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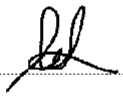
Signature:  **Date:** 10/07/2020

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Investigating the Methods of Patent Foramen Ovale Detection

Hanna Van der Giessen

A thesis submitted for the degree of

Masters of Health Science.

University of Otago,

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Abstract

A patent foramen ovale is a remnant of the foetal circulation that exists between the right atrium and the left atrium of the heart. It is estimated to be present in 1 in 4 adults in the general population. The presence of this residual hole can allow the passage of microemboli into the arterial system, which can then travel to the brain causing an ischemic stroke. A subtype of ischemic stroke is a cryptogenic stroke, which occurs when the initial cause of ischemia cannot be determined. The presence of a patent foramen ovale is more common in patients with cryptogenic stroke, suggesting a relationship. Currently, the most common method of detection for a patent foramen ovale is transthoracic echocardiography, which is often followed by transoesophageal echocardiography if the remnant is suspected and requires further investigation. An alternative method of diagnosis is transcranial Doppler, which has demonstrated diagnostic superiority in some studies. The use of all three imaging techniques are commonly utilized clinically, but the optimal method is still under debate.

In this thesis, the two bedside techniques transthoracic echocardiography and transcranial Doppler were applied to a case series of three patients with suspected patent foramen ovale that were referred on to the cardiology unit after an ischemic stroke, or possible cryptogenic stroke. Additionally, a systematic review and meta-analysis was undertaken to assess the status of the two diagnostic techniques in literature.

The case studies uncovered some significant pitfalls and advantages of both transthoracic echocardiography and transcranial Doppler, while still diagnosing all three case studies positively with a patent foramen ovale.

The systematic review and meta-analysis found that the sensitivity of the two bedside techniques changed when the gold standard reference technique, transoesophageal echocardiography, was included. Prior to its inclusion, the two techniques had similar levels of sensitivity, but on inclusion, transcranial Doppler appeared to be the optimal technique for the ruling in of a patent foramen ovale, while transthoracic echocardiology appeared to be the optimal technique for the ruling out of a patent foramen ovale.

Discrepancies between the detection techniques continue to emerge throughout literature, indicating the need for further investigation into the determination of the optimal technique, as well as methods that can be applied to optimize each technique. The treatment of a patent foramen ovale can be lifesaving, but an accurate initial diagnosis is needed to facilitate making the right treatment decision.

Acknowledgements

Firstly, I would like to give an enormous thank you to my three supervisors, Gillian Whalley, Luke Wilson, and Sean Coffey. All three of you have given me an incredible amount of guidance, encouragement, and feedback. You have given me the support and confidence to push my ideas further, while giving me the space and independence to let them grow. To Gillian especially – thank you for all of your patience, and your understanding, it was so constructive to my learning.

Secondly, I would like to thank the Otago Medical School for funding my project. I would not have been able to undertake this Master's thesis without a scholarship, and for that, I am eternally grateful!

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Lastly, because I have too many family and friends to thank and subsequently list, here (in Figure. I) are all the people that have helped me through this wonderful journey! Kia kaha.

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Abbreviations

AAo	Ascending Aortic Arch
CI	Confidence Interval
CPAP	Continuous Positive Airway Pressure
CS	Cryptogenic Stroke
CT	Computerized Tomography
Dao	Descending Aortic Arch
EF	Ejection Fraction
FN	False Negative
FP	False Positive
HDEC	Health and Disability Ethics Committee
IAS	Interatrial Septum
ICC	Intraclass Correlation
IVC	Inferior Vena Cava
LA	Left Atrium
LV	Left Ventricle
MCA	Middle Cerebral Artery
MCAv	Middle Cerebral Artery velocity
MOOSE	Meta-analysis of Observational Studies in Epidemiology
MRI	Magnetic Resonance Imaging
OUHEC	Otago University Human Ethics Committee
PE	Paradoxical Embolism
PFO	Patent Foramen Ovale
PMD-TCD	Power Mode Transcranial Doppler
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RA	Right Atrium
RLS	Right-to-Left Shunt
ROC	Receiver Operating Characteristic
RoPE	Risk of Paradoxical Embolism
RV	Right Ventricle

SVC	Superior Vena Cava
TAPSE	Tricuspid Annular Plane Systolic Excursion
TCD	Transcranial Doppler
TIA	Transient Ischemic Attack
TN	True Negative
TOE	Transoesophageal Echocardiography
TP	True Positive
TTE	Transthoracic Echocardiography

Chapter One: Introduction

Stroke

Stroke is one of the most devastating and debilitating neurological diseases that affects over 9,000 New Zealanders every year.[1] The most common form of stroke is an ischemic stroke, which is caused by the blockage or occlusion of a cerebral blood vessel by a microemboli and accounts for ~80% of all strokes.[2] The less common form of stroke is a haemorrhagic stroke, which typically involves the rupturing of a cerebral blood vessel, and the consequential blood leakage into the brain, leading to swelling and cell death.[3] A cryptogenic stroke (CS) is a stroke of unrecognised or unknown origin.[4] Around 40% of ischemic strokes are classified as cryptogenic, where no root cause has been determined following check-ups and testing.[5] A transient ischemic attack (TIA) is commonly referred to as a 'mini-stroke', due to its effects lasting for an acute period of time (<24 hrs), rather than permanently.[6] A CS is known to be transitory or reversible, meaning it was initiated by a risk factor that can be reduced, avoided, or abolished to prevent the stroke from ever reoccurring.[7] A patent foramen ovale (PFO) is a remnant of the foetal circulation that allows the passage of deoxygenated blood into the arterial system, and is therefore a potential mechanism for cryptogenic stroke.

Risk Factors for Ischemic Stroke

There are a variety of risk factors for ischemic stroke, but the largest risk factor is age, with the probability of stroke doubling every ten-years once an individual has reached 55-years of age.[8] Other clinical risk factors include, but are not limited to: health conditions such as diabetes, obesity, heart disease, and hypertension, as well as cigarette, alcohol, and recreational drug use.[3] Lastly, the male sex is a risk factor for stroke, with the incidence of stroke being 1.5 times higher in males compared to females.[6]

What is a Patent Foramen Ovale

A PFO is a common remnant of the foetal circulation that exists between the right atrium (RA) and left atrium (LA).[9] The failed fusion of the flap-like structure called the septum primum to the septum secundum leaves a tunnel between the two layers of the septum, clinically referred to as a PFO (Fig. 1).[10] Depending on the anatomy and the difference in pressure between the RA and LA, the flap-like structure may allow various degrees of right-to-left shunt (RLS).[10] A

RLS is characterised by the movement of venous blood into the arterial circulation.[11] The diameter of a PFO can vary from 1 to 19mm, with the average diameter increasing with age.[12] Studies estimate that 1 in 4 adults in the general population have a PFO.[13,14] Autopsy studies investigating the presence of a PFO demonstrate that the prevalence in the general population is close to 25%, but found that incidence decreased with age (34.3% in the first three decades of life, compared to 25.4% in the fourth through eight decades, and 20.2% during the ninth and tenth decade).[15] The prevalence of PFO appears to increase in individuals with other conditions such as CS, sleep apnoea, decompression sickness, or migraine.[16,17]

1. Anatomy of a PFO

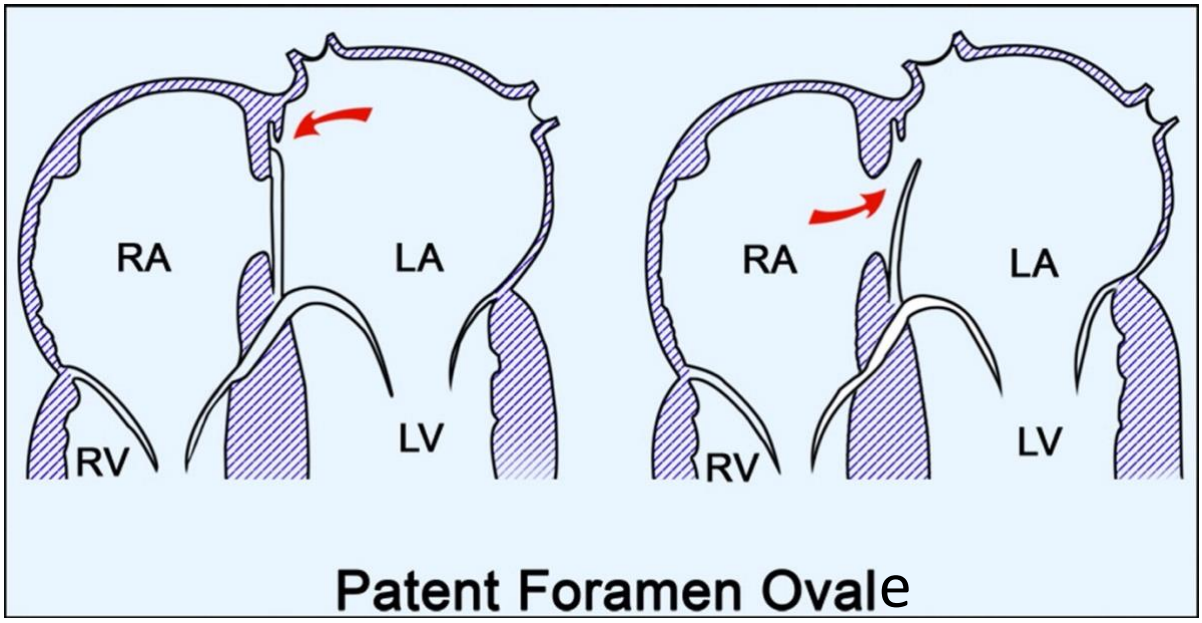


Figure 1:A schematic of the structure of a PFO. Abbreviations: PFO = patent foramen ovale, RA = right atrium, RV = right ventricle, LA = left atrium, LV = left ventricle. Image reprinted from 'Patent foramen ovale: a new disease?, Drighil et al., Figure 1, Copyright (2007), with permission from Elsevier. Licence number: 4865060470694

Ischemic Strokes and Patent Foramen Ovale

A PFO provides a route for venous blood and therefore microemboli into the arterial system and has been linked to CS.[18] It can also cause systemic embolism, which is when a microemboli gets trapped in the vascular supply of an organ, causing dysfunction.[19] Under normal circumstances, the lungs would filter out any venous microemboli,[17] but in the presence of PFO it is possible for microemboli to travel into the left heart, through the aorta and up to the brain.[20] When a microemboli passes from the venous to the arterial system, it is called a paradoxical embolism (PE).[21] These microemboli can then block one of the cerebral arteries causing an

ischemic stroke.[19] The prevalence of a PFO in CS patients has been shown to range from 24% to 59%.[22–25] Specifically, young patients (<55 years) who experience a CS have a higher prevalence of PFO (between 48-56%) compared to patients experiencing a stroke of recognised origin (4-20%).[4,26,27] It has also been reported that medium to large PFOs (≥2 mm) are more common in patients presenting with CS (26% of total PFOs found were medium or large) compared with stroke of known origin (6% of total PFOs found were medium or large).[28] Other mechanisms of PE such as the presence of a pulmonary arteriovenous fistula, atrial septal defect, or an atrial septal aneurysm can also cause a stroke.[13] One particular study by Mas et al., investigated young CS patients (<55 years) and found that 36% had a PFO, 1.7% had an atrial septal aneurysm, and 8.5% had both a PFO and an atrial septal aneurysm.[29] Paradoxical embolism as a result of CS tends to be more common in patients that have an absence of the traditional risk factors for ischemic stroke (older age, obesity, hypertension etc.).[30] The Risk of Paradoxical Embolism (RoPE) score has been developed to help identify the likelihood of the CS being caused by a PFO.[31]

The Risk of Paradoxical Embolism Score

The RoPE score was developed in 2013 by Kent et al., and involves a 10-point system used to stratify patients with CS and predict the contribution of the PFO to the presenting condition (Table 1).[31] It is used to ensure that the cause of CS is correctly attributed to a PFO, rather than the PFO being an 'innocent bystander' or incidental finding, which, given the prevalence of a PFO in the general population being up to 25%, may be likely.[30] The RoPE score considers variables such as age, presence of a cortical infarct on imaging, and the absence of factors such as diabetes, hypertension, smoking, and prior ischemic event. It calculates a 10-point score that predicts likelihood of the PFO being causal to the CS, as well as the risk of recurrent stroke.[31] The validity of this score was explored by Prefasi et al., in 2016 who concluded that a RoPE score of 7 was the cut off for validity.[32] They found that in a cohort of 58 CS patients, a RoPE score of 7 or under indicated a 0% chance of the CS being attributable to a PFO, compared to a 71.1% chance with a RoPE score higher than 7.[32]

Table 1: RoPE score calculator

Characteristic	Points	RoPE score
No history of hypertension	1	
No history of diabetes	1	
No history of stroke or TIA	1	
Non-smoker	1	
Cortical infarct on imaging	1	
Age, years		
18-29	5	
30-39	4	
40-49	3	
50-59	2	
60-69	1	
≥70	0	
Total score (sum of individual points)		
Maximum score (a patient <30 years with no hypertension, no diabetes, no history of stroke or TIA, non-smoker, and cortical infarct)		10
Minimum score (a patient ≥70 years with no hypertension, diabetes, prior stroke, current smoker, no cortical infarct)		0

Legend 1: The characteristics used to calculate the RoPE score and the value assigned to each. Available online at https://www.mdcalc.com/risk-paradoxical-embolism-rope-score. Abbreviations: RoPE = Risk of Paradoxical Embolism, TIA = transient ischemic attack

Migraine and Patent Foramen Ovale

A migraine headache can be characterised by the reoccurring symptom of a headache, as well as nausea, vomiting and other neurological-type dysfunctions.[10] The mechanism and pathophysiology behind migraine is yet to be fully understood,[33] yet studies show that there is a relationship between migraine and PFO. Zito et al found that 57% of patients with migraine had a PFO,[34] and West et al., found that 79% of patients with migraine and CS had a PFO.[24] Giardini et al., conducted a study investigating the effects of PFO closure on migraine with aura patients (recurring migraine paired with sensory disturbances) and found that after a follow-up within 2-years, migraine with aura was eliminated in 83% of the patients, and a

further 8% felt as though there was a reduction of symptoms.[35] On the contrary, one recent study by Mattle et al., also investigated the closure of PFO in migraine with aura patients, and found the closure to have no effect on the patients reoccurring migraines.[36] It is obvious that there is a relationship between migraine and PFO, but the mechanism to which still requires investigation.

