fibre density, and by reducing susceptibility of the thrombus to fibrinolysis independent of acute phase inflammatory effects(623, 624).

The mean hsCRP and D-dimer concentrations were also higher in cancer patients. Fifty nine percent (59%) of patients with cancer had hsCRP concentrations and 54% had D-dimer concentrations above the upper limit of normal at baseline assessment compared with 8% and 4% of volunteers without cancer, respectively.

5.8 The Ang-Tie-2 pathway
The current study showed significantly higher concentrations of Ang-1 and Ang-2 in the plasma of the cancer cohort than in the non cancer cohort. These findings add further evidence to the view that cancer induces a proinflammatory and pro-angiogenic environment within its host(170, 625, 626).

Changes in the angiopoietin-Tie-2 pathway have previously been demonstrated in cancer patients(432, 444, 448, 627, 628). Although the Ang-1:Ang-2 ratio has been published as a prognostic marker in myeloma, there have not been any published studies investigating possible relationships between ratios of Ang-1, Ang-2 and sTie-2 and cancer-associated thrombosis(440, 441). The presence of malignancy is associated with increased plasma concentrations of Ang-2 and
sTie-2(442, 443). In contrast to Engin et al., the mean Ang-1 concentration was higher in cancer patients without VTE and the mean sTie-2 concentration was higher in cancer patients diagnosed with baseline VTE in comparison to volunteers without cancer. The increased concentration of Ang-1 in cancer patients may be a host response, in order to maintain endothelial cell quiescence and counteract Ang-2 promotion of angiogenic and inflammatory processes although this has not been proven. It is also recognised, however, that thrombin promotes release of Ang-1 from platelets which might explain why Ang-1 concentrations would be raised in cancer patients who have higher mean concentrations of circulating thrombin and higher platelet counts(629).

Cancer patients with VTE had higher mean Ang-2 and sTie-2 concentrations but similar Ang-1 levels at baseline compared to those without VTE. These findings have also been found in patients with acute coronary syndrome(630). Increased levels of Ang-2 and sTie-2 suggest that the vascular endothelial lining is activated in cancer patients prior to chemotherapy administration. This is likely due to Ang-2 release from tumour-associated and host endothelial cells, partly stimulated by increased thrombin levels in cancer patients, to encourage angiogenesis and inflammation(402, 631). Increased Ang-2 production may also be induced by hypoxia and tumour angiogenesis(632). Increased concentrations of the Tie-2 receptor are not only associated with endothelial cell upregulation but also with tumour associated monocytes/macrophages recruited to sites of vascular EC damage/activation(633). The soluble Tie-2 portion of the receptor may then be cleaved by tumour-derived proteases in response to VEGF, increasing plasma concentrations(634, 635). It is not known if high sTie-2 concentrations may alter the bioavailability of circulating Ang-1 and Ang-2 (which have similar affinity for the soluble receptor) in cancer patients with VTE.

This is the first study to identify changes in the plasma levels of components of the Ang-Tie-2 pathway in cancer patients on chemotherapy (decreased Ang-1, Ang-1:Ang-2 and Ang-1:sTie-2 ratios and increased Ang-2 and sTie-2). It is also the first study to find an association between the development of cancer associated VTE on chemotherapy with a decrease in the Ang-2:sTie-2 ratio. These plasma findings will need validation in a larger prospective study.
A further increase in plasma Ang-2 and a reduction in Ang-1 and the Ang-1:Ang-2 ratio were seen post chemotherapy administration in patients who did not develop VTE within 100 days. This may be in response to endothelial damage caused by chemotherapeutic agents (506, 636). In the presence of other stimuli, such as VEGF and TNFα, angiogenesis and inflammation are further induced (410, 411). Ang-1 promotes endothelial cell (EC) survival through the PI3K pathway, inhibiting apoptosis and Ang-2 expression, and resultant EC activation. Reduction in Ang-1 may, therefore, coincide with increased Ang-2 expression (410). Platelet-derived Ang-1 may explain the reduction in Ang-1 concentrations in patients on chemotherapy, as these patients may become thrombocytopenic during each treatment cycle, and so Ang-1 levels may correlate with the platelet count. Further studies investigating the role of Ang-1, and plasma concentrations during chemotherapy treatment are required.

At follow up out to 100 days, mean plasma Ang-1 concentrations had also decreased in chemotherapy patients with VTE compared with baseline findings. Mean Ang-2 and sTie-2 concentrations remained at similar levels to baseline findings (although a non significant decrease in Ang-2 and an increase in sTie-2 were seen). These results show that chemotherapy is associated with changes in markers of angiogenesis and inflammation as well as coagulation. Furthermore, as described earlier, a decrease in the Ang-2:sTie-2 ratio is a candidate marker for VTE development on chemotherapy that warrants further investigation.

5.9 Coagulation variables
The TGA also identified differences between cancer patients and non cancer volunteers. Cancer patients produced more thrombin and higher peak levels of thrombin. Of note, cancer patients took longer to initiate coagulation (longer lag time) than volunteers without cancer but, once the process had commenced, the generation of thrombin was more rapid than in non cancer patients and both cohorts reached peak thrombin levels at similar times.

Differences in coagulation variables were also found at the baseline assessment between cancer patients diagnosed with VTE and cancer patients without VTE. D-dimer, INR and soluble P-selectin were higher in patients with VTE whereas antithrombin (AT) levels were lower. However, both INR and AT levels
remained within the laboratory normal limits and would not have been flagged as of concern clinically. Liver function tests were more deranged and the hsCRP level was higher. VTE patients had lower haemoglobin levels. Individual variables of the TGA were similar between the cohorts. Ratios of these variables with AT, however, were higher in patients with VTE, with the exception of the lag time, suggesting that these ratios may be useful in differentiating between cancer patients with and without VTE. Following multivariate and ROC curve analysis, however, only D-dimer and ECOG performance status were found to be independently associated with VTE at baseline. All the variables found on univariate analysis correlated highly with each other including the TGA variables and AT.

5.10 The TGA and VTE risk/diagnosis

In this study, changes in TGA variables and ratios of these variables with the endogenous anticoagulants, antithrombin or thrombomodulin, were not independently associated with increased VTE risk or VTE development in cancer patients. It is, however, not possible to draw clear conclusions on the TGA’s clinical utility in cancer associated thrombosis as this is the first study to document some of these findings and relatively small numbers of patients were recruited. The results are also at odds with findings from the CATS group who found that peak thrombin was independently associated with VTE development(584). Further research in this area with larger patient cohorts will be required.

To date, the TGA has been a time consuming test, fraught with inconsistencies in the acquisition and preparation of plasma samples in the preanalytical phase(609, 637). However, if these processes are standardised, the assay, once initiated, is fully automated and can analyse multiple samples simultaneously (allowing storage of samples prior to analysis if necessary) without further manual input until the analysis is completed. It produces immediate results in the form of a thrombin generation curve with a number of quantifiable variables, extrapolated from the curve by computer software. From a research perspective, the TGA is an interesting test as its aim is to directly measure thrombin levels rather than surrogate markers and give a global picture of the coagulation
process. Our study results, using this assay, have added weight to the previously published literature indicating that cancer induces a consistently procoagulant state in its hosts. TGA results from this study show that cancer patients produce more thrombin than individuals without cancer. Cancer patients are slower in initiating coagulation/thrombin production compared to those without cancer but, once they do, the coagulation process appears more efficient with a more rapid propagation phase of thrombin production as quantified by the velocity index. This resulted in higher peak blood levels of thrombin and total amounts of thrombin produced over time as characterised by the endogenous thrombin potential (ETP).

It is unclear as to why the initiation of coagulation is slower in cancer patients. It may be hypothesised that increased procoagulant activity, in the presence of a cancer, stimulates upregulation of circulating endogenous anticoagulants in the blood to counteract this. Alternatively, the same mechanism that upregulates procoagulant activity might also upregulate anticoagulant activity. This would result in delayed thrombin formation in cancer patients, as it takes longer to overcome the higher levels of anticoagulants than in people without cancer who may have lower circulating levels of endogenous anticoagulants. Our study, however, did not identify significant differences in plasma levels of AT or thrombomodulin (TM) between cancer and non-cancer patients but ratios of TGA variables to AT and TM did differ. We did not measure levels of activated protein C and Tissue Factor Pathway Inhibitor, due to the lack of widely available and validated assays, but measurement of these endogenous anticoagulants may shed light on the prolonged lag time in cancer patients. Cancer procoagulant (CP), through its activation of Factor X, as well as increased levels of Tissue Factor (TF) and cytokines may contribute to the rapid propagation phase in cancer patients and the increase in thrombin production (40, 170).

Following chemotherapy administration, blood markers in cancer patients changed significantly. An increase in mean AT levels and lowering of the mean peak thrombin, velocity index and ETP were seen in patients within 100 days of follow up, regardless of whether VTE developed or not. This suggests that chemotherapy reduced thrombin production in cancer patients, possibly by
reducing tumour cell bulk and proliferation, but this will require further research. Patients diagnosed with VTE, however, also exhibited these changes and so it is unclear from these results why some cancer patients develop VTE and why others do not.

5.11 D-dimer

D-dimer consistently showed the strongest associations with VTE risk and diagnosis, although the most effective cut-off level varied depending on its application as a marker for VTE prediction or diagnosis. The limit for D-dimer at the CHL is <250 ng/mL in the blood of non cancer patients presenting with clinically suspected VTE. Any result of ≥250 ng/mL would prompt further investigation. D-dimer is not currently routinely used in the assessment of cancer patients who tend to have high levels of circulating D-dimer secondary to their cancer. In this study, 51.4% of chemotherapy patients without VTE at baseline assessment (n=138), had D-dimer levels ≥250 ng/mL (67.1% with metastatic disease and 33.8% with local/locally advanced disease).

Results from this study suggest that a D-dimer of ≥270 ng/mL would aid in the diagnosis of baseline VTE in cancer patients, prior to commencing chemotherapy, with a sensitivity of 80-86% and specificity of 50-60%. As a predictor for VTE development within 100 days on chemotherapy, a D-dimer level of ≥270 ng/mL has a sensitivity of 80% and specificity of 64%. As a diagnostic marker of VTE development on chemotherapy within 100 days, a one-off measurement of ≥355 ng/mL would have a sensitivity of 100% and specificity of 75%. If a D-dimer is obtained prior to chemotherapy initiation and is repeated within 100 days, any increase in the level appears to be associated with the presence of VTE. At second assessment, an increase in the D-dimer, compared to baseline, of 6.5 ng/mL or more would aid the diagnosis of VTE with a sensitivity of 90.9% and specificity of 54.7%. An increase of 130 ng/mL or more would indicate the presence of VTE with a reduced sensitivity of 72.7% but increased specificity of 76.8%. As many cancer patients present with higher than normal D-dimers at baseline, a higher upper limit of normal will need to be identified in this patient group to guide decision making. As has been seen in the RAM analysis of this study, normal ranges and percentile cut offs may vary,
depending on the population being investigated. Our study suggests that a cut
off D-dimer level of $\geq 270$ ng/mL or more may aid the diagnosis of VTE prior to
chemotherapy initiation, and may predict for the development of VTE on
chemotherapy within 100 days. Any increase in D-dimer measured
subsequently during chemotherapy may indicate the development of VTE,
especially if the resultant D-dimer is $\geq 355$ ng/mL. These findings should be
further studied prospectively but do provide some insight into how to interpret
D-dimer levels in cancer patients. Raised D-dimer levels have tended to be
ignored in cancer patients to assist with VTE diagnosis but this study suggests
that it may, in fact, be a useful tool with appropriately identified cut-offs.