Other Shunts Similar to a Patent Foramen Ovale

Congenital malformations such as an atrial septal defect, atrial septal aneurysm, or a pulmonary arteriovenous fistula can also facilitate a RLS. These defects can be intracardiac (atrial septal defect or atrial septal aneurysm) or intrapulmonary (pulmonary arteriovenous fistula), and despite clear anatomical differences often end up causing the same health conditions. An atrial septal defect is a congenital heart disease and is known to be present in up to 0.2% of children.[37] While an atrial septal defect and a PFO exist in the same anatomical area (the septum), an atrial septal defect is a fixed opening of the septum which allows the passage of blood from one atria to the other without the need of a pressure gradient like a PFO.[38] An atrial septal aneurysm on the other hand, is more common than an atrial septal defect and is estimated to be present in around 1% of the population, but this proportion is increased when the patient presents with a condition such as a stroke or migraine.[39] It can be characterised by the bulging of remnant septal tissue into both the left and right atria.[40] The most common intrapulmonary shunt is a pulmonary arteriovenous fistula, which is a vessel malformation causing a connection between the pulmonary circulation and the systemic circulation, allowing deoxygenated blood to bypass the lungs.[41] An autopsy study showed only 3 in 15,000 individuals had the malformation.[42]

Optimisation of Diagnostic Tests

Sensitivity and specificity are measurements commonly used to evaluate a clinical test. Sensitivity refers to the tests ability to correctly identify patients with the condition, while specificity refers to the tests ability to correctly identify patients without the condition.[43] Both are measured as a percentage, and are usually measured using a gold standard reference test.[43] A gold standard test, also known as a reference test, is a test that is independent of the index tests that can indicate the true condition state of the patient.[44] It is used to compare the diagnostic abilities of the index tests and to calculate sensitivity and specificity.[44] Sometimes the reference test is unavailable or imperfect, so other mathematical modelling methods can be

used to estimate sensitivity and specificity, such as the maximum likelihood estimation and Bayesian inference.[45]

Diagnosis of Patent Foramen Ovale

There are three imaging methods that are clinically used to detect a RLS for the diagnosis of a PFO. Transthoracic echocardiography (TTE) directly images the heart and is most commonly used by cardiologists as the initial diagnostic test.[9] Transcranial Doppler (TCD) is often used by neurologists, which instead of imaging the heart directly, measures the blood velocity profile in the cerebral arteries.[46] Transoesophageal echocardiography (TOE) is the current clinical gold standard for diagnosis and is used after positive or suspected diagnosis of PFO from either TTE or TCD.[47]

Valsalva Manoeuvre

The Valsalva manoeuvre is a provocation that is commonly used concurrently with the three diagnostic imaging methodologies for PFO diagnosis. It can improve RLS visualisation and thus PFO diagnosis by up to 28%.[48] The Valsalva manoeuvre involves four phases; (1) the patient forcefully expires against a closed glottis which briefly increases the intrathoracic pressure, initially reducing venous return; (2-early) while the patient is still forcefully expiring venous return continues to decrease and as a result stroke volume drops resulting in a decrease in blood pressure; (2-late) the activation of the baroreflex increases the heart rate and subsequent blood pressure; (3-release) the patient releases abdominal pressure and blood flow returns to the heart, resulting in a decrease in intrathoracic pressure and the normalization of blood flow; (4) blood pressure overshoots as the heart refills.[49] The change in intrathoracic pressure during the Valsalva manoeuvre produces a positive right-to-left pressure gradient between the RA and the LA that would prompt a RLS if a PFO were present.[50] A well performed and well timed Valsalva manoeuvre is crucial for PFO detection as it prompts the contrast to move from the RA into the LA where it can then be imaged, either directly by TTE, or indirectly in the middle cerebral artery (MCA) by TCD.[51] If the Valsalva manoeuvre is performed at the wrong moment, the patient may miss the window when the LA is opacified, which may result in a RLS without contrast that could lead to a false negative result. There are several different procedures for the provocation of the Valsalva manoeuvre. Some clinicians use a taught Valsalva manoeuvre (where the patient bears down against a closed glottis or thumb in mouth);[48] some use a calibrated device (such as a sphygmomanometer);[52] some use

a coughing technique;[53] and some use abdominal compression paired with a Valsalva manoeuvre.[54] However, the choice and use of each method does not appear to be standardised.[52] Using a calibrated device can mimic a Valsalva manoeuvre by helping the patient maintain a constant intrathoracic pressure by blowing into a tube attached to a sphygmomanometer.[52,55] However, it is hard to know whether the pressure maintained on the sphygmomanometer is from the closure of the palate or the elevated intrathoracic pressure, although changes in heart rate and blood pressure can give indication to a genuine change in intrathoracic pressure.[56] Some clinicians apply abdominal compression during the Valsalva manoeuvre which further increases intrathoracic pressure, in turn decreasing venous return and left atrial pressure, enhancing the right-to-left pressure gradient that is needed to provoke a RLS.[51] This method is particularly useful for patients unable to perform an adequate Valsalva manoeuvre due to health conditions such as obesity, or if they were undergoing a TOE that required partial sedation.[54] Abdominal compression can improve the accuracy of TOE,[54] and can improve the sensitivity of TTE from 85% to 99% compared to a normal Valsalva manouevre.[51]

Saline Injection

Agitated saline is an inexpensive and effective contrast agent that is injected into the venous circulation during TTE, TCD and TOE for PFO diagnosis. The contrast is composed of microbubbles and is highly reflective of ultrasound compared to blood (which does not reflect ultrasound). The movement of blood that includes contrast can then be directly visualised using TTE, TOE or TCD.[57] A PFO is believed to be present if the contrast is seen in the LA, left ventricle (LV) or MCA. This is because the PFO provides a route for the contrast to cross the interatrial septum into the arterial system. If no PFO was present, the microbubbles would be filtered out by the lungs and no contrast would be visualised in the systemic circulation. The contrast's efficacy is improved when a small amount of blood (1 ml) is mixed into the saline.[58,59] The sensitivity of TTE and TCD is also improved by giving repeated saline injections.[60,61] The injection site has traditionally been via the brachial vein in the arm,[62] but higher sensitivity for PFO detection using TCD has been demonstrated when the agitated saline is injected via the femoral vein (50%) compared to the brachial vein (18%).[63] This is believed to be related to the direction of the inflow of the venous blood into the RA. Venous blood from the upper limb enters the RA via the superior vena cava, and directly traverses the tricuspid valve. Whereas femoral venous blood enters into the RA via the inferior vena cava and is directed towards the atrial septum. Blood from inferior vena cava is therefore more likely to

pass through the PFO if present.[63–65] The Eustachian valve, is another remnant of the foetal circulation located at the entrance of the inferior vena cava into the RA.[17] During foetal circulation it directs blood flow towards the foramen ovale, but if present in an adult would help to keep the foramen ovale patent.[17] The presence of this valve impairs the inflow of saline from the superior vena cava if injected by the arm. However, if saline is injected via the femoral vein, the contrast comes into the RA through the inferior vena cava and is directed right at the septum.[66] This has been shown to increase the reliability of detection.[66] Since TCD, TTE and TOE are all relatively similar in the way they detect a RLS (i.e. they require the agitated saline to pass through the PFO), it is likely that a change in injection site would also increase sensitivity of these methods, which has been demonstrated in studies by Hamann and Gin.[62,63] However, given the additional complexity and potential risk associated with femoral venous cannulation, this technique is likely to remain restricted to patients with a high suspicion of PFO but negative initial imaging studies.

Transthoracic Echocardiography

Transthoracic echocardiography (TTE) uses an ultrasound probe applied to the chest wall that uses high frequency sound waves to take still or moving images of the heart and its internal features. It can be used to assess and measure a variety of suspected problems, such as valve dysfunction, heart muscle contraction, and abnormal heart size and rhythm.[67] It is commonly used for the diagnosis of PFO by either imaging the interatrial septum directly, or paired with agitated saline contrast which can image the highly reflective microbubbles travelling between atria.[68] The TTE with agitated saline injection is typically the first diagnostic test implemented due to its ease of use, relatively low cost, and proven diagnostic ability. In some cases, microbubbles do traverse from the RA to LA at rest, confirming the presence of a PFO. This would usually occur within the first three cardiac cycles after opacification of the RA.[69] However, if this does not occur, the patient is then asked to perform the Valsalva manoeuvre (Fig. 2). The shunt is assessed during the first three to four heart beats following the release phase of the Valsalva manoeuvre.[48] The timing is important, since the presence of an intrapulmonary shunt will also allow microbubbles to pass into the arterial circulation, but with a time delay.[70] In the absence of a PFO, no opacification of the LA is observed, but if a PFO is present, the severity of the shunt can be graded based on the number of microbubbles observed

traversing the foramen ovale as mild (<10 microbubbles), moderate (10-20 microbubbles), or severe (>20 microbubbles).[48]

2. TTE Four chamber view of the heart at rest and during a bubble study

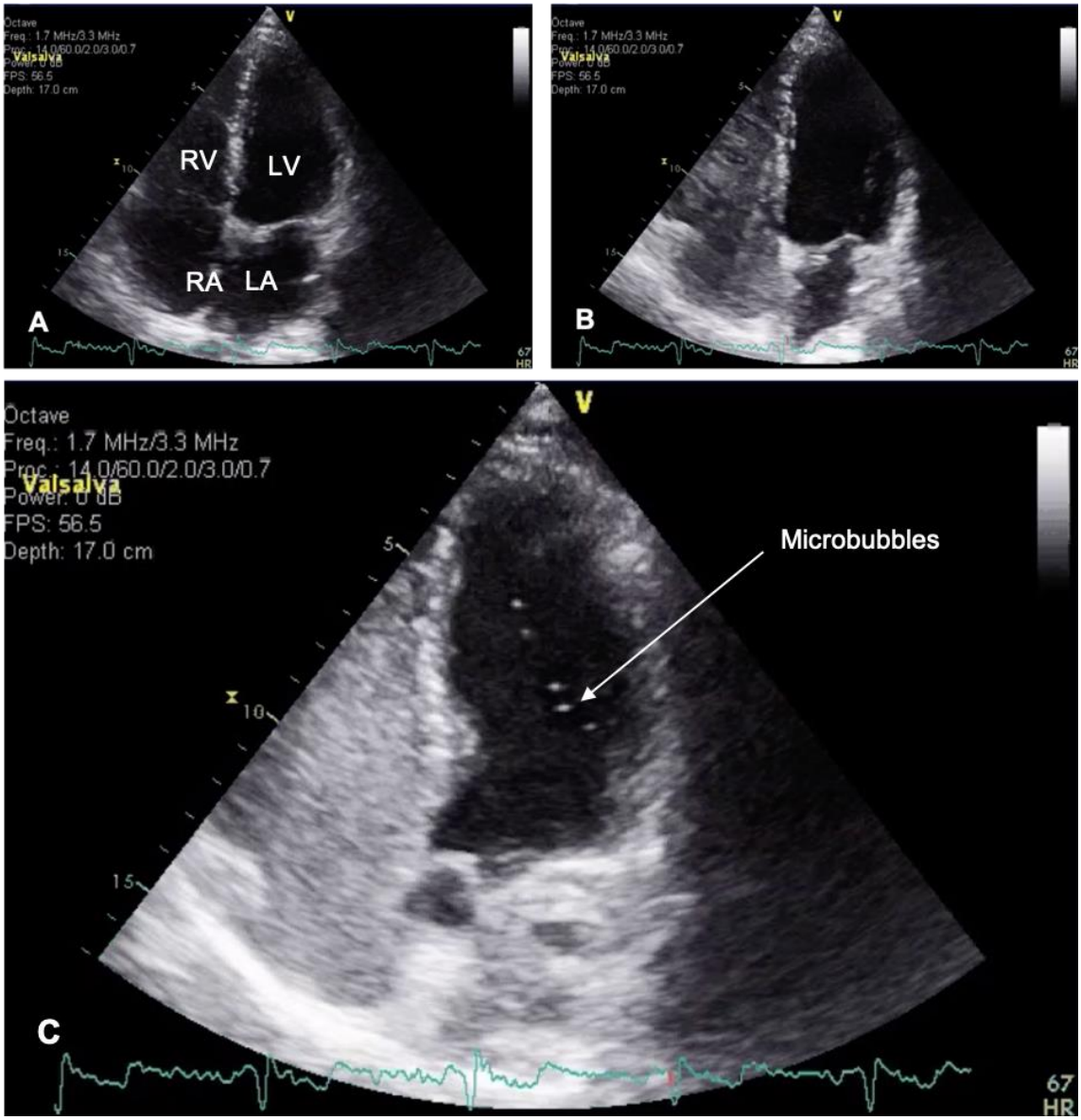


Figure 2: (A) Transthoracic echocardiogram four chamber view of the heart at rest prior to the agitated saline injection. (B) Opacified right heart during the agitated saline injection. (C) Multiple microbubbles indicated by the arrow can be seen in the left atrium and some have moved into the left ventricle following the release phase of the Valsalva manoeuvre. Abbreviations: TTE = transthoracic echocardiography, RV = right ventricle, LV = left ventricle, RA = right atrium, LA = left atrium. Image obtained with permission from the Southern District Health Board.

Transcranial Doppler

Transcranial Doppler (TCD) is an ultrasound technique that images a chosen cerebral blood vessel using an ultrasound probe applied to the temporal bone window of the head. It is becoming increasingly popular for the assessment of cerebral blood flow change, vascular stenosis, or to detect embolic signals within the arteries.[71] TCD can also be used to clinically diagnose several cerebrovascular disorders, such as vasospasm, sickle cell disease, or brain death.[71] The TCD imaging method for the non-invasive detection of PFO involves insonation of the major cerebral arteries, typically the MCA, with a standalone Doppler transducer to assess blood velocity profile. The Doppler system can be used for unilateral and/or bilateral imaging. The portable 2 MHz pulsed wave Doppler transducers are held securely in place with a specialised headband over the temporal bone window and are manipulated to optimise the blood velocity signal of the cerebral vessel of interest. This is visualised as a red band of blood flow signal if the blood is flowing towards the transducer, and a blue band if the blood is flowing away from the transducer[72] (Fig. 3). Modern TCD systems can display multiple vessels at once at different depths. The top screen displays the power M-Mode™ TCD (PMD-TCD; 33 sample gates) of all the vessels within the sampling depth range field. The yellow reference line on the top portion of the screen is the depth (in mm on the y-axis) at which the spectrogram (single-gate) velocity waveform on the bottom of the screen is derived, insonating the artery of interest (Fig. 3). The combination of PMD-TCD and the spectrogram increases the sensitivity of TCD for diagnosing a PFO.[73] If a RLS is present at rest or during the Valsalva manoeuvre, the clinician will visualise the microbubble contrast and hear audible *blips*. A RLS of any type is considered present if at least one microbubble is recorded with TCD within 25 seconds of injection of the agitated saline.[74] The 'rule of nine' was developed in 2010 by Lange et al., which helps the clinician differentiate between intracardiac and intrapulmonary shunts by considering three markers in the case of a positive test such as number of microbubbles, as well as the latency time and duration time of the microbubbles.[75] The RLS and size of the potential PFO is graded based on the number of microbubbles: grade 1 (1 – 10 microbubbles), 2 (11 – 30 microbubbles), 3 (31 – 100 microbubbles), 4 (101 – 300 microbubbles), and grade 5 (>300 microbubbles)[46] (Fig. 4).

3. TCD with and without Microbubbles

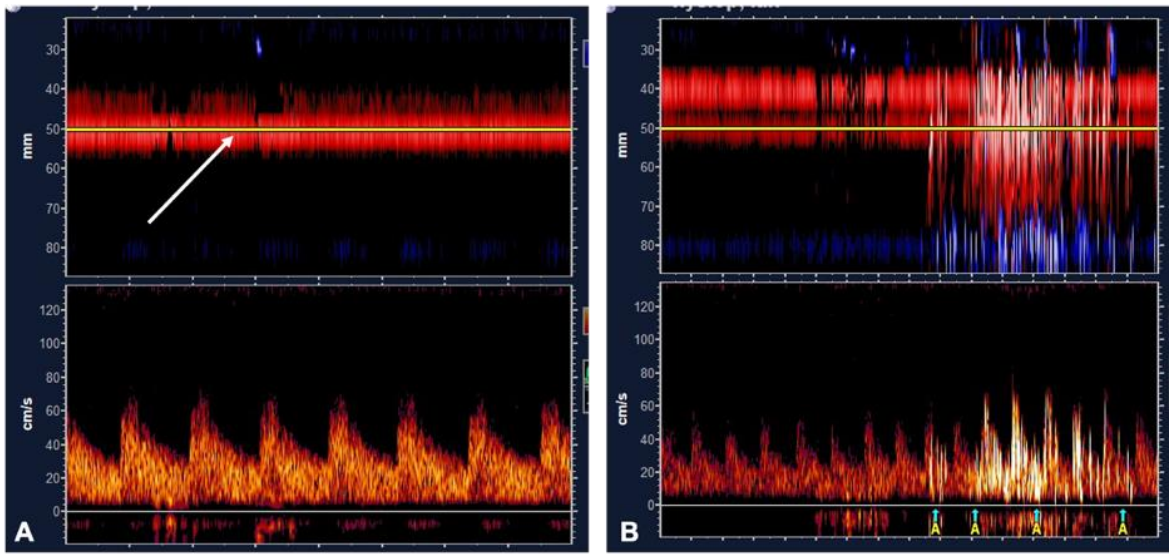


Figure 3: The arrow indicates the vessel of interest which is being insonated by the probe at the depth of the yellow reference line. (A) shows the vessel with no microbubbles present, and (B) with microbubbles (visualised as white disruption through the vessel of interest). Image obtained with permission from the Southern District Health Board.

4. The Five grades of PFO severity using TCD

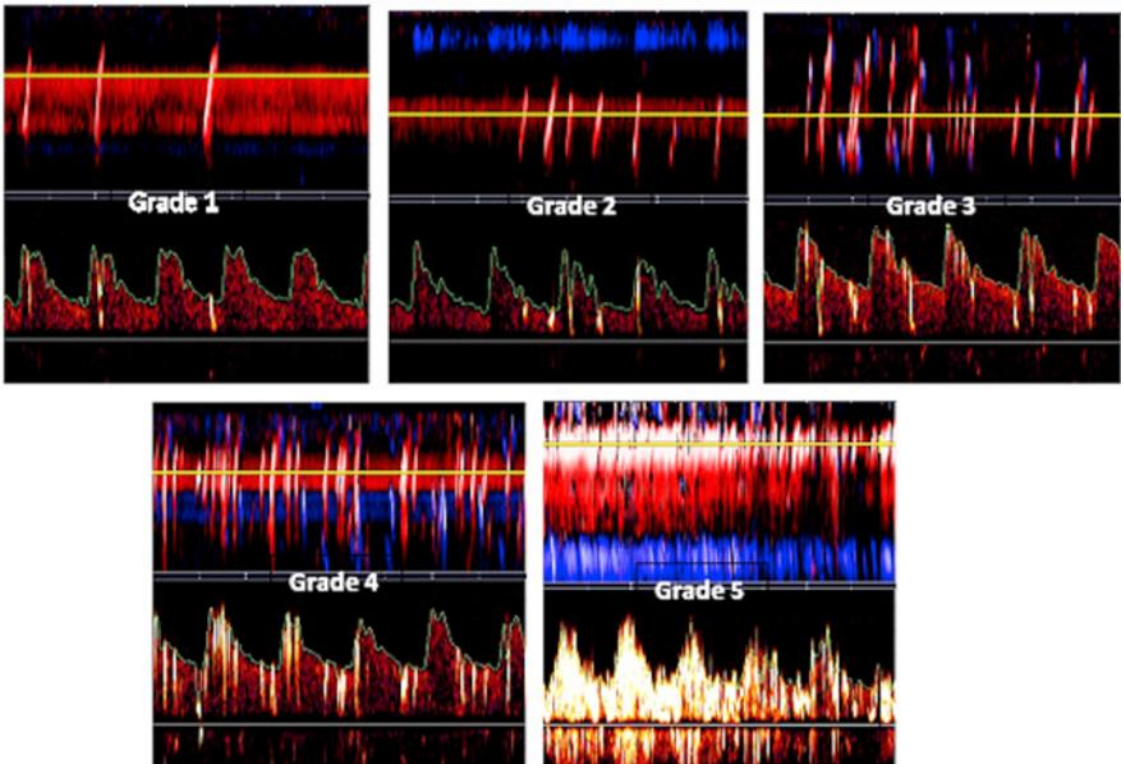


Figure 4: TCD screen shots of the 5 different grades. The presence of contrast passing through a vessel is obvious both visually (as a white mark running through the signal) and audibly. Abbreviations: TCD = transcranial Doppler. Image reprinted from 'Transcranial Doppler is Complementary to Echocardiography for Detection and Risk Stratification of Patent Foramen Ovale', Tobe et al., Page No.4, Copyright (2016), with permission from Elsevier.

Transoesophageal Echocardiography

Transoesophageal echocardiography (TOE) is commonly used for assessment of heart size, valve function, vegetation, as well as valvular regurgitation or stenosis.[76] It is considered the gold standard method of PFO diagnosis.[50] It provides high resolution imaging of the atria and interatrial septum, as the transducer (3-8 MHz) is placed in the oesophagus within close proximity to the heart.[69,77] TOE is often used following a positive or probable TTE or TCD to confirm the presence of a PFO, and to exclude the presence of additional defects, especially atrial septal defects.[77] During a TOE, patients must swallow the ultrasound probe, and sedation if often needed to minimise discomfort. Once the transducer is in position, the septum can be viewed from multiple angles allowing the best possible view for the presence or absence of a PFO (Fig. 5A). If discovered, the PFO and the associated anatomy can be assessed for suitability for transcatheter closure.[70] As in a TTE and TCD, agitated saline may be injected (Fig. 5B) and the patient may be asked to perform the Valsalva manoeuvre if no bubbles traverse at rest. Anaesthetic or sedation is normally required for a TOE and this can limit the patient's ability to perform a Valsalva manoeuvre, potentially impacting the sensitivity of this method.[73] The same shunt grade as TTE is used to assess the RLS during the TOE.

5. TOE of the heart during a Bubble Study

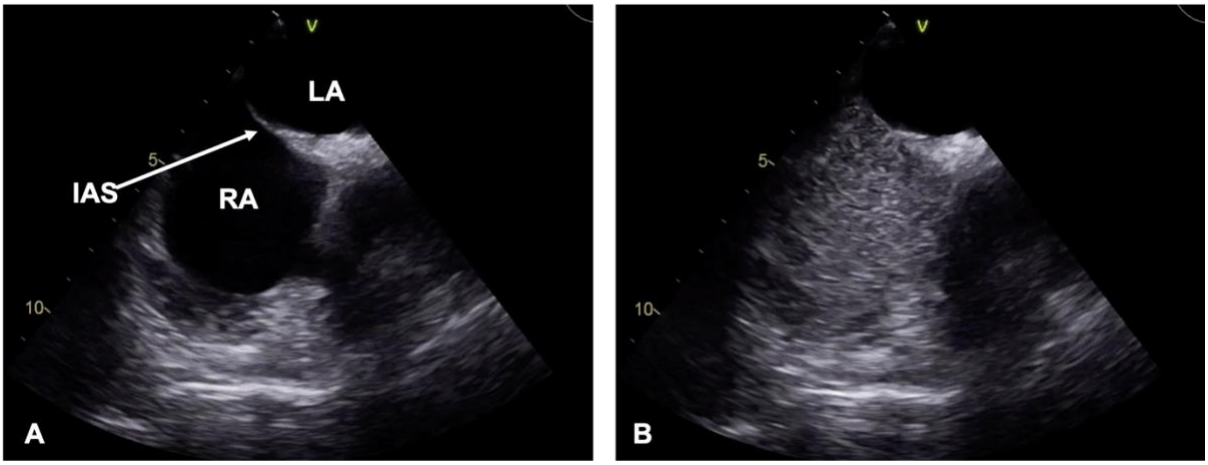


Figure 5: The heart visualised using TOE before (A), and after (B) the injection of agitated saline. Abbreviations: TOE = transoesophageal echocardiography, IAS = interatrial septum, RA = right atrium, LA = left atrium. Image obtained with permission from the Southern District Health Board.

Status of the Detection Techniques in Current Literature

There is debate around which method of detection is most sensitive and most specific for PFO detection, and studies show a significant amount of variation. Separate meta-analyses

investigating the sensitivity and specificity of each individual technique found TCD to be the most sensitive (97% for TCD, versus 88% for TTE and 89% for TOE). Contrastingly, the same studies found TTE to be the most specific (97% for TTE, versus 93% for TCD and 91% for TOE).[16,78,79] The implications of these variations can result in the false positive or false negative diagnoses of PFO. The clinical implications of a false negative diagnosis can result in the recurrence of a cerebral event due to the PFO not being identified, and therefore the correct method of treatment (if necessary) may not be made. A false positive diagnosis may result in the patient undergoing a TOE for further assessment, which comes with risk (such as damage to the throat or oesophagus).[70]

Head-to-Head comparison of TTE and TCD

Despite advocacy and professional preference for one technique over the other, few studies have conducted head-to-head comparison of these techniques. The diagnostic capabilities of TTE and TCD seem to be reasonably even, but there are methodological differences. While TTE images the heart and therefore the contrast directly, TCD images the MCA. TTE is reliant on the visualisation of microbubbles, whereas TCD can not only visualise a microbubble travelling through the MCA, but audible *blips* can also be heard.[80] TOE is often used as a gold standard reference technique against TTE or TCD, but very few studies have compared all three imaging techniques (TTE, TCD, TOE) for PFO detection in the same cohort (Table 2).[48,69,81,82] Of those that have, Gonzalez-Alujas et al., used the presence of a PFO in two of the three techniques as the gold standard.[48] They found that the sensitivity and specificity of TTE (100% and 100%, respectively) was superior to TCD (97% and 98%, respectively).[48] It is worth noting that the sensitivity of TOE was significantly lower (86%) than what might be anticipated compared to the other techniques.[48] In some cases, TOE did not detect a PFO when both TTE and TCD did resulting in a false negative diagnosis in more than 10% of the cohort.[48] In contrast, Maffe et al., reported that TOE was the optimal test. TOE detected the highest prevalence of PFO compared to TTE and TCD (PFO detected; N=62/75 vs N=55/75 and N=53/75).[69] The sensitivity of TTE and TCD was 89% and 85%, and specificity 100% and 95%, compared to TOE as the gold standard (100% and 100%).[69] The lower specificity for TCD may be due to the method detecting other malformations such as a pulmonary arteriovenous fistula,[83] or venous air embolism,[84] which would lead to a false positive diagnosis of a PFO.[82] Tullio et al., found that TCD was more sensitive than TTE. This was then attributed to suboptimal TTE image quality, led to false negative results.[81] This study was conducted in

the early 1990s and since then we have seen significant advancements in ultrasound technology, which may lessen the occurrence of false negatives using either technique.[81]

Table 2: Sensitivity and Specificity of TTE and TCD when compared to TOE

		MAFFE ET AL., 2010[69] (N=75)	GONZALEZ-ALUJAZ ET AL., 2011[48] (N=134)	TULLIO ET AL., 1993 (N=49)[81]	NEMEC ET AL., 1991 (N=32)[82]
TTE	**Sensitivity**	89%	100%	67%	54%
	Specificity	100%	100%	100%	94%
	PPV	100%	100%	100%	88%
	NPV	65%	100%	100%	74%
TCD	**Sensitivity**	85%	97%	78%	100%
	Specificity	90%	98%	100%	100%
	PPV	98%	99%	100%	100%
	NPV	53%	93%	100%	100%

Legend 2: TTE = transthoracic echocardiography, TCD = transcranial Doppler, TOE = transoesophageal echocardiography, PPV = positive predictive value, NPV = negative predictive value

Advantages and Pitfalls of Transthoracic Echocardiography

Transthoracic echocardiography can be used by the bedside, is relatively inexpensive, non-invasive, easy to use, and is a safe method of detection.[85] However, it still comes with pitfalls (Table 3). A meta-analysis investigating the diagnostic ability of TTE found that it had a pooled sensitivity of 88%, and pooled specificity of 97%.[78] To compromise on the lower level of sensitivity some studies claim that TTE is more sensitive when a cut off of <5 microbubbles is used.[51] While this technique retains a high level of sensitivity and specificity in general, it may not be suitable for the assessment of smaller shunts.[78] Image quality tends to be good in patients that are not overweight, but can be impaired when excess tissue lies between the heart and the probe.[78] Image quality can also be affected by inflated lungs caused by exaggerated inspiration prior to the Valsalva manoeuvre.[86] Suboptimal image quality can also make it difficult for the clinician to differentiate between an atrial septal aneurysm, atrial septal defect, or PFO.[13] However, the use of second-harmonic imaging has shown great improvements in the identification of smaller shunts and microbubbles.[77] An additional advantage of TTE is the

ability to differentiate between intrapulmonary shunts and intracardiac shunts. When using TTE the contrast travelling through the PFO will appear within the first three cardiac cycles,[69] whereas if the shunt is due to a intrapulmonary shunt, bubbles are likely to appear after four or more cycles.[87]

Advantages and Pitfalls of Transcranial Doppler

Like TTE, TCD comes with significant advantages and pitfalls (Table 3). The average sensitivity of TCD tends be between 93% and 100%,[68] however it tends to be less specific (78% - 100%).[53,88] While TCDs primary pitfall is the inability to differentiate between intracardiac and intrapulmonary shunts, this can be negated by considering the timing of microbubble appearance. The 'rule of 9' is used to help with specificity, as it allows the clinician to identify the presence of a PFO if at least nine microbubbles pass through the MCA and appear within 9 seconds of injection.[75,89] By evaluating the number of microbubbles travelling through the MCA, TCD is also able to assess the potential harm of the shunt. The 'curtain effect' (characterised by so many microbubbles passing through the vessel of interest that no single microbubble can be distinguished) usually presents in patients that have had a CS.[89] This can help clinicians distinguish between a 'harmful' RLS that can be caused by a mechanism such as a PFO which may cause further cerebrovascular events (such as a stroke), rather than a more 'innocent' shunt which may be an incidental finding, or is unlikely to cause recurrence due to an absence of presenting risk factors.[89] Some advantages of TCD include the combination of visual and audible cues to detect microbubbles, it being cost effective, safe and easy to perform, and unlike TTE and TOE, inflated lungs does not affect image quality.[85] Another prominent disadvantage of TCD is the reliance of a temporal bone window. Studies show that the window is missing in 10-20% of the population, and that the absence appears to be even more common in females, the elderly community, and Black and Asian populations.[71] Alternatives to the temporal window have been proposed such as the orbital window,[90] vertebral artery,[91] and vertebrobasilar circulation monitoring,[80] all which all have competitive sensitivities and specificities for PFO detection compared to the temporal window. Another pitfall of TCD is its inability to visualise the efficiency of the Valsalva manoeuvre. Unlike TTE, TCD images the MCA, and therefore the user is unable to directly assess the interatrial septum to observe if the Valsalva manoeuvre prompted a shunt, and more importantly, a shunt that included blood with contrast.[85]

Advantages and Pitfalls of Transoesophageal Echocardiography

TOE is generally considered to be the gold standard technique for PFO detection. However, Stafford et al., cumulated studies that were in agreement with the combination of TOE and TCD as a gold standard reference technique for PFO detection.[68] They found that the highest accuracy for PFO diagnosis was observed when the two techniques were combined.[68] Due to the close proximity to the heart, TOE has excellent image quality and therefore holds a significant advantage in the assessment of the septum for a specific diagnosis (i.e. PFO, atrial septal defect, atrial septal aneurysm, or intrapulmonary shunt) (Table 3). It is also able to assess the area for suitability for repair as it can grade the size and severity of the PFO clearly.[85] However, TOE tends to have a wider range of sensitivity (48 – 100%) and specificity (83 – 100%) than other techniques, and has a higher rate of false negatives.[68,92,93] Due to the semi-invasive nature of the technique it can cause injuries to the throat, oesophagus, and stomach.[86] This means the patient is often at least partially sedated, which can prevent the patient from performing an adequate Valsalva manoeuvre.[85] This makes TOE more susceptible to the false negative results and is its primary pitfall.[34,48,68,85]

Table 3: Advantages and disadvantages of TTE, TCD, and TOE for PFO diagnosis

	ADVANTAGES	DISADVANTAGES
TTE	• Easy to use • Low cost • Non-invasive • Safe • Differentiate between intracardiac and intrapulmonary shunt	• Suboptimal image quality • Lower sensitivity than TCD • Imaging window may effect image quality • Can be affected by obesity or inflated lungs
TCD	• Easy to use • Low cost • Non-invasive • Safe • More sensitive • Visual and audible cues • Not affected by suboptimal image quality	• Cannot differentiate between intracardiac shunt types • If timing is not regulated cannot differentiate between intracardiac and intrapulmonary shunts • Less specific • Patient may have the absence of a temporal window • Cannot assess the septum directly
TOE	• Superior image quality • Able to assess the septum directly for shunt size, severity and suitability for repair • Can differentiate between intracardiac shunts	• Invasive procedure • Sedation is often needed • Impairs the ability to perform an adequate Valsalva manoeuvre • Is known to diagnose false-negatives.

Legend 3*: Abbreviations: TTE = transthoracic echocardiography, TCD = transcranial Doppler, TOE = transoesophageal echocardiography*

Treatment

If left untreated, a PFO can cause recurring issues, such as CS or systemic embolism.[94] The rate of recurrent CS with no form of treatment is approximately 6% to 8% annually (with or without a PFO).[12] When the PFO is treated, whether that be medically or using a closure device, the rates of recurrence have been shown to decrease to 2% to 4% annually.[12] The RoPE score is a measure that helps determine the causal effect of the PFO to CS, and the risk of recurrence. A high RoPE score is indicative of the CS being attributable to the PFO due to the absence of traditional risk factors for ischemic stroke. However, a high RoPE score also indicates a rate of recurrence similar to that of a treated PFO (e.g. RoPE score of 10 suggests a 2% rate of TIA/stroke recurrence in the next 2 years).[13,94] This is thought to be due to the traditional risk factors of ischemia (i.e. hypertension and smoking) being a more common, and therefore more likely mechanism of recurrence than paradoxical embolism through a PFO.[94] It has also been shown that larger PFOs may indicate a lower risk of recurrent CS compared to smaller PFOs. This is thought to be due to larger shunts being the obvious cause of CS, with smaller shunts (otherwise known as 'innocent shunts') indicating the likelihood of a different, unknown mechanism causing the initial stroke, which could go on to cause a recurrent event.[95] Because the mechanism behind recurrent CS after positive PFO diagnosis is still being discussed, some patients are treated for PFO while others are not.