5.12 Study strengths
Although this single-centre study was small, the results can be applied to “real
world” clinical oncology practice. It was designed specifically to investigate
VTE development in ambulant cancer patients with prospectively collected data.
The patients were treated at a tertiary referral cancer centre, and had been
diagnosed with a good cross section of solid tumour malignancies or myeloma.
Patients were screened when they came for staging scans, or were seen in the
outpatient clinic at the time of scheduled treatment appointments to improve
study compliance. It would not have been possible to prespecify standard time
points for study assessments as requirement for restaging and appointments
varied depending on cancer primary, stage, treatment intent and chemotherapy
regimen as well as patient condition. This meant that patients were seen in
follow up at varying time points during a chemotherapy cycle.

Study investigators and patients were blinded to blood and radiological results
until after clinical assessments were completed, and stored plasma samples were
anonimised to blind the laboratory investigator to patient data at the time of
batched ELISA analysis. All laboratory and radiology staff were blinded to
patient data at the time of blood analysis/radiological investigation and
reporting. All radiological imaging was reported twice by experienced radiology
staff specialising in ultrasound and/or computed tomography scan reporting and
blinded to each other’s report.
5.13 Study limitations

There are a number of limitations to this study. Smaller numbers of patients were accrued than had been planned at study development. This was because the study’s recruitment and progress were impaired markedly by the Christchurch and Canterbury earthquakes which occurred between September 2010 and December 2011. This PhD study, in fact was supposed to commence on the day of the large earthquake of February 22\textsuperscript{nd} 2011 and was understandably postponed, eventually recruiting the first patient in July 2011. The resulting damage to infrastructure, loss of clinical staff and reduction in the Christchurch population significantly reduced the numbers of participants recruited, and severely limited laboratory and radiological investigations that could be performed during the first eighteen months.

The smaller numbers of patients have impacted on the statistical power for testing associations and so results from this study will need validation in larger cohorts. Additionally, most of the statistical tests undertaken in this thesis are considered hypothesis generating and as a consequence no formal adjustment has been made to the type I error rate to allow for multiple statistical comparisons. For this reason statistically significant results are interpreted with appropriate caution and in the context of the published literature. Additionally, given the small sample sizes associated with some of the effects being tested, a largely conservative strategy has been adopted in terms of the statistical analysis. For the most part univariate analyses have been undertaken using standard robust techniques, one-way ANOVA, chi-square tests, Mann-Whitney U tests and non-parametric approaches including Kaplan-Meier estimation, log-rank tests and ROC curves. This strategy is in accord with a conservative and cautious approach to the analysis and interpretation of this observational study.

Only eight of the 15 patients who developed suspected VTE in follow up had a full study assessment as patients had often received anticoagulation before they could be seen. The study, however, is strengthened by the fact that only robust data were analysed from the eight patients fully assessed, and is not compromised by potentially unreliable and incomplete data from the clinical notes in the seven patients who were not assessed. It was not possible, with the
small number of VTE events, to analyse the effects of specific cancer primaries, morphologies or chemotherapeutic regimens and make meaningful conclusions. It was, however, notable that a high proportion of the patients with gastric and gastrooesophageal adenocarcinoma, undertaking ECF chemotherapy, developed VTE. These cancers are recognised to have a strong association with VTE development and the ECF regimen is acknowledged to be associated with higher rates of thrombosis than sister regimens such as EOX and EOF(193, 486).

Analysis of variables in follow up such as components of the complete blood count will have been affected by chemotherapy depending on when blood samples were obtained. This will have limited their clinical utility in identifying patients with VTE. The study has, however, shown temporal changes of potential blood markers for thrombosis during chemotherapy treatment, some of them for the first time (decreases in sAng-2:Tie-2 ratio and TGA variables). The ELISAs chosen for this study were commercially available, had been characterised and standardised and have been used in published research on cancer patients. It must be acknowledged, however, that antiplatelet therapies (aspirin and/or clopidogrel), routinely taken by some participants in this study, may have influenced the assay results. It is beyond the scope of this study to clarify these effects and would require further research. Many cancer patients are routinely on these medications for cardiovascular risk (ischaemic heart disease, stroke prevention) and so real world practice will need to reflect on clinically meaningful findings, taking this into account. Excluding these patients from clinical trials investigating VTE will severely limit the clinical utility of the findings and remove a potentially high risk group from the studies. Future, appropriately powered studies could prospectively stratify for the use of antiplatelet drugs to allow subgroup analyses of patients on these drugs compared with patients not taking them.

Membrane-bound TM is primarily responsible for protein C activation and so analysis of circulating soluble TM may not appropriately identify the importance and influence of this pathway on thrombin activity. Further research should focus on measuring plasma concentrations of circulating activated protein C as this directly interacts with thrombin. Reliable assays for activated protein C are still to be developed and/or validated.
Patients were screened for PE using CTPA and for limb DVT/SVT using compression ultrasound, the current gold standard radiological imaging tests. Despite the design of the study, the number of patients who developed VTE may still have been under reported as a result of appropriate ethical limitations on the use of CT scans. We did not screen patients for PE/abdominal DVT if they did not require a staging CT scan as part of routine clinical practice. This approach minimised radiation exposure to patients and volunteers without cancer and was an important factor in reducing the number of CT scans performed on patients undergoing adjuvant therapy. Routine screening for cerebral vein/sinus thrombosis with CT was not undertaken. Most patients underwent routine ultrasound scans of the lower limbs but not of the upper limbs, which were only imaged if there was clinical concern for thrombosis. Volunteers without cancer did not undergo ultrasound scanning due to resource limitations as a result of the earthquake.

Line (CVAD) associated DVTs were included with other DVTs in our study analyses. In future appropriately powered studies, these could be analysed separately to assess the relative effects of the line, the cancer and chemotherapy in cancer patients.

Not all study patients went on to receive chemotherapy as this could not be predicted prior to recruitment, clinical assessment and diagnostic radiological imaging but this reflects the normal clinical scenario in this regard. Many patients left the study after consent and baseline assessment because they subsequently required radiation therapy, hormonal therapy, surgery and/or did not undertake chemotherapy during the study period (n=37). Some patients withdrew consent (n=11) due to deterioration in health or inability to attend study assessments or were lost to follow up (n=20) because they transferred care to another cancer centre and could not be followed up in Christchurch. This recruitment approach, however, ensured the collection of robust prospective clinical data that minimised the confounding impacts of other cancer treatment modalities.
5.14 Conclusion

This prospective study has highlighted that cancer patients are at high risk of VTE development, including ambulant patients on chemotherapy, confirming previously published, predominantly retrospective, database analyses. The VTE may be present prior to commencement of chemotherapy and potentially complicate treatment or be exacerbated by the treatment. Many of these events are clinically unsuspected prior to the diagnosis being made and may be associated with significant clot burden. Poorer survival was seen in patients with VTE (especially those diagnosed with lobar PE or DVT of the lower limb and abdomen) compared with patients without VTE. Screening cancer patients for VTE is currently the only way of accurately diagnosing VTE but this is neither cost effective nor feasible in resource-constrained health systems.

It could be argued that we have not yet found appropriate prevention nor treatment approaches for cancer-associated venous thrombosis. Future prospective, double-blind, randomised studies will be required investigating novel anticoagulants targeting factor Xa and thrombin as well as investigating the exogenous administration of commercially produced endogenous anticoagulants such as antithrombin to elucidate their efficacy.

Thromboprophylaxis studies should screen cancer patients for unsuspected VTE at baseline as these patients should be entered into a separate study investigating their management. Many clinicians question the clinical relevance of these thrombotic events, however, this study has shown that patients diagnosed with unsuspected VTE at baseline exhibit poorer survival than those without VTE, despite anticoagulation, and that the clot burden can be significant, challenging the assumption that unsuspected VTE tend to be small and unlikely to impact on patient outcome. In contrast, patients diagnosed with unsuspected VTE on chemotherapy treatment after baseline assessment, did not exhibit poorer survival compared with chemotherapy patients who did not develop VTE at all. This remains to be explained, but these patients were treated with anticoagulation which may have been effective in managing this cohort, suggesting that the pathophysiological mechanisms for VTE development may be different when chemotherapy is administered to a cancer patient. Sample
sizes were, however, small and so validation of these findings will be required in larger populations.

Current RAMs fail to identify those at highest risk, though identify the 10-15% at low risk. The Christchurch population-adapted scores appeared to improve the original models but this was not clinically meaningful in identifying patients at high risk for developing VTE. The CHCH PC Khorana/Ay score appeared to be most reliable in identifying patients at low risk. All parameters within this score are easily and widely measured around the world apart from sP-selectin. This study, however, suggests that sP-selectin could be removed from the model as its concentration closely correlates with D-dimer concentrations which are also measured in the model. This is in contrast to previous publications and requires further study (as described in sections 1.57 and 1.58, pages 145-149). Larger studies may also identify improved RAMs from mathematical models of novel biomarkers.

The D-dimer, Ang-2:sTie-2 ratio and ECOG performance status appear to be markers of thrombosis risk and/or diagnosis in cancer patients undergoing chemotherapy. Further work on these markers will be required in order to validate their utility in clinical practice, as results from this study are purely hypothesis generating. An appropriately powered, larger, prospective, multi-centred clinical study would be the next logical step in validating these findings.

This study should screen patients, at baseline assessment, for PE and DVT and record the presence of relevant risk factors for VTE to allow analysis and adjustment for multiple comparisons such as ethnicity, antiplatelet therapies, chemotherapy regimen, cancer stage and performance status. It should also screen for VTE at predefined timepoints enabling detection of unsuspected VTE and more accurate VTE rates, and it would be able to follow patients for outcomes such as survival and VTE recurrence rates.

The TGA has, so far, indicated a procoagulant state in cancer patients, but has not shown promise in this study in aiding either VTE prediction or diagnosis. It will be important for other studies to validate these findings with larger sample sizes. It would be interesting to relate plasma concentrations of activated protein
C to the TGA as has been attempted in this study with antithrombin and plasma thrombomodulin.

The high cumulative incidence of VTE in cancer patients on chemotherapy supports the need for further prospective studies. In the meantime, clinicians must maintain a high level of suspicion for VTE development and a low threshold for investigating VTE.
Chapter 6
Appendices
Appendix 1- Ethics approval letter for study

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Email: uppersouthb_ethicscommittee@moh.govt.nz

12 April 2011

Dr Anthony Rhaman
11A Finnsarby Place
Sumner
Christchurch 8081

Dear Dr Rhaman

Re: Ethics ref: URB/11/02/005 (please quote in all correspondence)
Study title: Venous thromboembolism in cancer patients about to commence chemotherapy
Investigators: Dr Anthony Rhaman, Associate Professor Bridget Robinson, Dr Sarah Gunningham, Dr Mark P Smith

This study was given ethical approval by the Upper South B Regional Ethics Committee on 12 April 2011. A list of members of the Committee is attached.