A PFO can be treated in several ways. The first involves PFO closure during open-heart surgery, which is extremely invasive and typically only takes place if the patient was undergoing open heart surgery for another reason. A much less invasive option is transcatheter closure of PFO (Fig. 6). In general, a catheter closure device accesses the RA via the femoral vein or the right internal jugular vein.[96,97] The transeptal sheath travels through the PFO, and the distal arm of the PFO closure device is deployed into the LA, preventing it from retracting back through the PFO. The proximal arm of the closure device, still located in the RA, is then deployed. This creates a clamp-like structure over the PFO, bringing the two layers of the septum together and essentially closing the PFO. Alongside closure techniques, medical therapy is also seen as a traditional, long-term preventative option.[18] Anticoagulants and antiplatelet agents such as warfarin or aspirin are commonly administrated to patients who are deemed to have a low-risk of a recurrent event.[5] Several studies have investigated the different methods of PFO treatment, and found that PFO closure has the best result in terms of decreased recurrence.[10,98] Khairy et al., found that over a 1-year follow up, the recurrence of medically

treated PFO patients ranged from 3.8-12%, while patients that had transcatheter closure had a recurrence range of 0-4.9%.[99] Long term studies also demonstrate the superiority of closure over medical treatment. Saver et al., found that the prevalence of recurrent CS after 6-years follow up was 2% in the PFO closure group, and 4.8% in the medically treated group.[100] Large scale studies my Mas et al., and Søndergaard et al., also suggest that PFO closure is superior to medical therapy.[101,102]

6. PFO Closure using Amplatzer device

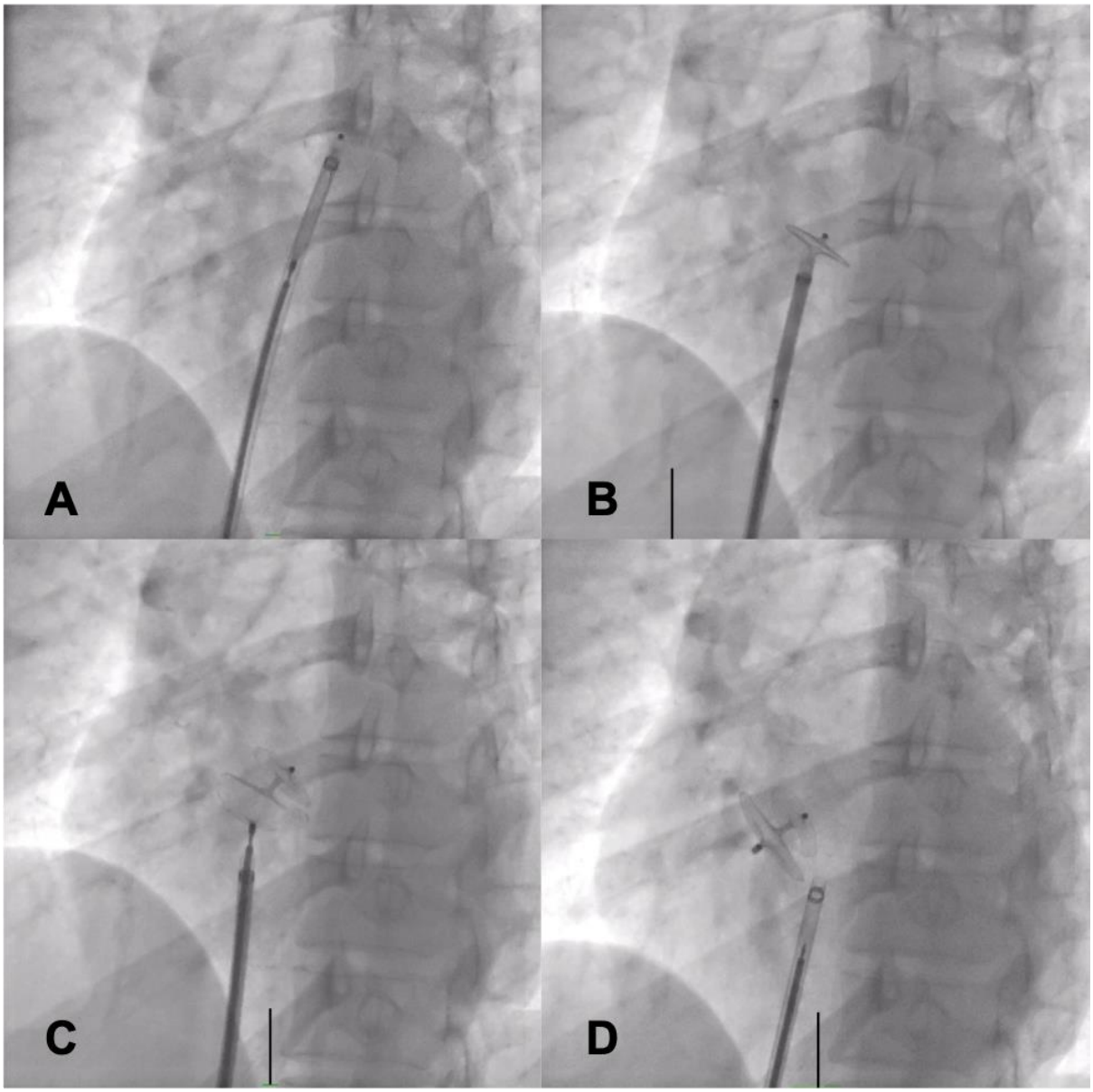


Figure 6: A-D The process of the Amplatzer device being put into place: A, the sheath is in place across the interatrial septum, with the dot indicating the distal end of the device just outside the sheath; B, the left atrial side of the device is deployed; C, the right atrial side of the device is deployed; D, the sheath is removed and the device is locked in place. Abbreviations: PFO = patent foramen ovale. Image obtained with permission from the Southern District Health Board.

Summary

The presence of a PFO is high in the general population, and higher in patients who present with CS and migraine. In young people with cryptogenic stroke, the diagnosis of a PFO indicates if and what treatment is appropriate. While there are several techniques to image this residual defect, more clarity is needed to establish the optimal diagnostic approach due to contrasting levels of sensitivity and specificity between the different methods. In this thesis I aim to compare the two bedside techniques TTE and TCD, through direct evaluation and a systematic review of the current literature for PFO diagnosis using these imaging tools.

Chapter Two: Clinical Study – Patent Foramen Ovale Detection Methods

Introduction

Patent foramen ovale (PFO) is estimated to be present in around 25% of the general population,[103] and 56% of patients with cryptogenic stroke (CS).[12] Different methods are used clinically to detect the presence of a PFO, such as transthoracic echocardiography (TTE), transcranial Doppler (TCD), and transoesophageal echocardiography (TOE). Studies have shown that all three methods are reliable for the detection of PFO, however, there are discrepancies between which method is most sensitive and most specific. While TOE is widely regarded as the gold standard with a recent meta-analysis demonstrating a sensitivity of 89%, and specificity of 91%,[16] both TTE and TCD challenge those statistics. In a meta-analysis by Ren et al., TTE was shown to have a weighted sensitivity of 88%, and weighted specificity of 97% when 16 eligible studies using TTE for the diagnosis of PFO were compared.[78] Mojadidi et al., did the same with TCD and found that it had a weighted sensitivity of 97% and a weighted specificity of 93% when 27 studies were compared.[79]

While there is debate within the literature as to which standard is considered the best, there are few head-to-head studies comparing each technique in the same patients. Maffè et al.,[69] González et al.,[48] and Zito et al.,[34] all compared the three techniques in their corresponding cohorts. The sensitivity and specificity of TTE were superior to TCD in the both studies by Maffè and González,[48,69] but the sensitivity of TTE was significantly lower than TCD in the study by Zito (94% vs. 55%).[34] Katsanos et al., conducted a meta-analysis comparing studies that used either technique against TOE to determine what may be the optimal technique and found similar results to Zito in terms of the sensitivity of TTE (pooled sensitivity of TTE 45% versus 96% for TCD).[93] While Katsanos et al., did not compare the two techniques in the same cohort, they analysed 35 studies, giving a very broad representation.[93]

Each technique has pitfalls and advantages which contribute to the discrepancies between their sensitivities and specificities. For example, TCD struggles to insonate the middle cerebral artery (MCA) when a patient has an insufficient temporal bone window, and TTE struggles to image the heart when the patient has an insufficient acoustic window due to a condition such

as obesity.[50,68] The use of the Valsalva manoeuvre is a common prompt that has shown to increase the sensitivity of all imaging modalities for PFO detection.[52] However, the application of the Valsalva manoeuvre is not consistent throughout literature, as there are multiple ways that it can be prompted. While some clinicians use a trained or untrained Valsalva manoeuvre,[104] others may use a coughing technique,[52] calibrated device,[73] or abdominal compression paired with the Valsalva manoeuvre to prompt a shunt.[54] Because the use of the Valsalva manoeuvre improves the sensitivity of each diagnostic test, the different levels of its application are likely to affect the sensitivity of PFO diagnosis between studies. The present clinical study will compare TTE to TCD in three case studies where patients have either had a stroke, or stroke like neurological symptoms. The null hypothesis of the present study is that there will be no difference between TTE and TCD as a bedside techniques for PFO detection, and the alterative hypothesis is that one of the methods will outperform the other.

Methods

Protocol

Prior to the commencement of this project an intricate protocol was established in order to outline the direction and rationale of this project, as well as describe the methods used. This protocol was used to obtain locality, Human Disability Ethics Committee (HDEC) ethical approval and Māori consultation. The full protocol can be found in the appendix.

Objectives

This project aimed to investigate the optimal process of PFO identification in patients being investigated for possible cryptogenic stroke. Specifically, the clinical sensitivity and specificity of TTE and TCD will be compared. If the patient is referred on both methods will be compared to the gold standard TOE. It also aimed to evaluate different areas where agitated saline can be imaged (interatrial septum, aortic arch, and MCA). This project aimed to provide important information about the optimal approach to PFO detection and assessment of future embolic risk by adding to the current evidence regarding PFO diagnosis.

Study Design

Eligible patients were either consented in the ward or were called prior to the appointment for a postal or email address so the patient information sheet and consent form could be sent to them. If the patient consented to this research study, the patient also had their MCA circulation

assessed with TCD during the bubble study for PFO screening in addition to the normal echocardiographic assessment (TTE, and TOE if referred on). The registered sonographer also took images of the aortic arch following a successful Valsalva manoeuvre using TTE alongside the routine imaging. The ethical application allowed for the recruitment of 40-60 patients, but due to COVID-19, and the resulting restriction of hospital access to clinical patients, a cohort of that size was not viable.

Recruitment

Patients were approached for recruitment into this study when they were initially referred to the echocardiology team for a TTE following a suspected CS or systemic embolism. This included outpatients, and inpatients in the wards. Usual clinical practice for young patients is to undertake a bubble study during the same inpatient appointment as the structural TTE. During recruitment, it was made clear to the patients that the additional TCD test was to be conducted on the same day as the TTE, to avoid a return visit to the hospital. Only patients that fit into the following criteria were approached for this study.

Inclusion

- Patients that have been referred to the echocardiography service for a routine TTE after a CS or systemic embolus.
- Over the age of 18.
- Willing and able to provide informed consent.
- Willing and able to comply with the study procedures.

Exclusion

- Have another identified potential cause of cardiac emboli other than PFO in their routine TTE (e.g. left ventricular thrombus, myxoma, vegetation on valve).
- Patients who are unable to perform the Valsalva manoeuvre.
- Pregnancy.

Informed Consent

Written informed consent was obtained prior to any data being collected. All participants were provided with a HDEC-approved participant information sheet and consent form that outlined the study rationale and expectations of them as participants. Additionally, as the head is regarded as Tapu (i.e. sacred) and cannot be touched in certain cultures, consent was granted by the participant following the complete understanding and agreement of the procedure that

would take place. They were then given the opportunity to ask questions, and if they chose to participate they signed the consent form. The consent process was documented, and the original consent form was filed in the research record. Participants were able to withdraw consent at any time and they were informed that the quality of their clinical care would not be adversely affected if they did not want to participate in this study.

Confidentiality

All study records and data collected during and after the study were stored in a secure area at the study institution. Each participant was given a unique study identifier that all the data was linked to. Data was held in a single central database. This database may be used for international comparisons in the future. If this were to be the case all analyses would be undertaken using the unique participant identifier and no information about participants would be released without their express permission. The only reason for doing so would be at the participant's request or as a requirement for clinical follow-up. The latter would only occur if the participant granted permission. When data is released (for example in publications) all patient identifiers will be omitted.

Risks and Benefits

A minor potential risk was discomfort from the pressure of the ultrasound transducer and so measures were taken at all times to ensure the patient felt comfortable. There is typically a small amount of transient pain associated with the intravenous cannula used with the agitated saline injection, however this is part of the patients routine TTE. Every effort was made to minimize any discomfort the patient felt.

The primary benefit produced of this study was adding to the literature regarding the management and assessment of CS patients, as well as the optimization of PFO detection methods. Patients that participated in this study also benefited from the additional assessment using TCD may have provided the clinician with more clarity for the diagnosis. This may have provided additional direction as to whether or not a TOE was to be conducted for further assessment regarding PFO closure.

Ethics

Ethics was sought via the HDEC expedited review pathway. Originally it was believed that Otago University Human Ethics Committee (OUHEC) (health) was needed, which is the

official Otago University ethics committee. This was declined after consideration and the application was escalated to HDEC. The final approval reference code is 19/CEN/201. Locality was also sought after and approved following HDEC ethical approval. This was approved under the project ID: 01590. Māori Consultation was sought, and support was given following the review of the research project.

Amendments

Once ethical approval was obtained, the time remaining for patient screening in this study was limited, and the number of in-patients that were eligible for the study were scarce. The decision was made to apply for an ethics amendment via HDEC to allow us to recruit out-patients that had already had their TTE completed, to invite them to attend for the TCD testing only. This amendment was provisionally approved, but by that time was no longer viable due restricted access to clinical patients for research personal as a result of COVID-19.

Personnel

Registered cardiac sonographers performed the TTE and I performed the TCD. As the testing was undertaken at the same time, blinding was not possible. However, when the testing was taking place I was focused on the TCD and ensuring the signal was strong throughout the Manoeuvre so I did not know the results of the TTE until they were given to me at a later date. The sonographer gave me the results of the TTE without knowledge of the TCD results. The sonographer was experienced and registered so the ability to conduct an adequate test for PFO presence was assumed. A reliability study in healthy volunteers was undertaken before testing the patients to establish technique proficiency, which is described prior to the procedures for the clinical patients. This involved 12 trial runs with participants, however, prior to this I had practised the technique thoroughly on volunteers and they were confident in my proficiency to perform the test.

Reliability Testing of Transcranial Doppler

Reliability testing was conducted on five healthy volunteers. Originally, participants were asked to come in three times over a period of months, however, due to COVID-19, only 2/5 participants were able to complete all three trials, while the other 3/5 completed two trials. Trials were conducted at the same time on separate days. Participants were asked to keep factors that may influence their blood pressure or heartbeat consistent over the three trials, such

as coffee ingested, or method of commute into work. For the setup of the TCD, around 20ml of gel was placed on the participant's temple just anterior to their ear. This was smoothed out to remove air bubbles within the gel that would disturb the signal. The headpiece was then placed over the participant's head, with the backstrap sitting low and near the external occipital protuberance. The headpiece was then tightened slightly, but not completely. 50ml of gel was put on top of the probe, which was then put into place on the headpiece. The headpiece was then tightened so it was secure, but not tight enough to cause the participant discomfort. Once the probe was pressed onto the participant's temporal bone window, it was turned on. The power of the ultrasound transducer was at 10% when it was turned on, but to obtain the optimal flow it was raised to 100% for the duration of the trial. The depth of the MCA is usually between 30-60mm deep if imaged from the temporal window.[105] The use of the audible sound signal was used to distinguish between different cerebral vessels, as the MCA emits a higher-pitched sound compared to the posterior cerebral artery.[106] Flow direction of the MCA was towards the probe (visualised as red). If the insonated vessel was deeper than 70mm, and was still flowing towards the probe it was deemed likely to be the posterior cerebral artery,[107] and the probe was readjusted in order to find the MCA. Once the optimal signal was obtained, the probe was tightened onto the headpiece so the signal would not be disturbed when the participant later performed the Valsalva manoeuvre. Participants were asked to remain still for 5 minutes while the baseline test was conducted.

The next stage involved the participants performing the Valsalva manoeuvre by blowing into a calibrated sphygmomanometer. A nose piece was put on them, and they were asked to take a large breath in, and then to blow out into the mouth piece maintaining 40 mmHg on the sphygmomanometer that they could see. They were asked to hold this exhalation for 10-15 seconds maintaining it at 40 mmHg. If the Valsalva manoeuvre was unsuccessful, the participants were asked to rest for 2 minutes before trying again to allow for the cerebral velocity measures to normalise. The TCD device (Spencer Doppler ST3, model PMD150, Redmond, WA, USA) was attached to an analogue-to-digital converter (Powerlab, ADInstruments, Dunedin, NZ). This was interfaced to a personal computer running specialised research software (Labchart 8, ADInstruments, Dunedin, NZ) for data collection and storage at a sampling frequency of 1 kHz for later analysis. The MCA flow velocity envelope was collected during the 5 minute baseline period, and across the Valsalva manoeuvre. The data measured included the mean (which was obtained by integrating the waveform across the entire cardiac cycle), systolic and diastolic MCA flow velocities. For the baseline test, the last 2

minutes of the 5 minute baseline period was analysed. Additionally, the lowest (for phase 2) and highest (for phase 4) beat were measured during the Valsalva manoeuvre.

Reliability Statistics

Reliability was conducted in order to determine the reproducibility of my ability to insonate the MCA over several different trials in five participants. A paired two tailed t-test was completed to test for differences between trials 1 and 2 using Excel 2016. No formal statistics were carried out investigating differences across all three trials as only 2/5 participants completed all three trials which resulted in inadequate statistical power to complete a repeated measured ANOVA. Additionally, a consecutive pairwise test from the analysis of reliability template by Hopkins (2015) was used to estimate the reproducibility.[108] The intraclass correlation coefficient (ICC), Pearson correlation coefficient, and the coefficient of variation were used to help determine the reliability.[108] Reliability testing was calculated between trials 1 and 2, and between trials 1, 2, and 3.

Valsalva Manoeuvre

The Valsalva manoeuvre was initially going to be performed by the patients using a calibrated device. However, in the first case study the patient blew against the device as instructed but the clinician indicated that the TTE image was distorted due to inflated lungs. Following this the decision was made to exclude the use of the calibrated device as a method of Valsalva manoeuvre for the remainder of the study. The standard Valsalva manoeuvre used by clinicians was then applied for the continuation of that case study. This involved a verbal prompt to bear down and force exhalation against a closed glottis or thumb in mouth, and was used for the rest of the case studies. In one case, abdominal compression was applied during a normal Valsalva manoeuvre.

Transthoracic Echocardiography

- Echocardiography was performed by a sonographer using standard echocardiographic machines (Vivid S6, E9 or E95, GE Ultrasound or SC2000Prime, Siemens Ultrasound). Images were recorded according to the recommendations of the American Society of Echocardiography. Views for left ventricular (LV) and left atrial (LA) assessment included the apical 4-chamber, apical 2-chamber, apical long axis and short-axis views. Images of the right ventricle (RV) and right atrium (RA) were recorded in the apical 4-chamber view.

- Saline was injected into the arm using a three-way tap, and two syringes of saline. In the three way tap the saline was mixed with a little bit of drawn up blood (1ml) to agitate the saline for optimal imaging. This approach was consistent for all participants.
- Following the injection of the agitated saline, the heart was continuously imaged and recorded as the patient performed the Valsalva manoeuvre.
- A PFO was positively identified on TTE if a bubble, or bubbles were seen in the left atrium or left ventricle and were graded as follows: mild (<10 microbubbles), moderate (10-20 microbubbles), or severe (>20 microbubbles)

Transcranial Doppler

- In most cases, a routine echo had already taken place, or a PFO was suspected, so the sonographer proceeded straight to a bubble study. Seeing as the patient had already given consent prior to this stage, I gave a brief re-explanation of the TCD procedure as I put the device on. The TCD device was set up and put on the same way as it was for the participants in the reliability study, and I used the probe to secure an optimal signal of the MCA. In some cases, it was harder than others and I had to ask for help from my supervisor to get a clear signal.
- Just prior to the injection of the agitated saline contrast, the record button was pressed on the TCD device. The patient was then asked to perform the Valsalva manoeuvre which was considered effective when there was a peak Doppler flow velocity reduction > 25% in the MCA. Once all necessary tests were made the TCD was turned off and the probe and headband were unattached and removed from the patient, who was then given a tissue to remove the gel. The TCD device was then packed up and removed from the room swiftly for sterilization and preparation before the next patient.
- The pulsed wave M-mode on the TCD device was used to assess the PFO and subsequent shunt in patients. The sweep settings were set on 4 seconds per frame. The TCD machine recorded both visual and audio recordings following the saline injection, and Valsalva manoeuvre. The presence of a microbubble could be visualised as a brightly coloured embolic track as it passed through the MCA. The PFO was graded as followed: grade 1 (1 – 10 microbubbles), 2 (11 – 30 microbubbles), 3 (31 – 100 microbubbles), 4 (101 – 300 microbubbles), and grade 5 (>300 microbubbles).

Transoesophageal Echocardiography

No patients in this study had a TOE in the time period of this study, either due to COVID-19, or because it was not clinically indicated.

RoPE Score

The Risk of Paradoxical Embolism (RoPE) score (Table 1, page 4) is used in patients with suspected cryptogenic stroke which are positive for PFO, to determine the probability of the CS being due to a PFO, and how likely recurrence is (whether through a PFO or due to another unrelated cause).[31] It was developed in 2013 by Kent et al., and was applied to all three case studies in the present study.

Results

Reliability Results

The middle cerebral artery blood velocities (MCAv) for the baseline, phase 2, and phase 4 measurements for the five participants is presented in Table 4. The t-test showed that the systolic, mean and diastolic MCAv (cerebral artery blood velocity) did not significantly differ between trial 1 and trial 2 ($p \geq 0.41$). The reliability during baseline and in response to the Valsalva manoeuvre is presented for the five participants on Table 5 (for trials 1 and 2), and Table 6 (for trials 1, 2, and 3). The ICC is moderate throughout the baseline activity of all measurements (systolic MCAv, Mean MCAv, and Diastolic MCAv) when trials 1 and 2 are just compared, and when trial 3 is included (ICC ranges from 0.72 to 0.87). Cicchtti et al., indicates that an ICC of over 0.7 is indicative of internal consistency for research.[109] The coefficient of variation is satisfactory for all results over all trials, indicating satisfactory absolute reliability.

Table 4: Middle cerebral artery flow velocities for each trial conducted for baseline, phase 2 and phase 4.

CONDITION		TRIAL 1			TRIAL 2			TRIAL 3		
	Participant	Systolic MCAv	Mean MCAv	Diastolic MCAv	Systolic MCAv	Mean MCAv	Diastolic MCAv	Systolic MCAv	Mean MCAv	Diastolic MCAv
BASELINE	1	73	45	32	80	47	33	90	52	38
BASELINE	2	102	68	49	82	54	38	97	64	46
BASELINE	3	90	61	44	95	63	43			
BASELINE	4	91	61	45	92	60	43			
BASELINE	5	103	65	47	109	71	52			
PHASE 2 LOWEST BEAT	1	60	37	28	74	41	29	73	44	35
PHASE 2 LOWEST BEAT	2	84	47	32	75	41	27	74	45	36
PHASE 2 LOWEST BEAT	3	75	49	39	82	56	44			
PHASE 2 LOWEST BEAT	4	97	58	43	84	47	35			
PHASE 2 LOWEST BEAT	5	85	52	35	75	40	32			
PHASE 4 HIGHEST BEAT	1	81	60	46	94	68	50	110	59	47
PHASE 4 HIGHEST BEAT	2	103	80	66	106	81	63	84	66	53
PHASE 4 HIGHEST BEAT	3	105	75	55	111	78	55			
PHASE 4 HIGHEST BEAT	4	103	66	43	92	71	59			
PHASE 4 HIGHEST BEAT	5	122	80	59	101	65	47			

Legend 4: Abbreviations, MCAv = Middle cerebral artery velocity

Table 5: Reliability Results between trials 1 and 2

RESULTS USING TRIALS 1-2		ICC	PEARSONS	COEFFICIENT OF VARIATION
BASELINE	Systolic MCAv	0.74	0.64	12.9%
	Mean MCAv	0.82	0.73	15.10%
	Diastolic MCAv	0.82	0.73	16.10%
PHASE 2	Systolic MCAv	0.47	0.63	13.00%
	Mean MCAv	0.41	0.35	15.50%
	Diastolic MCAv	0.85	0.77	18.20%
PHASE 4	Systolic MCAv	0.46	0.45	11.50%
	Mean MCAv	0.5	0.44	10.90%
	Diastolic MCAv	0.29	0.26	14.90%

Legend 5: Baseline measurement was taken over the last 2 minutes of the 5 minute baseline period. Phase 2 measured the lowest beat, while phase 4 measured the highest beat during the Valsalva Manoeuvre. Abbreviations: ICC = intraclass correlation coefficient, MCAv = middle cerebeal artery velocity

Table 6: Reliability Results between trials 1, 2 and 3

RESULTS USING TRIALS 1, 2 & 3 (MEAN ICC)		ICC	PEARSONS	COEFFICIENT OF VARIATION
BASELINE	Systolic MCAv	0.78	0.64	12.30%
	Mean MCAv	0.87	0.73	15.10%
	Diastolic MCAv	0.87	0.73	15.80%
PHASE 2	Systolic MCAv	0.55	0.63	12.40%
	Mean MCAv	0.51	0.35	14.70%
	Diastolic MCAv	0.87	0.77	17.10%
PHASE 4	Systolic MCAv	0.12	0.45	12.50%
	Mean MCAv	0.58	0.44	10.80%
	Diastolic MCAv	0.38	0.26	14.50%

Legend 6: Baseline measurement was taken over the last 2 minutes of the 5 minute baseline period. Phase 2 measured the lowest beat, while phase 4 measured the highest beat during the Valsalva Manoeuvre. Abbreviations: ICC = intraclass correlation coefficient, MCAv = middle cerebeal artery velocity

Case Study Results

Due to COVID-19, this study only managed to recruit 3 patients prior to the hospital being closed to non-essential users. The attributes and results of each of these case studies will be explained in depth below, and the general characteristics and findings can be found on Table 7.

Table 7: Characteristics, findings and decisions of the three case studies in the present study

	CASE 1	CASE 2	CASE 3
AGE (YEARS)	55	61	52
SEX	Female	Male	Female
HEIGHT (CMS)	164cm	174cm	157cm
WEIGHT (KG)	57kg	95kg	94kg
CLINICAL PRESENTATION	Multiple Neurological symptoms and chest pain	Ischemic stroke	Multiple Neurological symptoms and collapse
HYPERTENSION	Yes – on Losartan 25mg	No	Yes
DIABETIC	No	No	Yes
SMOKER	No	No	Yes
OTHER RISK FACTORS	Dyslipidaemia – on Atorvastatin 10 mg	Dyslipidaemia – on Atorvastatin 40 mg Overweight Family history of myocardial infarction	Dyslipidaemia – on Atorvastatin 20 mg Overweight Obstructive Sleep apnoea on CPAP
ROPE SCORE	5	5	3
ECG FINDINGS	No abnormal findings	No abnormal findings	Ventricular Trigeminy on presentation but reverted back to normal rhythm on follow up
GENERAL ECHO FINDINGS	No LV systolic dysfunction EF 55-60% Mildly dilated LA TAPSE 2.5cm	No abnormal findings EF 55% Mildly dilated LA TAPSE 2.7cm	Reduced ejection fraction (45-50%) Wall motion abnormalities (inferior, inferior lateral and possible anterior)
PFO YES/NO	Yes	Yes	Yes
TTE RESULT	Mild shunt	Severe shunt	Severe shunt
TCD RESULT	Grade 1 (mild shunt)	Grade 5 (severe)	N/A
DECISION	PFO incidental finding	PFO closure using amplatzer	PFO incidental fining

Legend 7: PFO = patent foramen ovale, CPAP = continuous positive airway pressure, LV = left ventricle, LA = left atrium, EF = ejection fraction, TAPSE = tricuspid annular plane systolic excursion.

Case Study #1

This 55 year old female patient who was of NZ/European ethnicity arrived to the echocardiography laboratory as an outpatient who had experienced multiple neurological symptoms and chest pain. A bubble study was conducted to assess possibility of CS through a PFO. The patient had no history of smoking and did not have diabetes. Losartan 25 mg was being used to treat hypertension, and Atorvastatin 10 mg was being used to treat dyslipidaemia. The echocardiogram revealed an ejection fraction (EF) of 55-60%, a mildly dilated LA, and normal RV size and function. The tricuspid annular plane systolic excursion (TAPSE), which is a parameter of global RV function, was normal at 2.5cm, and the RV systolic pressure was normal at 22mmHg+RA (about 5mmHg). The RoPE score for this patient, if a CS was found to be the cause of dysfunction, was 5. It took a few minutes to locate the MCA and secure a strong signal using the TCD. Initially the sphygmomanometer was used for the provocation of the Valsalva manoeuvre, but after the first attempt the clinician in charge of the TTE observed inflated lungs which obstructed the image of the TTE. From there a non-calibrated Valsalva manoeuvre was used. The aortic arch was imaged for the presence of microbubbles with none seen. TCD showed the passage of a small number of potential microbubbles and graded the shunt a 1 (1 – 10 microbubbles) (Fig. 7A). TTE also showed the passage of microbubbles into the LA and LV, and the shunt was classed as mild (<10 microbubbles) by the clinician using the TTE grading scale (Fig. 7B). Later on, the patient underwent a structural magnetic resonance imaging (MRI) to identify the presence/absence of a cortical infarct, none of which was found. The PFO was then later deemed to be an incidental finding as there was no stroke on the follow-up MRI.

7. TCD and TTE in Case 1

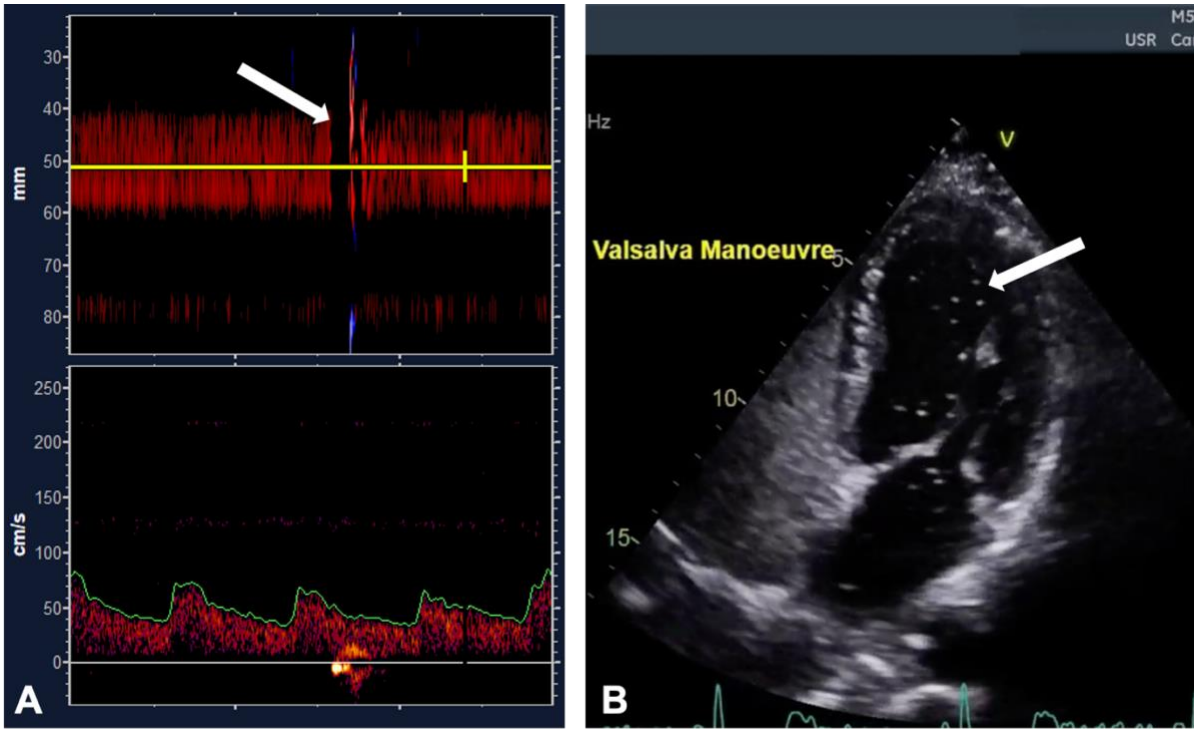


Figure 7: (A) TCD of the middle cerebral artery, arrow points at a potential microbubble travelling through the artery. (B) TTE of the heart (apical 4-chamber view), arrow indicates a microbubble in the LV, indicating the presence of a PFO. Abbreviations: TCD = transcranial Doppler, TTE = transthoracic echocardiogram, LV = left ventricle, PFO = patent foramen ovale.