Approved Documents
— Consent Form for patients with cancer commencing chemotherapy version 2 dated 14 March 2011
— Consent Form for Healthy volunteers version 2 dated 14 March 2011
— Information Sheet for Health Volunteers version dated 11 March 2011
— Information Sheet for Cancer Control version dated 14 March 2011
— Information Sheet for patients with cancer commencing chemotherapy version dated 14 March 2011
— Screening document for volunteers without cancer version 1 dated 14 March 2011
— Screening document for patients with cancer commencing chemotherapy version 1 dated 14 March 2011
— Screening document for patients with cancer not commencing chemotherapy version 1 dated 14 March 2011

This approval is valid until 1 December 2014, provided that Annual Progress Reports are submitted (see below).

Access to ACC
Appendix 2- Patient information sheets and consent forms

7.1 Information sheet for chemotherapy patients

Canterbury DHB
District Health Board
Te Poari Hauora o Waitaha

VENOUS THROMBOEMBOLISM IN CANCER PATIENTS COMMENCING CHEMOTHERAPY

INFORMATION SHEET FOR CANCER PATIENTS DUE TO UNDERGO CHEMOTHERAPY

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. You may choose not to take part in the study without giving any reason, and there will be no disadvantage to you of any kind. We thank you for considering our request. This information sheet contains 5 pages. Please ensure you have all 5 pages.

This study has received ethical approval from the Upper South B Regional Ethics Committee, ethics reference number URB/11/02/005.

What is the Aim of the Project?
This study is being undertaken by Dr Anthony Rahman, a trainee in Medical Oncology as part of his PhD research project.

Venous thromboembolism (VTE) describes the development of blood clots in the venous system of the body. These, most commonly develop in the legs (deep vein thrombosis or DVT) and in the lungs (pulmonary embolism or PE.) The risk of developing VTE is much higher in people with cancer than in healthy individuals and this risk increases further when cancer patients are treated with chemotherapy. VTE can be life-threatening and has been ranked as the second biggest killer of cancer patients worldwide. This is partly due to the fact that VTE can be difficult to recognise and many patients may not show any outward signs to suggest they have developed VTE.

This project will investigate the number of patients affected by PE and DVT at 3 time points during their chemotherapy treatment at the Christchurch Hospital Oncology or Haematology departments. It aims to develop a clinical tool that will recognise which cancer patients are at high risk of developing VTE. This could then be used to guide doctors in giving medication to prevent the development of VTE.
Why are you being asked to be involved?
Cancer patients newly referred to the Oncology or Haematology departments at Christchurch Hospital for chemotherapy treatment will be recruited to this study. Patients of all ethnicities and cultures are encouraged to participate.

Inclusion criteria (must be “Yes”)
1. Newly referred ambulant patients with a histological/ cytological diagnosis of cancer
   OR
   ambulant cancer patients with recurrent or progressive disease about to commence chemotherapy/ chemoradiation.
2. Male or female aged 18 years or older.
3. Written (signed) informed consent.
4. Eligible for staging CT of the chest with contrast and ultrasound of the lower limbs
   OR
   eligible for ultrasound of the lower limbs only.

Exclusion criteria (must be “No”)
1. Patients on anticoagulation (heparins, factor Xa inhibitors and oral vitamin K antagonists) or treated with anticoagulation in the last 4 weeks.
2. Patients with a known clotting disorder or thrombophilia.
3. Pregnant or lactating women.
4. Previous chemotherapy in the last 3 months.
5. Previous radiation treatment in the last 3 months.
6. Surgery in the last 2 weeks (excluding insertion of central venous access device).
7. Hypersensitivity to contrast media for the CT scan (PE prevalence study) cohort.
8. Patients unable to comply with the protocol.

What will Participants be Asked to Do?
Should you agree to take part in this project, you will be asked to attend 3 assessments in Oncology outpatients, in addition to your usual care:

1. First appointment with Oncology/Haematology doctor.
2. 6 to 12 weeks into chemotherapy treatment or after first appointment.
3. 6 months after first appointment.

During each assessment you will undergo a 30 to 60 minute consultation with a doctor or nurse specialist, including discussion of your medical history, a clinical examination, an electrocardiogram (ECG) or tracing of your heart rhythm, and blood samples will be taken to look for factors/markers that may suggest the development of VTE. A small number of patients with a venous access device in place, will be asked to provide 3 extra blood samples (1 teaspoonful per sample) around the first dose of chemotherapy to look at the early effects of chemotherapy and medication on blood cells.

If you are found to have a PE or DVT is detected as a result this study, your doctor will decide whether this should be treated. If this is suspected during the course of your follow up, you may be asked to see the study team for an additional assessment.

We are aiming to recruit 500 cancer patients on chemotherapy treatment, who will each be asked to undergo up to 3 CT scans of the chest (see below for an explanation of this) to look for PE and 3 ultrasound scans to look for DVT in the lower limb at each of the 3 time points described above. Each CT scan will take approximately 40 minutes while ultrasound scans take only 20 minutes to perform. Due to unforeseen circumstances you may have to wait in the Radiology department for your CT and ultrasound scans. In patients with a peripherally inserted central catheter (PICC) line in place for chemotherapy administration an ultrasound scan of the arm will be performed to look for upper limb DVT.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

**What are the possible risks?**

**Blood Tests:** While rare, the risks of blood tests can include fainting and/or pain, bruising, swelling, or rarely infection where the needle is inserted.

**ECGs:** Some people may experience skin irritation from the ECG electrodes or pain when removing the electrodes.

**CT Scans:** CT Scans involve exposure to X-ray radiation. Although the amount of X-ray radiation exposure is higher than a typical X-ray, the risk of harmful effects from a single exam is very small. The dye that is injected into a vein for the scan is usually well tolerated. However, occasionally people feel dizzy or
Venous thromboembolism in cancer patients undertaking chemotherapy

What are the potential benefits?
There is no guarantee that you will receive any benefit from taking part in this study. By taking part in this study, you may contribute information about venous thromboembolism and benefit other patients in the future.

What Data or Information will be Collected and What Use will be Made of it?
All information collected from you will be confidential and securely stored in such a way that only individuals involved in the study will be able to gain access to it- Professor Bridget Robinson, Dr Sarah Cunningham, Associate Professor Chris Frampton, Dr Mark Smith, Dr Margaret Currie, nursing and clerical staff involved in the research and Dr Anthony Rahman.

The data will not be linked directly to your name as you will be assigned a unique study identification number to keep your information as confidential as possible. The clinical data will include your gender, age, ethnicity, cancer history (if relevant), medical and surgical history, weight, height, findings of your clinical examination, medications and the results of blood samples and scans analysed during the study period.

The data will be used to provide information on the prevalence and incidence of DVT and PE in the Christchurch cancer population and assess whether this increases on chemotherapy. Information provided will also be used to develop a predictive model evaluating a cancer patient’s risk of developing DVT and PE when about to commence chemotherapy. This model can then be applied to future studies into prevention of DVT and PE using anticoagulant (anti clotting) treatments.

At the end of the project any personal information or raw data on which the results of the project depend will be retained in secure storage for ten years, after which it will be destroyed. Blood and tissue samples are required to be disposed of by standard disposal methods (incineration) under the Healthcare Waste Management Standard NZS 4304:2002. You may choose to have your sample(s) disposed of with the appropriate karakia (blessing).
Costs: You will not be charged for taking part in this study. The office visits, physical examinations, and other procedures associated with the study will be at no cost to you.

Payment for taking part in the study: You will not be paid for taking part in this study.

Compensation: In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation, and Compensation Act 2001. ACC cover is not automatic, and your case will need to be assessed by ACC according to the provisions of the Injury Prevention, Rehabilitation, and Compensation Act 2001. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors, such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses, and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.

You are also advised to check whether participation in this study would affect any indemnity cover you have or are considering, such as medical insurance, life insurance and superannuation.

Voluntary participation and early withdrawal
Taking part in this research study is up to you. You may refuse to take part or you may stop participating in the study at any time for any reason. The study doctor may remove you from the study at any time for medical reasons or if you fail to follow the instructions given to you. The study could also be stopped by the Ethics Committee ahead of schedule. If you do not wish to take part, leave or are asked to leave the study early you will not lose any benefits that you would otherwise have and your present or future medical care will not be affected.

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Dr Anthony Rahman or Professor Bridget Robinson
Department of Medicine Department of Medicine
Phone 03 364 0020 (pager 8829) Phone 03 364 0361
7.2 Information sheet for volunteers without cancer

VENOUS THROMBOEMBOLISM IN CANCER PATIENTS
COMMENCING CHEMOTHERAPY

INFORMATION SHEET FOR
HEALTHY VOLUNTEERS WITHOUT CANCER

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request. This information sheet contains 5 pages. Please ensure you have all 5 pages.

This study has received ethical approval from the Upper South B Regional Ethics Committee, ethics reference number URB/11/02/005.

What is the Aim of the Project?
This study is being undertaken by Dr Anthony Rahman, a trainee in Medical Oncology as part of his PhD research project.

Venous thromboembolism (VTE) describes the development of blood clots in the venous system of the body. These, most commonly develop in the legs, termed deep vein thrombosis or DVT and in the lungs, termed pulmonary embolism or PE. The risk of developing VTE is much higher in people with cancer than in healthy individuals and this risk increases further when cancer patients are treated with chemotherapy. VTE can be life threatening and has been ranked as the second biggest killer of cancer patients worldwide. This is partly due to the fact that VTE can be difficult to recognise and many patients may not show any outward signs to suggest they have developed VTE.

This project will investigate the number of patients affected by PE and DVT at 3 time points during their chemotherapy treatment at the Christchurch Hospital Oncology or Haematology departments and also further develop a clinical tool to recognise which cancer patients are at high risk of developing VTE. This could then be used to guide doctors in giving medication to prevent the development of VTE.
**Why are you being asked to be involved?**

To assess the effect of chemotherapy on cancer patients we require 50 healthy volunteers without cancer to be a control group. We are looking for thirty-five patients aged 50-70 years and fifteen patients outside of this age group. All ethnicities and cultures are encouraged to participate. Our target group (cancer patients receiving chemotherapy) can then be compared with this control group to help us to recognise any differences that may be caused by chemotherapy agents.

**Inclusion criteria**

1. Individual is ambulant.
2. Male or female aged 18 years or older.
3. Written (signed) informed consent.

**Exclusion criteria**

1. Volunteers previously assessed and treated in the Oncology/Haematology service.
2. Patients on anticoagulation (heparins, factor Xa inhibitors and oral vitamin K antagonists) or treated with anticoagulation within the last 4 weeks.
3. Patients with a known clotting disorder or thrombophilia.
4. Pregnant or lactating women.
5. Previous chemotherapy.
7. Surgery in the last 2 weeks.
8. Previous malignancies within 10 years except in situ cervix or non-melanoma skin cancers.
9. Patients unable to comply with the protocol.

**What will Participants be Asked to Do?**

1. Should you agree to take part in this project, you will be asked to attend 1 assessment in Oncology outpatients.

During the assessment you will undergo a 30 to 60 minute consultation with a doctor or nurse specialist including discussion of your medical history, a clinical examination, an electrocardiogram (ECG) or tracing of your heart rhythm and blood samples will be taken to look for factors/markers that may suggest the development of VTE.