Case Study #2

This 61 year old male who was of NZ/European ethnicity arrived to the echocardiography laboratory as an outpatient after an ischemic stroke. A bubble study was undertaken to assess for the presence of a PFO. The patient had family history of myocardial infarction, and was overweight. Atorvastatin 40mg was being used to treat dyslipidaemia. The patient did not have hypertension, had no history of smoking, and did not have diabetes. The structural echocardiogram revealed an EF of 55%, a mildly dilated LA, and normal RV size and function. The TAPSE was normal at 2.7cm, and the RV systolic pressure could not be assessed, but appeared normal. This patient had a RoPE score of 5, which indicated a 34% chance of the stroke being due to a PFO, and a 7% chance of a similar recurrent event. A subcostal view using colour Doppler of the interatrial septum displayed movement of venous blood from the RA to the LA, presumably through a PFO (Fig. 8). During one of the contrast injections the clinician used abdominal compression to provoke a Valsalva manoeuvre, which improved the sensitivity of both techniques compared to a normal Valsalva manoeuvre (Fig. 9 and 10). TTE showed the passage of microbubbles into the LA and LV during normal Valsalva manoeuvre

and when abdominal compression was applied during the Valsalva manoeuvre, and the shunt was classed as severe (>20 microbubbles) by the cardiologist using the TTE grading scale (Fig. 9A and 9B). TCD indicated the presence of a grade 2 PFO (11 – 30 microbubbles) during the normal Valsalva manoeuvre (Fig. 10A), but demonstrated a grade 5 PFO (shower curtain, >300 microbubbles) when abdominal compression was applied (Fig. 10B) The aortic arch was also visualised for the presence of any microbubbles travelling to the periphery, and 2 microbubbles were detected travelling down the aortic arch. This patient was referred on for AMPLATZER closure of PFO which was occluded (25mm) successfully. TOE was not used to assess or confirm the presence of a PFO prior to this.

8. TTE of the IAS in Case 2

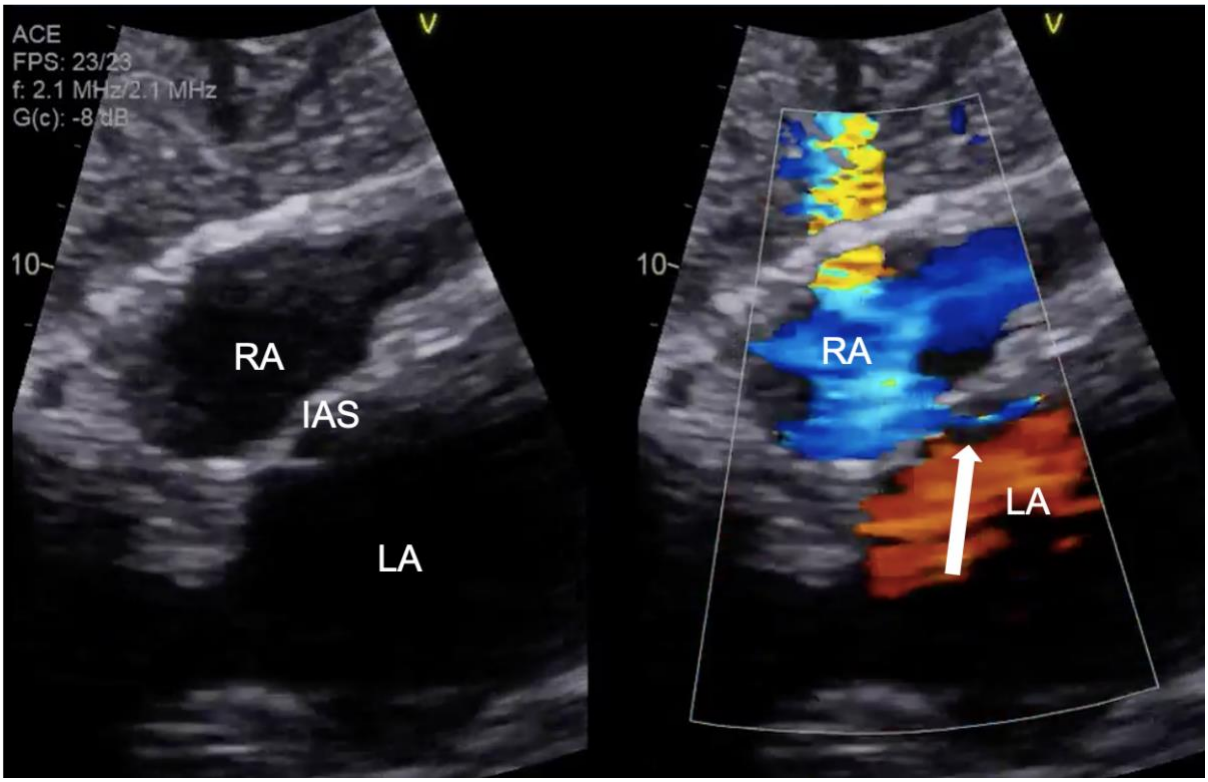


Figure 8: Subcostal view using TTE of the IAS, where venous blood (blue) can be seen travelling from the RA into the LA, and mixing with arterial blood (red). Abbreviations: TTE = transthoracic echocardiography, IAS = interatrial septum, RA = right atrium, LA = left atrium

9. TTE in Case 2

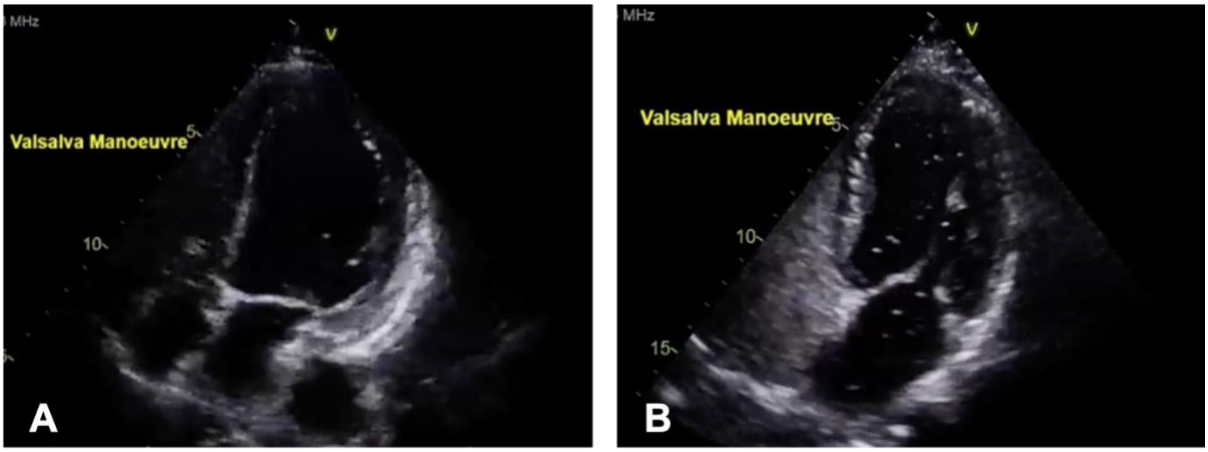


Figure 9: TTE of the heart during (A) normal Valsalva manoeuvre, (B) Valsalva manoeuvre with abdominal compression. Abbreviations: TTE = transthoracic echocardiography

10. TCD in Case 2

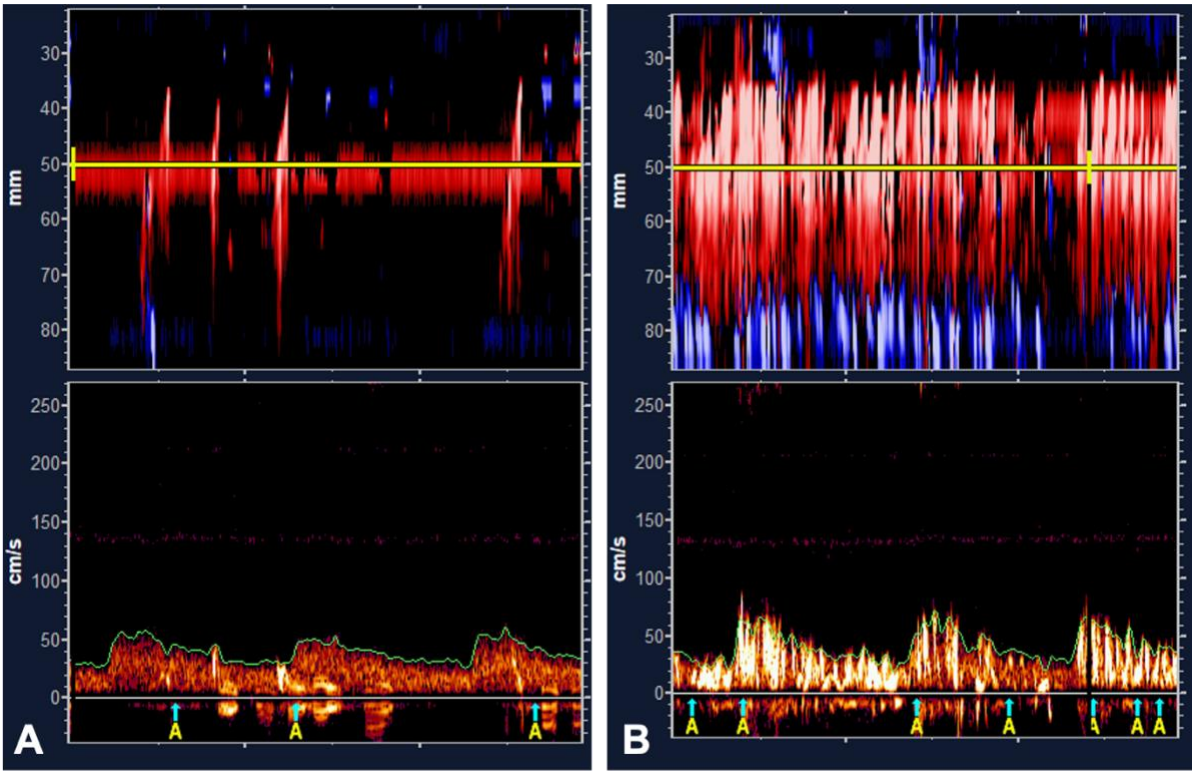


Figure 10: TCD imaging of the middle cerebral artery. (A) Microbubbles visible during normal Valsalva manoeuvre, (B) Shower 'curtain' of microbubbles observed during abdominal compression paired with the Valsalva manoeuvre. Abbreviations: TCD = transcranial Doppler

Case Study #3

This 52 year old female who was of NZ/European ethnicity arrived at the echocardiography laboratory as an inpatient with neurological symptoms and collapse. The previously conducted computerised tomography (CT) scan and MRI showed no relevant abnormalities but a PFO was still suspected. The patient had hypertension, and diabetes, was a smoker, and was overweight. The patient also had obstructive sleep apnoea which was being treated with continuous positive airways pressure (CPAP). Atorvastatin 20mg was being used to treat dyslipidaemia. The echocardiogram revealed an EF of 45-50%, and the patient appeared to have wall motion abnormalities (inferior, inferior lateral and possible anterior). The RoPE score for this patient, if a CS was found to be the cause of dysfunction, was 3. No cerebral vessel velocity signals could be found, which was likely to be due to a poor temporal bone window. TCD was therefore unsuccessful in this patient. TTE showed the passage of microbubbles into the LA and LV at both rest (Fig. 11A) and during the Valsalva manoeuvre (Fig. 11B), and the shunt was classed as severe (>20 microbubbles) using the TTE grading scale. The aortic arch was imaged and several microbubbles were seen travelling to the periphery (Fig. 12). The PFO was then later deemed to be an incidental finding as there was infarct on the MRI, and the symptoms the patient presented with were likely to have been caused by a separate risk factor (such as hypertension).

11. TTE in Case 3

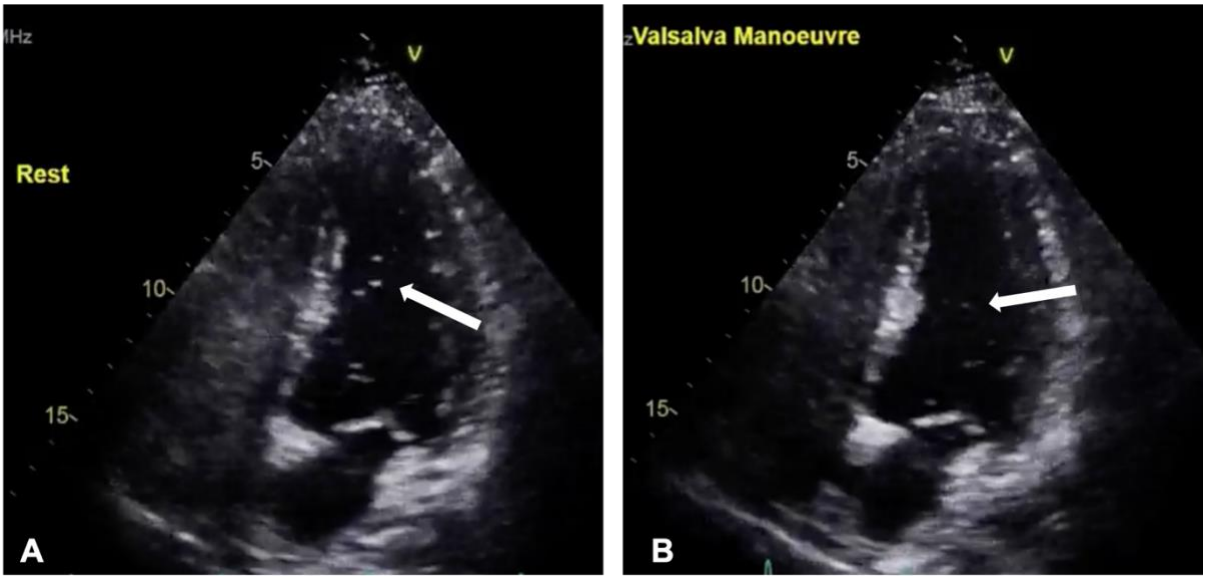


Figure 11: Transthoracic echocardiogram of the heart. (A) Arrow indicates microbubbles in the left ventricle at rest, (B) indicates microbubbles in the left ventricle during the Valsalva manoeuvre. Abbreviations: TTE = transthoracic echocardiography

12. TTE of the Aortic Arch in Case 3

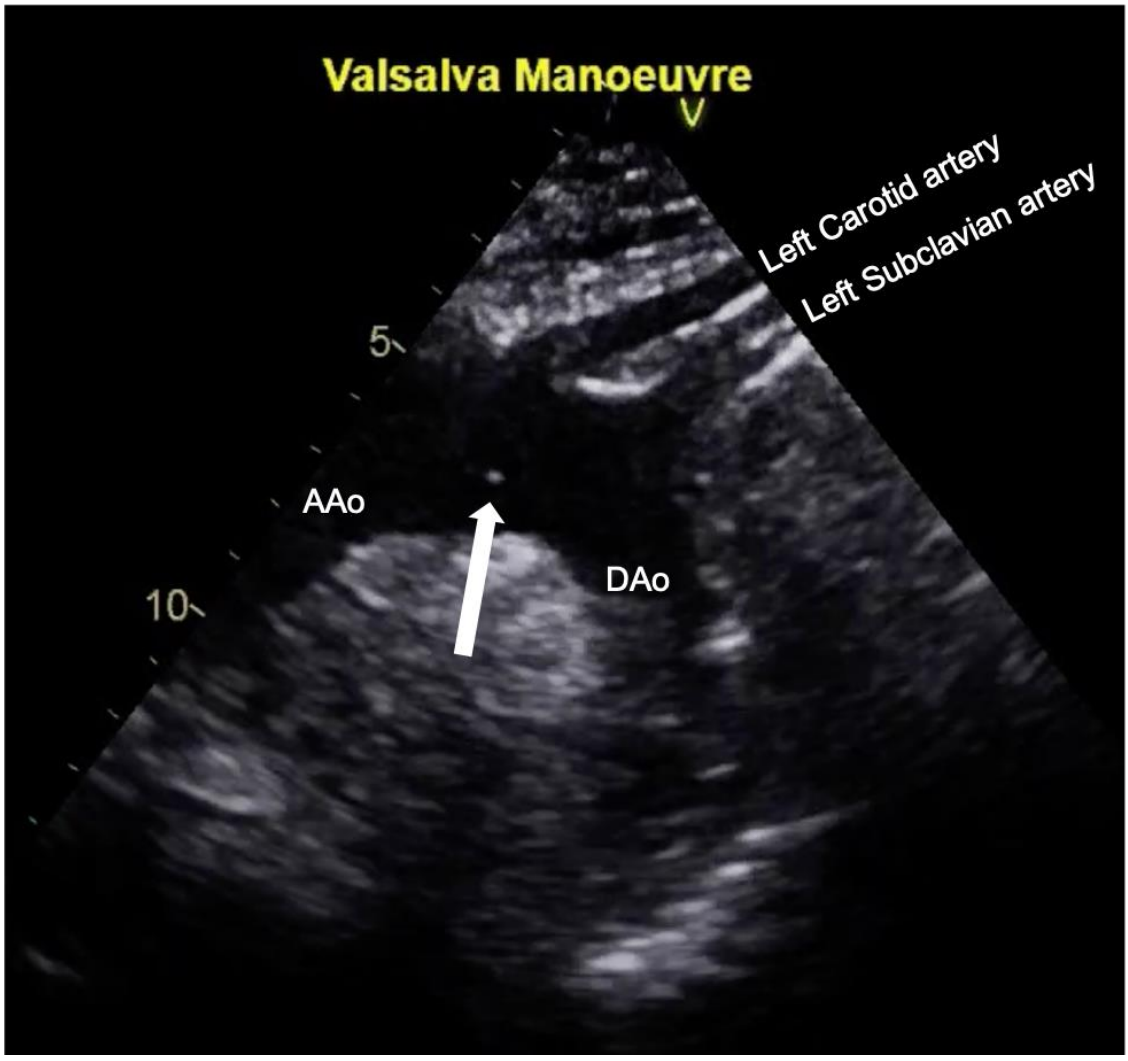


Figure 12: View of the aortic arch using TTE. The arrow indicate the microbubble as it travels through the arch, past the left carotid artery and subclavian artery, before travelling down to the periphery. Abbreviations: TTE = transthoracic echocardiography, AAo = ascending aortic arch, DAo = descending aortic arch

Discussion

Reproducibility Results

The reliability testing demonstrated the reliable insonation of the MCA in the same participants over a series of trials. This allows us to assume that the arteries insonated in the present study are correct and accurate. The ICC indicates the 'relative reliability', and estimates the consistency of multiple measurements taken on separate occasions in an individual.[110] The coefficient of variance is an indicator of the amount of error associated with those multiple measures, and indicates the absolute reliability.[111] These assessments were made to ensure that any variance within the data comes from between the participants, not between the variables

measured (i.e. the MCA of one participant may be deeper than the MCA of the other, so this testing would indicate differences between separate tests on the same participants, not between participants). The ICC for the baseline measurements was satisfactory, indicating satisfactory reproducibility. However, the ICC is reduced during phase 2 and phase 4 compared to baseline in both sets of results over all measurements, which is likely to be due to differences in the Valsalva manoeuvre. The Valsalva manoeuvre is a dynamic physiological response, and therefore it commonly has a variable physiological response associated with it. Additionally, patients often contract their head muscles when performing the Valsalva manoeuvre which can disturb the flow signal and subsequent velocity value. Despite the more moderate ICC over the Valsalva manoeuvre, it still indicates the ability to maintain a good quality velocity signal across and following a Valsalva manoeuvre which is critical for the detection of microbubbles if a right-to-left shunt (RLS) is present. Lastly, the ICC appeared to increase over all of the measurements when comparing results from just trials 1 and 2, to trials 1, 2, and 3. This is indicative that the ICC would have continued to improve should all trials have occurred three times as planned.

Similarities between the Cases

The prevalence of a PFO in CS patients is higher than that of the general population (40% vs 25%),[26] especially in young (<55 years) CS patients (up to 55%).[88] We report three patients with suspected CS or confirmed ischemic stroke who were referred on to the cardiology department for a bubble study for investigation of a possible PFO. Despite the similarity in age (ranges from 52 to 61 years), the three patients had varying risk-factors and pre-existing health conditions (see Table 7). All three patients were found to have a PFO, and both TTE and TCD were in eventual agreement with the determination of shunt severity in all patients, except case 3 where TCD was unsuccessful due to an absent temporal bone window.

The degree of shunt determination in case 1 was relatively consistent between TTE and TCD, with both methods grading the shunt as mild from their respective shunt grades. While this consistency is positive, studies have shown that smaller shunts are often indicative of an 'innocent shunt' that poses no further threat.[13] This was later confirmed when the MRI showed no cortical infarct, and the PFO was deemed to be an incidental finding. Case 1 had a RoPE score of 5, indicating a 34% chance of the presenting condition to be due to PFO, and a 7% chance of general recurrence. Although case 1 did not end up presenting with a infarct on

imaging, this reinforces the importance of the RoPE score and how it may shape the decisions the clinician makes in terms of discovering a potentially innocent PFO vs a potentially dangerous PFO, and how to go forward with treatment.[94]

Studies have shown that a RoPE score of above 7 is highly indicative of the PFO being causal to the CS.[32] However, other studies have indicated that a lower rope score (1-7) is related to an increased chance of recurrence compared to a RoPE score of >7 (12.9% vs 5.4% respectively), especially in patients that present with a transient ischemic attack (TIA).[95] This is thought to be due to other present risk factors for stroke being more likely to cause embolism compared to a RLS through a PFO.[94] While the RoPE score for patient 2 was also 5 (regarded as a low RoPE score), the shunt was classed as severe. Since the patient presented with ischemic stroke on MRI, and demonstrated a large RLS using both methods, as well as when imaging the interatrial septum, the decision to close the PFO was made. When compared to case 3, who also had a severe shunt diagnosed by TTE, case 2 presented with less risk factors that may have attributed to the initial event, therefore making the case for closure much more compelling. While case 3 still presented with a large RLS, the RoPE score was 3, and the pre-existing health conditions (i.e. was a smoker, had diabetes and hypertension) meant the chances of the PFO being causal to the CS were unlikely. It also indicated that the chances of recurrent stroke through a mechanism unrelated to a PFO were much more likely, and therefore the closure of the PFO was unlikely to make a difference.

While the RoPE score is relatively new and is still being applied in up-and-coming studies, its relevance in this present clinical study is high as it indicates the importance of a correct and accurate PFO diagnosis. If TTE and TCD are not correctly identifying the attributes of a PFO, the subsequent decisions made by the clinician may not be the most suitable for the patient. This highlights the need for further investigation into what may be the optimal method of PFO diagnosis, and the exposure of the pitfalls and advantages of each technique so they can be adapted to appropriately.

Challenges of both Techniques

All three cases exposed both positive and negative attributes of TTE and TCD. While case 1 was the most consistent in terms of grading the severity of the PFO, TTE was impaired by inflated lungs when the sphygmomanometer was used to provoke the Valsalva manoeuvre.

TTE can be impaired by over inflation of the lungs in a patient, due to them taking too large of a breath in prior to the Valsalva manoeuvre,[86] as may have been the case in this particular patient. Some studies have shown that the sphygmomanometer is a good method of provoking a change in interatrial pressure to mimic the Valsalva manoeuvre,[112] but other studies have criticised it.[52] When using a sphygmomanometer the patient is required to maintain a 40mmHg pressure. However, it is impossible to know whether that is being maintained by palatal closure or elevated intrathoracic pressure.[52] In this particular case the inflated lungs was likely to have been caused by exaggerated inspiration prior to the patient using the sphygmomanometer, which did not affect TCD and its ability to grade the shunt. However, if the Valsalva manoeuvre was not able to prompt a proper change in intrathoracic pressure, this would have affected both TTE and TCD as the chances of microbubbles shunting through the PFO were much less likely. More research into the use of a sphygmomanometer is needed in order to establish its application in a clinical setting.

Prior to abdominal compression, case 2 was graded as severe using TTE, but only as mild using TCD. This is directly indicative of how the two grading scales are not aligned. While a severe shunt using TTE only requires more than 20 bubbles to be visualised in the left heart, a severe shunt using TCD (i.e. grade 4 or 5) requires more than 100 microbubbles to travel through the MCA. In case 2, around 25 microbubbles were seen using both methods during the normal Valsalva manoeuvre, but the shunts were graded on opposing ends of the spectrum. However, when abdominal compression was used, the grade of TTE did not change (as it was already classed as the highest grade prior to compression), but the TCD grade escalated to a grade 5 shunt. This indicates the importance of a proper Valsalva manoeuvre to give the most accurate indication of grade as possible, and highlights a potential downfall of TTE as the threshold for a 'severe' shunt is relatively low.

Case 3 exposed one of the major pitfalls of TCD as we were not able to locate the MCA. TTE imaged the heart without problems and indicated the presence of a severe shunt. Studies have shown that the absence of a temporal bone window is more common in females than males (females 67% vs males 35% in patients with an absent temporal bone window),[113] and is believed to be present in about 10% of individuals.[80] Alternatives such as the transorbital approach,[90] or submandibular approach[114] can be utilised, however this had not been practiced by myself, or had undergone reliability testing prior to the commencement of this study, and therefore could not be used.

Alternative Imaging planes, Peripheral Emboli

Microbubbles travelling to the periphery were seen in both case 2 and 3. Imaging the aortic arch is a novel technique used in this study to investigate the presence of microbubbles travelling to areas other than the brain. TTE is able to directly image these microbubbles in the aortic arch itself. TCD is unable to do this as any microbubbles visible in the MCA have already deviated from the arch and travelled upstream. Microbubbles travelling downstream after a RLS through a PFO poses a threat to other areas of the body such as the kidney and limbs.[115] The ability of TTE to image these bubbles directly is a major advantage over TCD, and although the presence of microbubbles in the MCA using TCD could assume the presence of microbubbles in the aortic arch as well, the direct visualisation of such is superior. Above all, imaging emboli as they move from the ascending aorta and down the descending aorta could potentially offer a diagnostic advantage for peripheral embolism and could be the focus of future work.

Limitations

None of the cases in the present study were referred on for a TOE. This was understandable in case 1 and 3 where the PFO was deemed to be an incidental finding and was less likely to cause further complications. However, case 2 did not undergo a TOE prior to closure. The use of TOE is common prior to the closure of a PFO, as it is used to assess and measure the area for the suitability of closure, and to ensure the right sized device is used. However, the decision not to go forward with a TOE was likely impacted by COVID-19. During the pandemic most patients that required a TOE were either rebooked or reconsidered due to the risk of an aerosol generating procedure, which would have put both the patients and clinicians at risk. As such, we were unable to compare TTE and TCD to a reference technique, and could not compare all three techniques within the same patient to determine if any of the methods had provided false negative or false positive results. Moreover, sensitivity and specificity are best calculated when matched against a reference technique, so the present study would have carried more significance were the TTE and TCD weighed against TOE. In regards to reproducibility, it would have been best to complete all three trials in all five reliability participants, but due to COVID-19 that was not possible. However, the presenting results give a satisfactory indication of reproducibility.

Future Research

To summarise, the three case studies all displayed the presence of a RLS through a PFO. The largest shunt was successfully closed, and the other two shunts were determined to be incidental findings that were unlikely to cause future recurrent events. TTE was able to diagnose all three shunts as positive, as well as image the aortic arch, but struggled when a sphygmomanometer was used to prompt a Valsalva manoeuvre due to inflated lungs. TCD gave a precise shunt grade for two of the three cases, but was not able to grade the third due to an insufficient temporal bone window. TTE and TCD differed in their grading of the normally provoked shunt in case 2, indicating that the two grading systems may not be perfectly aligned. However, they were well matched when abdominal compression was used to provoke the Valsalva manoeuvre. A larger scale study is needed comparing TTE to TCD in CS patients, or patients presenting with the symptoms of a CS. The use of TOE as a reference technique would also shed light on each technique and indicate the presence of any false positive or false negative results. The RoPE score should be used and critiqued as we further understand how well it is aligned with clinicians and the choices they make regarding the contribution of the PFO to the presenting symptoms, as well as the likelihood of it causing recurrence. A follow up period of several years would be beneficial as this would enable the assessment of success of treatment (if taken) and recurrence in relation to PFO presence.

Chapter Three: Systematic Review and Meta-analysis

Introduction

Thus far in the present thesis, we have discussed a patent foramen ovale (PFO), its prevalence, and ischemic events it can contribute to such as cryptogenic stroke (CS) and migraine. We have reviewed the three primary methods of PFO diagnosis; transthoracic echocardiography (TTE), transcranial Doppler (TCD), and transoesophageal echocardiography (TOE) and their use in current literature, as well as their pitfalls and advantages. Additionally, we have applied the two bedside techniques to a group of case studies in a clinical application to directly assess their diagnostic ability in patients with ischemic stroke or suspected CS. The case studies uncovered both advantages and pitfalls of TTE and TCD, further adding to the conversation comparing these two techniques in current literature. The presence of a right-to-left shunt (RLS) through a PFO is associated with CS, a type of stroke that has an unknown source of embolism and therefore the cause of the stroke-like symptoms is unknown.[5] The association between PFO and CS is still under debate,[94,116,117] as well as the methods used to diagnose a PFO.[85] While the general level of sensitivity and specificity of TTE and TCD is high, studies comparing both techniques against a gold standard reference technique (TOE) indicate there is still inconsistency between TTE and TCD in regards to the optimal techique.[48,69,81,82] Meta-analyses have been conducted that compare TTE to TOE,[78] and others that compare TCD to TOE.[79] If these two meta-analyses are compared side to side, TCD appears to be the most sensitive technique, while TTE the more specific. Katsanos et al., conducted a meta-analysis which compared different studies using TTE or TCD alongside the reference technique TOE, and came to the same conclusion.[93] While the results of these meta-analyses draw the same generalised conclusion, they do not consider studies where TTE and TCD were performed in the same cohort of patients making a direct comparison between TCD and TTE impossible. The present systematic review and meta-analysis will analyse studies using TTE and TCD in the same cohort of patients with or without a reference technique. This will shed light on the current status of TTE and TCD and their abilities to make the most accurate diagnosis in the same group of patients, with the same presenting symptoms and risk factors, and investigate the effect that TOE has on the diagnostic ability of either technique when introduced as a reference standard. This systematic review and meta-analysis will include studies that assess

patients with possible CS or migraine-like symptoms that are therefore undergoing a bubble study for PFO presence.

Rationale

There is inconsistency within the literature regarding the optimal test for PFO diagnosis. TTE and TCD are often used interchangeably between different studies, with or without a gold standard measurement of reference (TOE). It is obvious that there is a relationship between PFO presence and CS or migraine, especially in the younger population (<55 years).[30] However it is still debated as to whether or not the relationship is causal.[30,52] Therapeutic remedies to treat a PFO have shown to decrease the prevalence of ongoing problems,[118] as well as reduce the chance of recurrent events,[21] so the accurate diagnosis and assessment of a PFO is crucial. This meta-analysis will review the previous literature involving the use of TTE, and TCD to diagnose a PFO in CS patients or migraine patients predominantly. It will include studies with or without TOE used as a gold standard. This meta-analysis will be conducted to help determine the current ability of these diagnostic techniques and gain insight as to which technique may have the upper hand for PFO diagnosis.

Methods

The present meta-analysis adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines,[119] and was developed according to the MOOSE (Meta-analysis of Observational Studies in Epidemiology) proposal.[120]

Eligibility Criteria

Studies involving patients with stroke or migraine as the main aetiology were included. Studies included required to have both TTE and TCD performed on the same cohort of human participants, and preferably a gold standard test for confirmation (i.e. TOE). These studies were graded as A quality. Studies that included TTE and TCD without external confirmation (i.e. no TOE) were included but graded as B quality. Studies were excluded if they recruited children or had less than 20 participants. This was because studies with a larger cohort of participants were a more reliable source of statistical significance in terms of pooled sensitivity and specificity. Additionally, there were no studies with under 20 participants, other than case studies, that made the inclusion criteria as they were excluded for other reasons. If a study involved a control group, the results from this group were omitted. If this was not possible the

study was omitted. Control groups were omitted as the study was focused on the assessment of a PFO due to a suspected CS, and the accuracy of the assessment techniques. Additionally, the controls were excluded to keep the data extraction consistent as only one of the final studies included involved controls.