If you are found to have a PE or DVT as a result of this study, your doctor will decide whether this should be treated.
Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

**What are the possible risks?**

**Blood Tests:** The risks of blood tests include fainting and pain, bruising, swelling, or rarely infection where the needle is inserted.

**ECGs:** Skin irritation from the ECG electrodes or pain when removing the electrodes is a possible risk.

**What are the potential benefits?**

There is no guarantee that you will receive any benefit from taking part in this study. By taking part in this study, you may contribute information about venous thromboembolism and benefit other patients in the future.

**What Data or Information will be Collected and What Use will be Made of it?**

All information collected from you will be confidential and securely stored in such a way that only individuals involved in the study will be able to gain access to it: Professor Bridget Robinson, Dr Sarah Cunningham, Dr Margaret Currie, Associate Professor Chris Frampont, Dr Mark Smith, nursing and clerical staff involved in the research and Dr Anthony Rahman.

The data will not be linked directly to your name as you will be assigned a unique study identification number to keep your information as confidential as possible. The clinical data will include your gender, age, ethnicity, cancer history (if relevant), medical and surgical history, weight, height, findings of your clinical examination, medications and the results of blood samples and scans analysed during the study period.

The data will be used to provide information on the prevalence and incidence of DVT and PE in the Christchurch cancer population and assess whether this increases on chemotherapy. Information provided will also be used to develop a predictive model evaluating a cancer patient's risk of developing DVT and PE when about to commence chemotherapy. This model can then be applied to future studies into prevention of DVT and PE using anticoagulant treatments.

At the end of the project any personal information or raw data on which the results of the project depend will be retained in secure storage for ten years, after which it will be destroyed. Blood and tissue samples are required to be disposed of by standard disposal methods (incineration) under the Healthcare Waste Management
You may choose to have your sample(s) disposed of with the appropriate karakia (blessing).

**Costs:** You will not be charged for taking part in this study. The office visits, physical examinations, and other procedures associated with the study will be at no cost to you.

**Payment for taking part in the study:** You will not be paid for taking part in this study.

**Compensation:** In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation, and Compensation Act 2001. ACC cover is not automatic, and your case will need to be assessed by ACC according to the provisions of the Injury Prevention, Rehabilitation, and Compensation Act 2001. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors, such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses, and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators.

If you have any questions about ACC, contact your nearest ACC office or the investigator.

You are also advised to check whether participation in this study would affect any indemnity cover you have or are considering, such as medical insurance, life insurance and superannuation.

**Voluntary participation and early withdrawal**

Taking part in this research study is up to you. You may refuse to take part or you may stop participating in the study at any time for any reason. The study doctor may remove you from the study at any time for medical reasons or if you fail to follow the instructions given to you. The study could also be stopped by the Ethics Committee ahead of schedule. If you do not wish to take part, leave or are asked to leave the study early you will not lose any benefits that you would otherwise have and your present or future medical care will not be affected.
If you have any questions about our project, either now or in the future, please feel free to contact either:-

Dr Anthony Rahman or Professor Bridget Robinson
Department of Medicine Department of Medicine
Phone 03 364 0020 (pager 8829) Phone 03 364 0361
### 7.3 Consent form for chemotherapy patients

**VENOUS THROMBOEMBOLISM IN CANCER PATIENTS COMMENCING CHEMOTHERAPY**

**CONSENT FORM FOR PARTICIPANTS WITH CANCER WHO ARE ABOUT TO COMMENCE CHEMOTHERAPY**

<table>
<thead>
<tr>
<th>Language</th>
<th>Consent to have an interpreter</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Māori</td>
<td>E kainga au i tūtū tangata un teo</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cook Island Māori</td>
<td>Ka inanga ana i tūtū tangata un teo</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Fijian</td>
<td>Au gaeru ma dasi wakadewa vosa veli</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nuean</td>
<td>Fia manako au ke fakasoga e tana tagata fakahokohoko lomu</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sāmoan</td>
<td>Ou te manako hā i sā te fakamata upe</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tokelauan</td>
<td>Ko au e fakau ke fakarau te gagana Peleti ki na gagana e na motu o te Fakarau</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oku ou fiamau ha fakatenina</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*Other languages to be added following consultation with relevant communities.*

I have read and I understand the information sheet dated **14 March 2011** for volunteers taking part in the study designed to investigate venous thromboembolism in cancer patients commencing chemotherapy. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

I have had the opportunity to use written support or a friend to help me ask questions and understand the study.

I understand that taking part in this study is voluntary (my choice), and that I may withdraw from the study at any time, and that this will in no way affect my future continuing health care.

I have had this project explained to me by **[Name]**.

I understand that any information I give in this study is confidential and that no material that could identify me will be used in any reports on this study.

I understand the compensation provisions for this study.

I have had time to consider whether to take part in the study.
I know who to contact if I have any side effects from the study.
I know who to contact if I have any questions about this study.

<table>
<thead>
<tr>
<th>Consent Item</th>
<th>Yes</th>
<th>No</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I consent to the researchers storing a specimen of my blood for its later use as a part of this study or another research subject to ethical approval by a New Zealand-accredited ethics committee.</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I consent to blood samples being destroyed at the end of the study</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A kānaka is to be performed when blood samples are to be destroyed</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>I consent to blood samples being sent overseas for tests not available in New Zealand, performed in collaboration with New Zealand researchers</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>I agree to my GP or other current provider being informed of my participation in this study.</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

5. I (Full name) hereby consent to take part in this study

<table>
<thead>
<tr>
<th>Field</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>Signature</td>
<td></td>
</tr>
<tr>
<td>Full names of researchers</td>
<td></td>
</tr>
<tr>
<td>Contact phone number for researchers</td>
<td></td>
</tr>
<tr>
<td>Project explained by</td>
<td></td>
</tr>
<tr>
<td>Project role</td>
<td></td>
</tr>
<tr>
<td>Signature</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
</tbody>
</table>

Notes:
1. A copy of the consent form is to be retained by each participant and (in the case of patients) a copy is to be placed in the medical file.

Study: VTE in patients having chemotherapy v2 14 March 2011
### 7.4 Consent form for volunteers without cancer

**VENOUS THROMBOEMBOLISM IN CANCER PATIENTS COMMENCING CHEMOTHERAPY**

**CONSENT FORM FOR PARTICIPANTS WHO ARE NOT COMMENCING CHEMOTHERAPY AND HEALTHY VOLUNTEERS WITHOUT CANCER**

<table>
<thead>
<tr>
<th>Language</th>
<th>Consent for Volunteers who are not Commencing Chemotherapy and Healthy Volunteers without Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter</td>
</tr>
<tr>
<td>Deaf</td>
<td>I wish to have a NZ sign language interpreter</td>
</tr>
<tr>
<td>Māori</td>
<td>E hiahia ana ahau ki te tahia ka whaka Māori ka whaka pakeha kore</td>
</tr>
<tr>
<td>Cook Island Māori</td>
<td>Ke i manganu au i te tanga au reo</td>
</tr>
<tr>
<td>Fijian</td>
<td>Au gawerewa me dua e vakadewinga rosa vei au</td>
</tr>
<tr>
<td>Niuean</td>
<td>Fia manakia au ko fakaaega o taha tagata fakahohonoko kupu</td>
</tr>
<tr>
<td>Samoan</td>
<td>Ou te mana ko i i se ta'amatua upu</td>
</tr>
<tr>
<td>Tokelau</td>
<td>Ko ou a fofoaki he tino ko faalili te gagana Palenia ki nga gaganu e na motu o te Fafekea</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oka ou famalu ha fakatulitea</td>
</tr>
</tbody>
</table>

Other languages to be added following consultation with relevant communities.

I have read and I understand the information sheet dated ______ for volunteers taking part in the study designed to investigate venous thromboembolism in cancer patients commencing chemotherapy. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

I have had the opportunity to use whānau support or a friend to help me ask questions and understand the study.

I understand that taking part in this study is voluntary (my choice), and that I may withdraw from the study at any time, and this will in no way affect my future and continuing health care.

I have had this project explained to me by ____________________________

I understand that my participation in this study is confidential, and that no material that could identify me will be used in any reports on this study.

I understand the compensation provisions for this study.

I have had time to consider whether to take part in the study.

I know who to contact if I have any side effects from the study.

---

Study: VTE in patients having chemotherapy  
14 March 2011  

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420 Venous thromboembolism in cancer patients undertaking chemotherapy
I know who to contact if I have any questions about this study.

I consent to the researchers storing a specimen of my blood for its later use as a part of this study or other research subject to ethical approval by a New Zealand accredited ethics committee. Yes ☐ No ☐

OR

I consent to blood samples being destroyed at the end of the study. Yes ☐ No ☐

A karakia is to be performed when blood samples are to be destroyed. Yes ☐ No ☐

I consent to blood samples being sent overseas for tests not available in New Zealand, performed in collaboration with New Zealand researchers. Yes ☐ No ☐

I agree to my GP or other current provider being informed of my Participation in this study. Yes ☐ No ☐

5. I (full name) hereby consent to take part in this study

| Date: | 
| Signature: | 
| Full names of researchers: | 
| Contact phone number for researchers: | 
| Project explained by: | 
| Project role: | 
| Signature: | 
| Date: | 

Notes:

1. A copy of the consent form is to be retained by each participant and (in the case of patients) a copy is to be placed in the medical file.

Study: VTE in patients having chemotherapy_v2 11 March 2011
Appendix 3- Clinical assessment sheets

8.1 Baseline visit clinical assessment sheet

Canterbury DHB
District Health Board
To Poari Hauora o Waitaha

Venous Thromboembolism in Patients about to Commence Chemotherapy

Patient Questionnaire (to be completed with Health Professional)

Baseline (Visit 1)  Date:

1. DEMOGRAPHIC DATA

Name: ........................................................................................................

NHI Number: ...........................................................................................

Research ID Number: ..............................................................................

Date of Birth: ..........................................................................................

Sex: □ Male  □ Female

Ethnicity  □ NZ European  □ Maori  □ Pacific Peoples  □

 □ Middle Eastern  □ Asian  □ Latin American  □

 □ African  □ Other

Date of First Specialist Assessment: ..........................................................

Date of consent to study: ...........................................................................

2. STUDY PARTICIPATION

Patient continuing on Venous Thromboembolism Study  Yes □  No □

If no, complete the section below

Patient Withdrawn Consent  Yes □  No □

Pulmonary Embolism (PE) diagnosed  Yes □  No □  Date ............

Deep Vein Thrombosis (DVT) diagnosed  Yes □  No □  Date ............

Lost to Follow up:  Yes □  No □

Death:  Yes □  No □  Date ............

Cause of Death: ..........................................................................................

Other:  Yes □  No □

#TE Study Patient Questionnaire – Baseline up to 13 April 2012
ETHICS REF: 1014/01/2001FS

422 Venous thromboembolism in cancer patients undertaking chemotherapy
3. CANCER

Primary cancer: .................................................................

Cell Type: ..............................................................................

Grade of cancer: .................................................................

Stage of cancer (I – IV): please state if clinical or pathological ........................................

TNM/FIGO Stage: ......................................................................

Recent Cancer-Related Surgery ≤ 6/52  Yes ☐  No ☐

Name of Surgery: ......................................................................

Purpose of Surgery  Curative ☐  Palliative ☐  Other ☐

Date of Surgery: ........................................................................

Intrapertioneal port inserted  Yes ☐  No ☐

Date of port insertion: ...........................................................

ECOG Performance status (0 – 4): Refer to attached sheet ..............................................

Karnofsky Performance status (0 – 100): Refer to attached sheet .......................................
<table>
<thead>
<tr>
<th><strong>History of Arrhythmia</strong></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History of Hypertension</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of Hypercholesterolemia</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of chronic venous insufficiency:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of venous thromboembolism:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of arterial thrombosis:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of diabetes mellitus:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of chronic obstructive pulmonary disease:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Recent bed rest ≥ 48 hours in last week:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Non Stop Travel ≥ 6 hours in last 2 weeks:</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**Date of Non Stop Travel:**

---

**Length (Hrs) of Non Stop Travel:**

| **Recent trauma/Collapse ≤ 4 weeks:** | Yes | No |
| **Leg swelling ≤ 4 weeks:** | Yes | No |
| **Leg pain ≤ 4 weeks:** | Yes | No |
| **Arm swelling ≤ 4 weeks:** | Yes | No |
| **Arm pain ≤ 4 weeks:** | Yes | No |
| **History of connective tissue disorder:** | Yes | No |
| **Blood transfusion(s) in last 4 weeks:** | Yes | No |
| **Platelet transfusion in last 4 weeks:** | Yes | No |
| **Hospital Admission > 1 day over last 4 weeks:** | Yes | No |

---

**5. FAMILY HISTORY**

| **Family history of Venous Thromboembolism** | Yes | No |
| **Family history of bleeding/coagulation disorders:** | Yes | No |

---

**6. SURGICAL HISTORY (not related to section 2)**

| **Recent non-cancer surgery within past 6 weeks** | Yes | No |
| **Previous Surgery within the last 2 years** | Yes | No |

**Name and Date of Surgery:**

---

**VTE Study: Patient Questionnaire – Baseline v6 13 April 2012**

---

424 Venous thromboembolism in cancer patients undertaking chemotherapy
7. MEDICATION HISTORY
(Especially important to list if on aspirin, NSAIDs, oral contraceptive pill, HRT, statin or proton pump inhibitor, hemopoietic agents, anticoagulants, steroids, tamoxifen or aromatase inhibitors).

**Medications**

1. Name: ____________________________
   Dosage: ____________________________ Frequency: ____________________________
   When commenced: < 1/12 □ 1 – 3/12 □ > 3/12 □

2. Name: ____________________________
   Dosage: ____________________________ Frequency: ____________________________
   When commenced: < 1/12 □ 1 – 3/12 □ > 3/12 □

3. Name: ____________________________
   Dosage: ____________________________ Frequency: ____________________________
   When commenced: < 1/12 □ 1 – 3/12 □ > 3/12 □

4. Name: ____________________________
   Dosage: ____________________________ Frequency: ____________________________
   When commenced: < 1/12 □ 1 – 3/12 □ > 3/12 □

5. Name: ____________________________
   Dosage: ____________________________ Frequency: ____________________________
   When commenced: < 1/12 □ 1 – 3/12 □ > 3/12 □

(if more than 5 medications, please write on separate sheet as provided)

Anticoagulation in the last 4 weeks: Yes □ No □
Taking exogenous oestrogen/progesterone: Yes □ No □

Drug allergies: ____________________________
8. SOCIAL HISTORY
Smoking Status: Current □ Ex-smoker □ Never □
Smoking Method: Tailormade □ Roll Your Own □ Cigar □ Pipe □
Number of packs per day: .................................................................
If Roll Your Own or Pipe Smoker - Number of grams per week: ........................................
Alcohol Consumption - Units per week: .........................................................
Current or recent pregnancy in last 4/52: Yes □ No □
Lives in own home: Yes □ No □
Lives in Rest home: Yes □ No □
Lives in Respite Care: Yes □ No □
How long in Respite Care: < 1/12 □ 1/12 □

9. CHEMOTHERAPY
Chemotherapy Drugs

Chemotherapy Commencement Date: ..............................................................
On granulocyte colony stimulating factor (G-CSF) Yes □ No □
On erythropoietic agent Yes □ No □

10. CVAD (CENTRAL VENOUS ACCESS DEVICE)
PICC: Yes □ No □
Hickman: Yes □ No □
Portacath: Yes □ No □
Date of Insertion: .........................................................................................
If PICC Right □ Left □
11. EXAMINATION AND INVESTIGATION FINDINGS

Temperature: ...................... °C

Pulse Rate: Per minute: ......................... Regular ☐ Irregular ☐

Lying Blood Pressure: .................................................................

Standing Blood Pressure: ...........................................................

Respiratory Rate: Per minute: ...................................................

Oxygen saturations on room air at rest: .........................................%

Heart Sounds: Normal ☐ Abnormal ☐

Comments: ...........................................................................................

Raised Jugular Venous Pressure: Yes ☐ No ☐

Right Ventricular Heave: Yes ☐ No ☐

Clear Chest to Auscultation: Yes ☐ No ☐

Comments: ...........................................................................................

Abdomen soft: Yes ☐ No ☐

Abdomen non-tender: Yes ☐ No ☐

Palpable abdominal mass/ organomegaly: Yes ☐ No ☐

Lower Limb Examination

Varicose Veins: Yes ☐ No ☐

Right ☐ Left ☐ Bilateral ☐

Lower Limb Edema: Yes ☐ No ☐

Right ☐ Left ☐ Bilateral ☐

Leg Ulcers Present: Yes ☐ No ☐

Right ☐ Left ☐ Bilateral ☐

Leg pain on palpation: Yes ☐ No ☐

Right ☐ Left ☐ Bilateral ☐
Lower Limb Diameter Measurements
Left - 5 cm below Tibial Tuberosity (cm) .................................................................
Left - 25 cm above Tibial Tuberosity (cm) .............................................................
Right - 5 cm below Tibial Tuberosity (cm) .............................................................
Right - 25 cm above Tibial Tuberosity (cm) ..........................................................

Upper Limb Diameter Measurements
Left - 5 cm below Medial Epicondyle (cm) ..........................................................
Left - 10 cm above Medial Epicondyle (cm) ..........................................................
Right - 5 cm below Medial Epicondyle (cm) ..........................................................
Right - 10 cm above Medial Epicondyle (cm) ..........................................................

Graded Compression Stockings: Yes [ ] No [ ]
If yes, number of days worn (upper or lower limb) ..............................................
Height .................................................................................................................. cm
Weight ............................................................................................................. kg
Body Mass Index ...............................................................................................  

12. BLOODS
Canterbury Health Laboratories (CHL) Bloods
Haemoglobin ........................................................................................................
Leukocyte Count .................................................................................................
Platelet Count .....................................................................................................
D-Dimer ...............................................................................................................  
CRP .....................................................................................................................
Thrombin Generation Assay ..............................................................................
Pregnancy (Serum Beta HCG): Positive [ ] Negative [ ]
Raised due to Germ Cell Tumour [ ] N/A [ ]
University of Otago MacKenzie Cancer Research Laboratory Blends

Tissue Factor: Date frozen: Time frozen:
Ang 1: Date frozen: Time frozen:
Ang 2: Date frozen: Time frozen:
P-Selectin: Date frozen: Time frozen:
Comments: Date frozen: Time frozen:

13. ECG

Date: 

ECG Normal: Yes: No
Rate ≥ 100 /min: Yes: No
Sinus Rhythm: Yes: No
Newly Diagnosed Atrial Fibrillation: Yes: No
Right Bundle Branch Block: Yes: No
Right Axis Deviation: Yes: No
ST changes: Yes: No
Right ventricular hypertrophy: Yes: No

14. IMAGING

Pleural effusion on chest imaging: Yes: No: N/A
Pulmonary atelectasis on chest imaging: Yes: No: N/A
Rounded heart shadow on chest imaging: Yes: No: N/A
PE seen on CTPA: Date: Yes: No: N/A
DVT seen on USS: Date: Yes: No: N/A

Health Professional Signature: 
Date Questionnaire Completed: 

VTE Study, Patient Questionnaire – Baseline v6 13 April 2012
Ethics Ref: UR28/1/02/009

Venous thromboembolism in cancer patients undertaking chemotherapy
8.2 Visit 2 clinical assessment sheet (within 100 days follow up)

Venous thromboembolism in Patients about to Commence Chemotherapy

Patient Questionnaire (to be completed with Health Professional)

Visit 2  Date:

1. DEMOGRAPHIC DATA

Name: .................................................................
NHI Number: ................................................................
Research ID Number: ..................................................

2. STUDY PARTICIPATION

Patient continuing on Venous Thromboembolism Study  Yes [ ]  No [ ]
If no, complete the section below

Patient Withdrawn Consent  Yes [ ]  No [ ]
Pulmonary Embolism (PE) diagnosed  Yes [ ]  No [ ]  Date: .............
Deep Vein Thrombosis (DVT) diagnosed  Yes [ ]  No [ ]  Date: .............
Lost to Follow up:  Yes [ ]  No [ ]
Death:  Yes [ ]  No [ ]  Date: .............
Cause of Death: ................................................................
Other:  Yes [ ]  No [ ]
Comments: ................................................................

3. CANCER

Primary cancer: ..............................................................
Cell Type: ................................................................
Stage of cancer (I - IV): .............................................
TNM Stage: ................................................................

Has patient upstaged since last assessment?  Yes [ ]  No [ ]
Recent Cancer-Related Surgery ± 6 months:  Yes [ ]  No [ ]
Name of Surgery: ........................................................

VTE Study Patient Questionnaire - Visit 2 v4 29 April 2012
Ethics Ref: DHE/10/2002
### 4. SYMPTOMS AND MEDICAL HISTORY

Since last assessment, have any of the following occurred?

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoptysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breathlessness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudden Onset</td>
<td></td>
<td></td>
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<tr>
<td>Gradual Onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest Pain</td>
<td></td>
<td></td>
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<tr>
<td>Pleuritic Chest Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td></td>
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<tr>
<td>Stroke / Transient Ischaemic Attack</td>
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<tr>
<td>Cardiac Failure</td>
<td></td>
<td></td>
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<tr>
<td>Bleeding / coagulation disorders</td>
<td></td>
<td></td>
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<tr>
<td>Varicose veins</td>
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</tr>
<tr>
<td>Arrhythmia</td>
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<tr>
<td>Hypertension</td>
<td></td>
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<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic venous insufficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td></td>
<td></td>
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<tr>
<td>Arterial thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
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<tr>
<td>Chronic obstructive pulmonary disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent bed rest &gt; 48 hours in last week</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 5. FAMILY HISTORY

Since last assessment, have any of the following occurred?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of Venous Thromboembolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of bleeding/coagulation disorders</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 6. SURGICAL HISTORY (not related to section 2)

Since Baseline, has the following occurred?

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cancer surgery within past 6 weeks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Name and Date of Surgery:  

<table>
<thead>
<tr>
<th>Information</th>
<th></th>
</tr>
</thead>
</table>

### 7. MEDICATION HISTORY

(especially important to list if on aspirin, NSAIDs, oral contraceptive pill, HRT, statin or thienopane acid, hormone-synthetic agents, anticoagulants, steroids, tamoxifen or aromatase inhibitors)

**Medications**

1. Name:  

**Dosage:**  

**Frequency:**
<table>
<thead>
<tr>
<th>Name</th>
<th>When commenced:</th>
<th>New Medication</th>
<th>Dose Change</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 1/12</td>
<td>No</td>
<td>Yes</td>
<td>1 - 3/12</td>
</tr>
<tr>
<td></td>
<td>1 - 3/12</td>
<td>No</td>
<td>No</td>
<td>&gt; 3/12</td>
</tr>
<tr>
<td></td>
<td>&gt; 3/12</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
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<td>1 - 3/12</td>
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<td>No</td>
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</tr>
<tr>
<td></td>
<td>&gt; 3/12</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
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<th>Dose Change</th>
<th>Frequency</th>
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<td>Yes</td>
<td>1 - 3/12</td>
</tr>
<tr>
<td></td>
<td>1 - 3/12</td>
<td>No</td>
<td>No</td>
<td>&gt; 3/12</td>
</tr>
<tr>
<td></td>
<td>&gt; 3/12</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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</tr>
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<tr>
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<td>&gt; 3/12</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Name</th>
<th>When commenced:</th>
<th>New Medication</th>
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<th>Frequency</th>
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<tbody>
<tr>
<td></td>
<td>&lt; 1/12</td>
<td>No</td>
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</tr>
<tr>
<td></td>
<td>1 - 3/12</td>
<td>No</td>
<td>No</td>
<td>&gt; 3/12</td>
</tr>
<tr>
<td></td>
<td>&gt; 3/12</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Venous thromboembolism in cancer patients undertaking chemotherapy
8. SOCIAL HISTORY

Since last assessment, have there been any changes to your social history?

Yes [ ]  No [ ]  If yes, complete this section

Smoking Status:  Current [ ]  Ex-smoker [ ]  Never [ ]

Smoking Method:  Tailormade [ ]  Roll Your Own [ ]  Cigar [ ]  Pipe [ ]

Number of packs per day: [ ]

If Roll Your Own or Pipe Smoker - Number of grams per week: [ ]

Alcohol Consumption - Units per week: [ ]

Current or recent pregnancy in last 4/52:  Yes [ ]  No [ ]

Lives in own home:  Yes [ ]  No [ ]

Lives in Rest home:  Yes [ ]  No [ ]

Lives in Respite Care:  Yes [ ]  No [ ]

How long in Respite Care:  < 1/12 [ ]  > 1/12 [ ]

9. CHEMOTHERAPY

Since last assessment, has there been any change to your chemotherapy drugs?

Yes [ ]  No [ ]

If yes, complete this section

(a) Stopped Chemotherapy:  Yes [ ]  No [ ]

(b) Changed Chemotherapy Regimen:  Yes [ ]  No [ ]

(c) Other: [ ]

Chemotherapy Drugs: [ ]
10. CVAD (CENTRAL VENOUS ACCESS DEVICE)
Since last assessment, has your original CVAD remained in situ? Yes ☐ No ☐
If no, please show reason for removal.
Infection ☐
Line blockage/No Blood Return ☐
Deep Vein Thrombosis ☐
Patient Preference ☐
Other ☐
Has your CVAD been replaced? Yes ☐ No ☐
If yes, was your CVAD replaced with any of the devices listed below?
PICC: Yes ☐ No ☐
Hickman: Yes ☐ No ☐
Portacath: Yes ☐ No ☐
Date of Insertion: ________________________________
If your CVAD is a PICC, please indicate insertion in right or left arm
Right Arm ☐ Left Arm ☐

11. EXAMINATION AND INVESTIGATION FINDINGS
Temperature: __________ °C
Pulse Rate: Per minute: __________ Regular ☐ Irregular ☐
Lying Blood Pressure: ________________________________
Standing Blood Pressure: ________________________________
Respiratory Rate: Per minute: __________
Oxygen saturations on room air at rest: __________ %
Heart Sounds: Normal ☐ Abnormal ☐
Comments: ____________________________________________
<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raised Jugular Veinous Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Ventricular Heave</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear Chest to Auscultation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen soft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen non-tender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpable abdominal mass/ organomegaly</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lower Limb Examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicose Veins:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Limb Oedema:</td>
<td></td>
<td></td>
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<tr>
<td>Right</td>
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<td></td>
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<tr>
<td>Left</td>
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<tr>
<td>Bilateral</td>
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<td></td>
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<tr>
<td>Leg Ulcers Present:</td>
<td></td>
<td></td>
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<tr>
<td>Right</td>
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<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg pain on palpation:</td>
<td></td>
<td></td>
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<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lower Limb Diameter Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left – 5 cm below Tibial Tuberosity (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left – 25 cm above Tibial Tuberosity (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right – 5 cm below Tibial Tuberosity (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right – 25 cm above Tibial Tuberosity (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Upper Limb Diameter Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left – 5 cm below Medial Epicondyle (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left – 10 cm above Medial Epicondyle (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right – 5 cm below Medial Epicondyle (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right – 10 cm above Medial Epicondyle (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graded Compression Stockings:</td>
<td>Yes [ ] No [ ]</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>If yes, number of days worn:</td>
<td>(upper or lower limb)</td>
<td></td>
</tr>
<tr>
<td>Height:</td>
<td>cm</td>
<td></td>
</tr>
<tr>
<td>Weight:</td>
<td>kg</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 12. BLOODS

#### Canterbury Health Laboratories (CHL) Bloods
- Haemoglobin: ........................................
- Leukocyte Count: ...................................
- Platelet Count: ....................................
- D-Dimer: ...........................................
- CRP: ................................................
- Thrombin Generation Assay:  
- Pregnancy (Serum Beta HCG): Pos[ ] Neg[ ]
- Raised due to Germ Cell Tumour [ ] NA [ ]

#### University of Otago MacKenzie Cancer Research Laboratory Bloods
- Tissue Factor: Date frozen: Time frozen:  
- Ang 1: Date frozen: Time frozen:  
- Ang 2: Date frozen: Time frozen:  
- P-Selectin: Date frozen: Time frozen:  
- Comments: ..............................................

### 13. ECG

#### Date: ..............................................
- ECG Normal: Yes [ ] No [ ]
- Rate: ≥ 100 / min: Yes [ ] No [ ]
- Sinus Rhythm: Yes [ ] No [ ]
- Newly Diagnosed Atrial Fibrillation: Yes [ ] No [ ]
- Right Bundle Branch Block: Yes [ ] No [ ]
### 14. IMAGING

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural effusion on chest imaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate-like atelectasis on chest imaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raised hemidiaphragm on chest imaging</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PE seen on CTPA 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DVT seen on USS 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Health Professional Signature: .................................................................

Date Questionnaire Completed: .................................................................
### 8.3 Visit 3 clinical assessment sheet (beyond 100 days follow up)

**Canterbury DHB**

**District Health Board**

---

**Venous Thromboembolism in Patients about to Commence Chemotherapy**

**Patient Questionnaire (to be completed with Health Professional)**

<table>
<thead>
<tr>
<th>Visit</th>
<th>Date</th>
</tr>
</thead>
</table>

#### 1. DEMOGRAPHIC DATA

- Name:  
- NHL Number:  
- Research ID Number:  

#### 2. STUDY PARTICIPATION

Patient continuing on Venous Thromboembolism Study:  

- Yes  
- No  

If no, complete the section below:

Patient withdrew Consent:  

- Yes  
- No  

Pulmonary Embolism (PE) diagnosed:  

- Yes  
- No  

Date:  

Deep Vein Thrombosis (DVT) diagnosed:  

- Yes  
- No  

Date:  

Lost to follow up:  

- Yes  
- No  

Death:  

- Yes  
- No  

Date:  

Cause of Death:  

- Other  

Comments:  

#### 3. CANCER

Primary cancer:  

Cell Type:  

Stage of cancer (I-IV):  

TNM Stage:  

Has patient upstaged since last assessment?  

- Yes  
- No  

Recent Cancer-Related Surgery ≤ 6 /52:  

- Yes  
- No  

Name of Surgery:  

---

*VTE Study Patient Questionnaire – Visit 3 v4 13 April 2012*

*Ethics Ref: URB11/02005*
### 4. SYMPTOMS AND MEDICAL HISTORY

Since last assessment, have any of the following occurred?

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoptysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breathlessness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If yes - Sudden Onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gradual Onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleuritic Chest Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke / Transient Ischaemic Attack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding/coagulation disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicose veins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhythmia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic venous insufficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent bed rest &gt;= 48 hours in last week</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Venous thromboembolism in cancer patients undertaking chemotherapy

5. FAMILY HISTORY
Since last assessment, have any of the following occurred?
Family history of Venous Thromboembolism  Yes ❑ No ❑
Family history of bleeding/coagulation disorders: Yes ❑ No ❑

6. SURGICAL HISTORY (not related to section 2)
Since Baseline, has the following occurred?
Non – cancer surgery within past 6 weeks  Yes ❑ No ❑

Name and Date of Surgery:........................................................................................................
........................................................................................................................................
........................................................................................................................................

7. MEDICATION HISTORY
(especially important to list if on aspirin, NSAIDs, oral contraceptive pill, HRT, statin or tranexamic acid, 
heparinoid agents, anticoagulants, steroids, immunosuppressors or aromatase inhibitors.)

Medications
1. Name:.....................................................................................................................

Dosage: .................................................. Frequency: .......................................
<table>
<thead>
<tr>
<th>When commenced:</th>
<th>&lt; 1/12</th>
<th>1 - 3/12</th>
<th>&gt; 3/12</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Medication</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Dose Change</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Name:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosage..........</td>
<td></td>
<td>Frequency:</td>
<td></td>
</tr>
<tr>
<td>When commenced:</td>
<td>&lt; 1/12</td>
<td>1 - 3/12</td>
<td>&gt; 3/12</td>
</tr>
<tr>
<td>New Medication</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Dose Change</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Name:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosage..........</td>
<td></td>
<td>Frequency:</td>
<td></td>
</tr>
<tr>
<td>When commenced:</td>
<td>&lt; 1/12</td>
<td>1 - 3/12</td>
<td>&gt; 3/12</td>
</tr>
<tr>
<td>New Medication</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Dose Change</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Name:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosage..........</td>
<td></td>
<td>Frequency:</td>
<td></td>
</tr>
<tr>
<td>When commenced:</td>
<td>&lt; 1/12</td>
<td>1 - 3/12</td>
<td>&gt; 3/12</td>
</tr>
<tr>
<td>New Medication</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Dose Change</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>
Venous thromboembolism in cancer patients undertaking chemotherapy

8. SOCIAL HISTORY
Since last assessment, have there been any changes to your social history?
Yes ☐ No ☐ If yes, complete this section

Smoking Status: Current ☐ Ex-smoker ☐ Never ☐

Smoking Method: Tailormade ☐ Roll Your Own ☐ Cigar ☐ Pipe ☐

Number of packs per day:..............................................................
If Roll Your Own or Pipe Smoker - Number of grams per week:...

Alcohol Consumption - Units per week:...........................................

Current or recent pregnancy in last 6/52: Yes ☐ No ☐
Lives in own home: Yes ☐ No ☐
Lives in Rest home: Yes ☐ No ☐
Lives in Respite Care: Yes ☐ No ☐
How long in Respite Care: < 1/12 ☐ > 1/12 ☐

9. CHEMOTHERAPY
Since last assessment, has there been any change to your chemotherapy drugs?
Yes ☐ No ☐
If yes, complete this section

(a). Stopped Chemotherapy: Yes ☐ No ☐
(b). Changed Chemotherapy Regimen: Yes ☐ No ☐
(c). Other:..................................................................................

Chemotherapy Drugs
10. CVAD (CENTRAL VENOUS ACCESS DEVICE)

Since last assessment, has your original CVAD remained in situ? Yes ☐ No ☐

If no, please show reason for removal:
- Infection ☐
- Line blockage/ No Blood Return ☐
- Deep Vein Thrombosis ☐
- Patient Preference ☐
- Other ☐

Has your CVAD been replaced? Yes ☐ No ☐

If yes, was your CVAD replaced with any of the devices listed below?
- PICC: Yes ☐ No ☐
- Hickman: Yes ☐ No ☐
- Portacath: Yes ☐ No ☐

Date of Insertion: ........................................

If your CVAD is a PICC, please indicate insertion in right or left arm
- Right Arm ☐
- Left Arm ☐

11. EXAMINATION AND INVESTIGATION FINDINGS

Temperature: ..................... °C
Pulse Rate: Per minute ......................... Regular ☐ Irregular ☐
Lying Blood Pressure: ........................................
Standing Blood Pressure: .................................
Respiratory Rate: Per minute .................................
Oxygen saturations on room air at rest: ........................................ %
Heart Sounds: Normal ☐ Abnormal ☐

Comments: ........................................
<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raised Jugular Venous Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Ventricular Heave</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear Chest to Auscultation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comments**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen soft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen non-tender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpable abdominal mass/ organomegaly</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lower Limb Examination**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicose Veins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Limb Oedema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg Ulcers Present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg pain on palpation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lower Limb Diameter Measurements**

- Left – 5 cm below Tibial Tuberosity: (cm)
- Left – 25 cm above Tibial Tuberosity: (cm)
- Right – 5 cm below Tibial Tuberosity: (cm)
- Right – 25 cm above Tibial Tuberosity: (cm)

**Upper Limb Diameter Measurements**

- Left – 5 cm below Medial Epicondyle: (cm)
- Left – 10 cm above Medial Epicondyle: (cm)
- Right – 5 cm below Medial Epicondyle: (cm)
- Right – 10 cm above Medial Epicondyle: (cm)
Graded Compression Stockings: Yes [ ] No [ ]

If yes, number of days worn (upper or lower limb) ..........................................................

Height: ........................................................................................................... cm

Weight: ........................................................................................................... kg

Body Mass Index: ...........................................................................................................

12. BLOODS

**Canterbury Health Laboratories (CHL) Bloods**

Haemoglobin: ...........................................................................................................

Leukocyte Count: .....................................................................................................

Platelet Count: ......................................................................................................

D-Dimer: ..................................................................................................................

CRP: .........................................................................................................................

Thrombin Generation Assay: ..................................................................................

Pregnancy (Serum Beta HCG): Positive [ ] Negative [ ] Raised due to Germ Cell Tumour [ ] N/A [ ]

**University of Otago MacKenzie Cancer Research Laboratory Bloods**

Tissue Factor: Date frozen Time frozen

Ang 1: Date frozen Time frozen

Ang 2: Date frozen Time frozen

P-Selectin: Date frozen Time frozen

Comments: ..............................................................................................................

13. ECG

Date: ......................................................................................................................

ECG Normal: Yes [ ] No [ ]

Rate > 100 / min: Yes [ ] No [ ]

Sinus Rhythm: Yes [ ] No [ ]

Newly Diagnosed Atrial Fibrillation: Yes [ ] No [ ]

Right Bundle Branch Block: Yes [ ] No [ ]
<table>
<thead>
<tr>
<th>Right Atrial Deviation</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST changes:</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Right ventricular hypertrophy</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

14. IMAGING

<table>
<thead>
<tr>
<th>Pleural effusion on chest imaging:</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate-like atelectasis on chest imaging</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Raised hemidiaphragm on chest imaging:</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>PE seen on CTPA 2: Date..................</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>DVT seen on USS 2: Date..................</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Health Professional Signature: ........................................................................................................

Date Questionnaire Completed: ...........................................................................................................
8.4 Blood request forms

**Canterbury Health Laboratories**

**RESEARCH REQUEST FORM**

<table>
<thead>
<tr>
<th>Surname</th>
<th>Given Names</th>
<th>City</th>
<th>Sample date, time</th>
<th>Requested by</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tony Rahman</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age or D.O.B.</th>
<th>Sex</th>
<th>Patient Number</th>
<th>R5602</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SEP** (Separation for Thrombin Generation Assay + 4 supplied cryovials – 1 ml in each)

PLEASE SEND SPECIMENS (& CRYOVIALS) DIRECTLY TO COAG LAB

1 x citrate – (for TGA) Please centrifuge, aliquot and place the rack on the bottom shelf of Coag-80 freezer.
4 x citrates – Please Double spin and aliquot 1 mL of plasma into each of the 5 supplied cryovials, freeze at -80.

Please leave a copy of the request form on the bench if specimen arrives out of hours.

RS503 VTE in Cancer Patients undergoing Chemotherapy

RS503 VTE in Cancer Patients undergoing Chemotherapy

Canterbury Health Laboratories

RESEARCH REQUEST FORM

<table>
<thead>
<tr>
<th>Surname</th>
<th>Given Names</th>
<th>City</th>
<th>Sample date, time</th>
<th>Requested by</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tony Rahman</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age or D.O.B.</th>
<th>Sex</th>
<th>Patient Number</th>
<th>R5602</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SEP** (Separation for Thrombin Generation Assay + 4 supplied cryovials – 1 ml in each)

PLEASE SEND SPECIMENS (& CRYOVIALS) DIRECTLY TO COAG LAB

1 x citrate – (for TGA) Please centrifuge, aliquot and place the rack on the bottom shelf of Coag-80 freezer.
4 x citrates – Please Double spin and aliquot 1 mL of plasma into each of the 5 supplied cryovials, freeze at -80.

Please leave a copy of the request form on the bench if specimen arrives out of hours.

RS503 VTE in Cancer Patients undergoing Chemotherapy

RS503 VTE in Cancer Patients undergoing Chemotherapy

Canterbury Health Laboratories
# Appendix 4 - Performance Status Scores

## 9.1 Karnofsky performance status score

### Karnofsky Performance Status Scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Normal, no complaints, no evidence of disease</td>
</tr>
<tr>
<td>90</td>
<td>Able to carry on normal activity, minor signs or symptoms of disease</td>
</tr>
<tr>
<td>80</td>
<td>Normal activity with effort, some signs or symptoms of disease</td>
</tr>
<tr>
<td>70</td>
<td>Cares for self, unable to carry on normal activity or to do active work</td>
</tr>
<tr>
<td>60</td>
<td>Requires occasional assistance but is able to care for most of personal needs</td>
</tr>
<tr>
<td>50</td>
<td>Requires considerable assistance and frequent medical care</td>
</tr>
<tr>
<td>40</td>
<td>Disabled, requires special care and assistance</td>
</tr>
<tr>
<td>30</td>
<td>Severely disabled, hospital admission is indicated although death not imminent</td>
</tr>
<tr>
<td>20</td>
<td>Very sick; hospital admission necessary, active supportive treatment necessary</td>
</tr>
<tr>
<td>10</td>
<td>Moribund, final processes progressing rapidly</td>
</tr>
<tr>
<td>0</td>
<td>Dead</td>
</tr>
</tbody>
</table>

The Karnofsky Performance Scale Index allows patients to be classified according to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the worse the survival for most serious illnesses.
## 9.2 ECOG performance status score

Venous Thromboembolism in Patients about to Commence Chemotherapy

**ECOG Performance Status**

<table>
<thead>
<tr>
<th>GRADE</th>
<th>WHO/ECOG</th>
<th>Tick ✓</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction</td>
<td>✓</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light housework, office work</td>
<td>✓</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours</td>
<td>✓</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self-care, confined to bed or chair more than 50% of waking hours</td>
<td>✓</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair</td>
<td>✓</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
<td>✓</td>
</tr>
</tbody>
</table>

ECOG – Eastern Cooperative Oncology Group  
WHO – World Health Organization
Appendix 5- Radiation Assessment Reports

10.1 Radiation risk assessment for 64 slice MDCT

21 January 2011
Tony Rahman
Oncology Registrar
Christchurch Hospital

Ref: Radiation Risk Assessment for clinical trial

Summary

This letter provides a radiation dose and risk assessment that has been requested to support a new clinical trial. The trial proposal is to substitute regular chest CT scan for CTPA CT scans in selected oncology patients in order to try to better detect pulmonary embolism.

The assessment concludes that for chest only CT scans there is negligible difference in patient dose. If the scan length is longer to include abdomen and pelvis then there could be a small additional radiation dose presenting minimal additional risk, this risk should be balanced against the potential benefits of the trial.

Determination of accurate dose per scan for future patients is not possible, there is a wide range in the doses received for any given CT scan as it is very dependent on patient size.

The calculations provided in this assessment are intended as an indication only for adult population.

Method of calculating effective dose

To determine any difference in radiation dose that the individual will receive as a result of receiving a CTPA scan instead of a regular CT scan, data from a selection of recent patients who had been scanned for each of the applicable protocols were examined. The dose length product for each scan was collected and average (mean) and standard deviation obtained.

An average effective dose was determined by applying a K factor to the DLP (AAPM report 56) for each different protocol so that comparisons could be made.

Protocols (CT scan types)

Patients in the trial could require chest only CT scans or to also include abdomen or abdomen and pelvis. The current protocol typically used for oncology patients includes a scan length from lung apices to the lesser trochanter and is referred to as ‘ONC CAP’. In order to make dose comparisons a similar CTPA protocol is required for examination, the ‘CTPA/Abdomen (regular)’ protocol which is a series of two CT scans, the first from lung apices to mid kidneys, followed by a second scan from the base of lung to pubic symphysis was selected.

Patients who had received the following CT scans were used to determine an average dose for each scan:

- CT chest (n=20)
- CTPA chest (n=21)
- ONC CAP (n=20)
- CTPA abdomen (regular) (n=19)
Table 1 below shows the average effective dose for each scan type, the standard deviation gives an idea of the range of doses received (the uncertainty in the effective dose).

<table>
<thead>
<tr>
<th>Scan type</th>
<th>Effective dose (mean) mSv</th>
<th>Standard deviation mSv</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT chest</td>
<td>7.8</td>
<td>3.7</td>
</tr>
<tr>
<td>CTPA chest</td>
<td>7.5</td>
<td>2.3</td>
</tr>
<tr>
<td>ONC CAP</td>
<td>17.7</td>
<td>6.1</td>
</tr>
<tr>
<td>CTPA abdomen (regular)</td>
<td>26.4</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Table 1

Discussion

From the results it can be seen that patients on the trial who have CTPA chest instead of CT chest would on average receive 0.2 mSv less radiation dose. For patients who require a longer scan including the abdomen, results indicate that they could receive on average an additional 7 mSv, however these results are not comparing identical scan parameters and caution should be taken. (The reason for some additional dose of the abdomen scan is that there is a region of overlap between the two scan components required due to contrast being in different phases.)

There is great variation between individual doses for the same scan (largely due to patient physical size variations), the calculated doses ranges for CTPA and regular CT scans overlap and could therefore be considered of similar magnitude.

Conclusions

There is no real difference in dose found between CT chest and CTPA chest scans.

For scans including abdomen area, by introducing a CTPA scan, there is potential for a small increase in radiation dose to the patient and estimates pessimistically suggest this could be around 0.7 mSv per scan. Patients typically receive three scans during the course of their treatment which could on average result in 26.1 mSv additional radiation dose.

The total lifetime risk of any hazard from 26.1 mSv of radiation is no greater than 0.13% (one in 770) [ICRP 103], this is less risk than the lifetime risk of fatality from drowning or homicide in New Zealand (each, 2 in 1000) [NRLCS].

Most radiation induced cancers take many years to develop (of the order of 20 years), and so are of particular concern when irradiating young healthy subjects. It is likely that the patients involved in this study will have an older age profile or shorter life expectancy than an average adult. When considering the risks it is very important to balance them against the benefits of the study, which may significantly improve or lengthen the patient’s life.

Please contact me if you have any queries regarding this assessment.

Kind regards,
Annalise Ronaldson
(Checked: Dr D O’Keefe)

__________________________
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10.2 Radiation risk assessment for 128 slice MDCT

MEMO

TO: Tony Rahman
FROM: Steven Muir
DATE: 7/1/14
SUBJECT: CT doses for oncology clinical trial

Introduction
CTPA doses were calculated in 2011 for a clinical trial to detect pulmonary embolism in oncology patients. Scans were then performed on the CT3 GE scanner. Most CTPA exams are now performed on the Siemens CT scanner installed in 2012 and so a check of the doses given by the new scanner was required to ensure they are still within the expected range.

Method
An Inteleviewer search for CTPA abdomen since July 2012 gave 24 results and the DLPs were recorded. CTPA Chest are more common and the last months results gave 24 results which were averaged. A k-factor of 0.015 was used to convert DLP to effective dose. A check of the k-factor using the Impact spreadsheet gave very similar results.

Results
The results are given in table 1. The dose for CTPA abdomen on the Siemens scanner was 15.8 mSv compared to 19.3 mSv on the GE CT3 scanner and 28.4 from the 2011 calculations. The dose for CTPA Chest on the Siemens scanner was 4.8 mSv compared to 8.4 mSv on the GE scanner and 7.8 mSv from the 2011 calculations.

Conclusions
The CT doses in this study have reduced 30-40% from the 2011 estimates when performed on the new Siemens scanner for both chest and abdomen CTPA procedures. GE CT3 doses for abdomen CTPA have reduced but the GE CT3 chest CTPA doses are slightly higher than the 2011 estimates.
Table 1. Dose report results

<table>
<thead>
<tr>
<th>Siemens flash CTPA DLP mGy.cm</th>
<th>GE CTPA abdomen DLP mGy.cm</th>
<th>CT Chest CTPA Siemens flash DLP mGy.cm</th>
<th>CT Chest CTPA GE VCT DLP mGy.cm</th>
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</table>

Average DLP: 1050.1 1200.7 318.5 563
Std Dev: 318.5 343.7 85.9 183

Effective dose (mSV): 15.6 19.2 4.8 8.44
Std Dev: 4.8 5.2 1.4 27

Steven Muir  
Medical Physics and Bioengineering Dept.
Appendix 6 Ultrasound protocols and patient information sheet

11.1 Ultrasound examination for deep venous thrombosis (DVT) assessment protocol utilising complete compression ultrasound technique (CCUS) and pulsed wave spectral image assessment of the Common femoral vein (CFV)

Complete compression ultrasound (CCUS) refers to the application of a suitable pressure so that the intramural pressure of the vein is overcome and the vein will collapse. The corresponding artery will remain open since a much larger amount of pressure is required to overcome the intramural pressure of the artery.

All CCUS techniques involve compression of the vein in true transverse section.

A compression manoeuvre resulting in the collapse of the vein proves that no thrombus is present in this venous segment. If a venous segment is not completely compressible the quality of the compression manoeuvre has to be checked. If the compression manoeuvre was adequate the respective venous segment is deemed to be an indication of the presence of thrombus.

A spectral waveform is obtained in the mid common femoral vein, a waveform showing phasicity and spontaneous forward flow excludes the present of significant venous thrombi from this point to the diaphragm. A non-phasic waveform suggests the presence of proximal thrombosis and the iliac veins and inferior vena cava (IVC) will then need to be examined.

The examination starts with the patient in the supine or semi recumbent position with venous assessment starting at the level of the CFV.

The CFV is evaluated via spectral assessment and CCUS. The examination is then continued along the length of the femoral vein (FV) using the CCUS technique. The Popliteal vein and tibial peroneal trunk (TPT) are similarly assessed via the CCUS technique.
11.1.1 Standard imaging series
CFV spectral trace obtained in longitudinal venous section

Deep femoral vein proximally with colour Doppler

Sapheno-femoral junction with colour

Split screen CCUS assessment of CFV, non compression / compression

Split screen CCUS assessment of FV, non compression / compression (proximal, mid, distal)

Split screen CCUS assessment of FV, non compression / compression (level of adductor canal)

Split screen CCUS assessment of popliteal vein, non compression / compression

Split screen CCUS assessment of TPT, non compression / compression

11.1.2 Ultrasound Equipment
Siemens ANTARES ultrasound unit

D.V.T setting

VFX9-4 transducer

11.1.3 Image recording/reporting
Protocol led image series are recorded as static images on the CDHB radiology PACS systems

A worksheet of findings is completed by the sonographer

Images are reported by VTE study radiologist
11.2 Upper limb venous duplex ultrasound protocol

11.2.1 Equipment
- Siemens ANTARES, VFX9-4 or VF13-5
- Small foot print multi frequency linear transducer preferred
- Curvy linear transducer may be of use in large patients
- Low flow colour settings
- Harmonics may be useful.

11.2.2 Patient Preparation
- Obtain history
- Observe limb (line placement, puncture site)
- Patient supine with bed flat is possible
- Patient head turned away from side being examined
- Arm abducted and supported

11.2.3 Technical considerations
- Use compression techniques where appropriate
- Colour Doppler
- Pulsed Doppler
- Light transducer pressure on superficial veins
- Optimise image

11.2.4 General Considerations
Upper limb veins should show:
- Spontaneous flow
- Phasic flow (upper limb flow increases with inspiration)
- Response to Augmentation
Competence

Upper limb veins will normally demonstrate a degree of pulsatility

11.2.5 Standard Imaging Series

- Internal jugular vein (IJV) in Transverse and Longitudinal with colour and Pulsed Doppler recorded.
- Gentle compression
- Subclavian in longitudinal section with Doppler waveforms recorded proximal, mid and distally.
- Axillary in long with waveform recorded
- Axillary in transverse with compression if possible
- Brachial veins. Compression in transverse with split screen recorded in prox, mid and distal
- Basilic vein in long in colour and with waveform recorded
- Cephalic vein in colour and with waveform recorded
- Labeled images to show extent of thrombus if present
- Observe if thrombus is attached or free
- Venous catheters or lines should be examined along their length to check for the presence of thrombus

11.2.6 Findings in Upper limb venous thrombosis

- Incomplete compression
- Persistent intramural filling defect with colour Doppler
- Absent or decreased transmitted cardiac pulsatility
- Abnormal response to respiratory maneuvers
- Large collateral veins
When thrombus or obstruction of the subclavian or axillary veins is present, the venous signals may be absent or continuous and non-phasic.

Increased flow may also be evident in the cephalic vein with obstruction of the axillary or subclavian vein

11.2.7 After the Examination

- Fill out appropriate worksheet
- Code U59
11.3 Patient information sheet

Patient information on ultrasound scans for the venous thromboembolism in cancer patients commencing chemotherapy study

(Ethics reference URB/11/02/005)

What is an ultrasound scan?

Ultrasound imaging, also called ultrasound scanning or sonography, involves exposing part of the body to high-frequency sound waves to produce pictures of the inside of the body. Ultrasound exams do not use x rays. Because ultrasound images are captured in real-time, they can show the structure and movement of the body's internal organs, as well as blood flowing through blood vessels. There are no risks involved with having an ultrasound scan.

Ultrasound imaging is a noninvasive medical test that helps physicians diagnose and treat medical conditions. Venous ultrasound provides pictures of the veins throughout the body. The most common reason for a venous ultrasound exam is to search for blood clots, especially in the veins of the leg. This condition is often referred to as deep vein thrombosis or DVT. These clots may break off and pass into the lungs, where they can cause a dangerous condition called pulmonary embolism. If the blood clot in the leg is found early enough, treatment can be started to prevent it from passing to the lung.

Do I need to do anything before the scan?

You should wear comfortable, loose-fitting clothing for your ultrasound exam. You may need to remove all clothing and jewellery in the area to be examined. You may be asked to wear a gown during the procedure but there is no other special preparation for a venous ultrasound.

What happens during the scan?

You are positioned lying face-up on an examination table that can be tilted or moved. A clear water-based gel is applied to the area of the body being studied to help the transducer make secure contact with the body and eliminate air pockets between the transducer and the skin. The sonographer (ultrasound technologist) or radiologist then presses the transducer firmly against the skin in various locations, sweeping over the area of interest or angling the sound beam from a farther location to better see an area of concern. No needles are involved during this procedure.
What happens after the scan?

When the examination is complete, you will be asked to dress and wait while the ultrasound images are reviewed. However, the sonographer or radiologist is often able to review the ultrasound images in real-time as they are acquired and the patient can be released immediately. If the scan is normal you can go home.

If a DVT or blood clot is found then a doctor will be required to see you to discuss treatment for this immediately after your scan which will increase the length of your appointment and you will be required to go to the Emergency Department of Christchurch Hospital for this assessment.

What do I do if I require more information or need to change my appointment?

Please contact Dr Tony Rahman at Christchurch Hospital between the hours of 0800 and 1600 on (03) 364 0640 and ask switchboard to contact him on pager 8829.

Thank you.
Appendix 7 Sample statistics used within this study (CD-ROM)

Please see the attached CD-ROM. Thank you.
Venous thromboembolism in cancer patients undertaking chemotherapy

33. Mann KG. Thrombin: can't live without it; probably die from it. Chest. 2003;124(3 Suppl):S4-S9.
50. Mousa SA, Mohamed S. Inhibition of endothelial cell tube formation by the low molecular weight heparin, tinzaparin, is mediated by tissue factor pathway inhibitor. Thrombosis and Haemostasis. 2004;92(3):627-33.
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Iles S. Clinical experience and pre-test probability scores in the diagnosis of pulmonary embolism. QJM. 2003;96(3):211-5.


Venous thromboembolism in cancer patients undertaking chemotherapy


Venous thromboembolism in cancer patients undertaking chemotherapy

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Kowalski E, Budzynski AZ, Kopec M, Latallo ZS, Lipinski B, Wegrzynowicz Z. Circulating fibrinogen degradation products (Fdp) in dog
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Venous thromboembolism in cancer patients undertaking chemotherapy


Venous thromboembolism in cancer patients undertaking chemotherapy