Information Sources

Online databases were used to yield all results. This included Web of Science, Scopus, PubMed, and Medline/Ovid.

Search Strategy

This study used an advanced Boolean search strategy in all databases and identified all studies before March 7, 2020, no limit was applied to the start date. This involved the following phrases: ("Echocardiography" OR "Doppler" OR "TTE" OR "transthoracic echocardiography" OR "transthoracic echocardiogram" OR "ultrasound" OR "TOE" OR "transoesophageal echocardiogram" OR "transoesophageal echocardiography"), ("TCD" OR "transcranial doppler" OR "doppler"), ("PFO" OR "patent foramen ovale" OR "RLS" OR "right-to-left shunt OR ASD" OR "Atrial septal defect"), ("Stroke" OR "cryptogenic stroke" OR "TIA" OR "transient ischemic attack" OR "cerebrovascular event" OR "ischemic stroke" OR "cerebral ischemia" OR "peripheral emboli" OR "paradoxical embolism"). Hits from all databases were included, and duplicates were removed. References of selected studies were also searched manually for articles that may have been missed in the initial search. No language restrictions were imposed at any stage of the selection.

Selection Process

After duplicates were removed, titles and abstracts were screened by three reviewers (myself (HVDG), supervisor (GAW), and supervisor (LW)). Studies read in full if 2/3 reviewers agreed that the title or abstract included the use of either TTE or TCD as a screening method for the detection of RLS or indicated the potential for the use of either technique. The final set of studies to be read in full were assessed by three reviewers (HVDG, GAW, and LW) and were included for extraction if 2/3 reviewers agreed that the study mentioned the use of both techniques on human participants.

Data Extraction Process

Data was extracted from eligible studies by two reviewers (HVDG and GAW) and any disagreements were resolved by consensus (HVDG, GAW, and LW). The data extracted from each study included first author, year of publication, country, main aetiology, mean age (years), number of subjects, proportion of females, proportion of subjects with PFO, and the true positive (TP), false positive (FP), true negative (TN), false negative (FN), sensitivity, and specificity of each technique (TTE and TCD). The methodological quality of each paper was graded with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.[121]

Risk of Bias

Studies were assessed for all forms of bias by 2/3 reviewers (HVDG and GAW). It was agreed upon that because this meta-analysis was investigating the diagnostic ability of the detection methods, not the prevalence of PFO in the population, selection bias would be present. To ensure that studies evaluated patients in a consecutive fashion, we assessed for red flags such as an extremely high prevalence of PFO in the cohort, as this would indicate only patients with a PFO were evaluated, not patients with a suspected PFO. Additionally, studies were excluded if the use of the Valsalva manoeuvre was not consistent between patients or methods. This aimed to prevent a bias towards one technique over another (e.g. normal provocation of the Valsalva manoeuvre using TCD, but abdominal compression with the Valsalva manoeuvre in TTE).

Data Synthesis

The pooled sensitivity, specificity, as well a receiver operating characteristic (ROC) curve, with a 95% confidence interval (CI), were separately estimated for TCD and TTE. The A grade studies were calculated against TOE, which was treated as the gold standard, and the B grade studies were calculated against each other, with a positive TCD being presumed as true-positive unless stated otherwise. All statistical analyses were performed with Review Manager (Revman) version 5.3 software (Copenhagen, Denmark, Nordic Cochrane Centre, Cochrane Collaboration, 2014)

Results

Study Selection

The search strategy yielded 2108 articles, and after the deletion of duplicates 1146 studies remained for the first assessment (title + abstract). After the first assessment, 961 studies were excluded based on the title and abstract. One hundred and eighty five studies were then retrieved for full text review, of these 173 were excluded as they either did not have TTE and TCD (n=71), had incorrect methodology (e.g. was a case study, or had too smaller cohort) (n=53), had insufficient data (e.g. mentions the right methods, but is focused on another area so did not provide the right data to extract) (n=42), were biased (n=5), or other (n=2). Finally seven grade A studies,[34,48,53,69,81,82,122] and five grade B studies[123–127] were included (Fig. 13).

13. PRISMA flow diagram of study selection

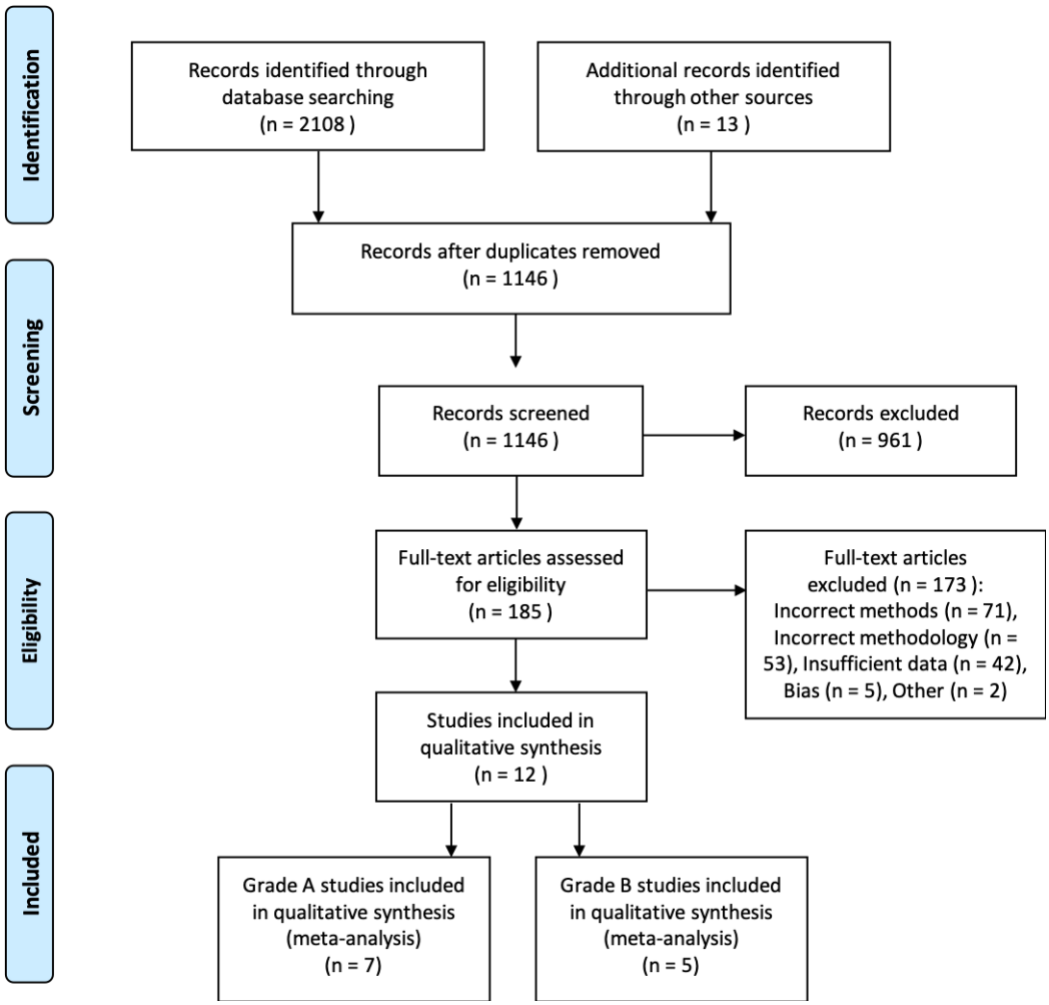


Figure 13: Flow chart diagram presenting the selection of eligible studies. Grade A = A study that included both TTE and TCD as an imaging method, with TOE used as a gold standard in the same patients. Grade B = A study that only uses TTE and TCD as an imaging method in the same patients. Abbreviations: PRISMA = preferred reporting items for systematic reviews and meta-analyses, TTE = transthoracic echocardiography, TCD = transcranial Doppler, TOE = transoesophageal echocardiography

Study Characteristics

The seven grade A studies included 537 patients (weighted average of 79% stroke/transient ischemic attack (TIA)), had a mean age of 51 years and was comprised of 51% females. The five grade B studies included 476 patients (weighted average of 75% stroke/TIA), had a mean age of 48 years and was comprised of 47% females. The characteristics of the included studies can be found on Table 8.

Table 8: Characteristics of included studies in the Meta-Analysis

Author	Year	Grade	Total Patients	Stroke/TIA Patients (%)	Mean age, years	Females (%)	Prevalence PFO (%)
Di Tullio et al[81]	1993	A	49	100	64	44	39
Nemec et al[82]	1991	A	32	68	50	56	41
Maffè et al[69]	2010	A	75	56	49	63	83
Albert et al[53]	1997	A	69	100	44	59	36
González-Alujas et al[48]	2011	A	134	89	46	44	69
Zito et al[34]	2009	A	72	69	49	54	65
Souteyrand et al[122]	2006	A	107	100	56	37	35
Puledda et al[127]	2016	B	97	100	40	38	52
Di Tullio et al[123]	1993	B	80	100	61	41	26
Teague et al[126]	1991	B	46	52	41	46	41
Itoh et al[124]	1994	B	30	100	55	40	57
Corrado et al[125]	2011	B	232	22	43	69	77

Legend 8: Grade A = A study that included both TTE and TCD as an imaging method, with TOE used as a gold standard in the same patients. Grade B = A study that only uses TTE and TCD as an imaging method in the same patients. Abbreviations: TTE = transthoracic echocardiogram, TCD = transcranial Doppler, TOE = transoesophageal echocardiogram, TIA = Transient Ischemic attack.

Quality Assessment

The quality assessment of included studies used the recommended 14-item checklist (Table 9).[121] Items 1, 2, 5, 6 and 7 scored well indicating the absence of bias in those areas. All items that are not depicted in green for "yes" are discussed in the legend other than items 12 and 13 which were classed as "unclear" as they were unreported. Item 7 ("Was the reference standard

independent of the index test (i.e. the index test did not form part of the reference standard)?") was true and correct in all studies, although one could argue that the efficiency of the Valsalva manoeuvre could influence the diagnostic accuracy of each test, if conducted at different times. The use and provocation of the Valsalva manoeuvre tended to be inconsistent between studies. Some studies made patients practice the Valsalva manoeuvre prior to the assessment,[125] others would only conduct it if no obvious PFO was seen at rest, and without prior practice.[126]

Table 9: The QUADAS 14 - point checklist for bias within included studies

The QUADAS 14 – Point Checklist for Included Studies	Di Tullio et al **(A)**	Nemec et al **(A)**	Maffé et al **(A)**	Albert et al **(A)**	González - Alujas et al **(A)**	Zito et al **(A)**	Souteyrand et al **(A)**	Pulledda et al **(B)**	Di Tullio et al **(B)**	Teague et al **(B)**	Itoh et al **(B)**	Corrado et al **(B)**
1. Was the spectrum of patients representative of the patients who will receive the test in practice?												
2. Were selection criteria clearly described?												
3. Is the reference standard likely to correctly classify the target condition?	**	**	**	**	**	**	**	**	**	**	**	**
4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?				†			†					
5. Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?												
6. Did patients receive the same reference standard regardless of the index test result?												
7. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?												
8. Was the execution of the index test described in sufficient detail to permit replication of the test?												‡
9. Was the execution of the reference standard described in sufficient detail to permit its replication?												‡

10. Were the index test results interpreted without knowledge of the results of the reference standard?		*							*			*
11. Were the reference standard results interpreted without knowledge of the results of the index test?		*							*			*
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?												
13. Were uninterpretable/ intermediate test results reported?												
14. Were withdrawals from the study explained?												×

Legend 9: A = A grade study that included both TTE and TCD as an imaging method, with TOE used as a gold standard in the same patients. B = B grade study that only uses TTE and TCD as an imaging method in the same patients. Abbreviations: QUADAS = Quality Assessment of Diagnostic Accuracy Studies, TTE = transthoracic echocardiogram, TCD = transcranial Doppler, TOE = transoesophageal echocardiogram, TIA = Transient Ischemic attack.

* This study did not mention blinding of results, so it was unclear to if this was done or not.

** This question is subjective to current literature and drives the overarching theme of this review. While the Grade A studies have reasonable ground to consider transoesophageal echocardiography (TOE) as the reference standard, Grade B studies are comparing transthoracic echocardiography (TTE) to transcranial Doppler (TCD) both of which are not considered the current reference standard, so the answer for item 3 is unclear.

† The time period between tests was not mentioned.

‡ The methods were not described in detail

× Nine patients did not have both methods (232 participants in study, full results for only 223 included in the analysis). Therefore, nine were unaccounted for.

Results of Grade A Studies

The forest plot for the A grade studies, which included TTE and TCD as detection methods for PFO prevalence against the reference technique TOE, can be seen in Figure 14. Generally, specificity was high for both TCD and TTE across studies, ranging from 0.92 to 1.00 for TCD, and remaining at 1.00 for TTE. TCD gave the highest number of FP diagnoses compared to TTE (1.3% vs 0% respectively), but TTE reported the highest number of FN diagnoses (10.6% for TTE compared to 3.7% for TCD). Overall, both methods were more likely to produce a FN than a FP result. When the weighted specificity of the two techniques were compared in a summary forest plot, there was no difference: specificity for TCD was 0.97 (95% CI 0.94-0.99) and specificity for TTE was 1.00 (95% CI 0.98-1.00) (Fig. 15). When the weighted sensitivities were compared the sensitivity was significantly lower in the TTE (0.81, 95% CI 0.76-0.85) studies compared to TCD (0.91, 95% CI 0.86-0.94) (Fig. 14). There was a trend towards higher sensitivity with later year of publication for TTE, but not TCD (Fig. 15). The ROC curve was similar for both methods but favoured TCD overall (Fig. 16A).

14. Forest plot of the Grade A studies

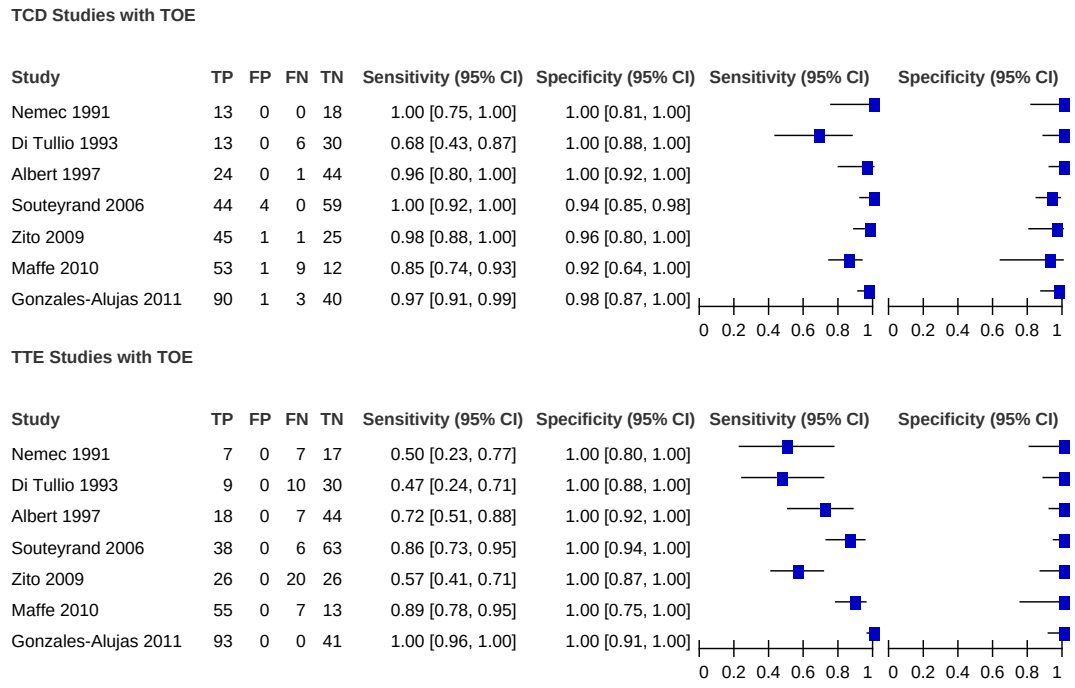
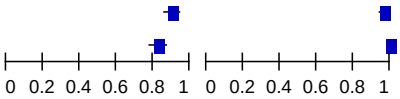


Figure 14: Forest plot of the sensitivity and specificity of each individual grade A study. Grade A = A study that included both TTE and TCD as an imaging method, with TOE used as a gold standard in the same patients. Abbreviations: TTE = transthoracic echocardiogram, TCD = transcranial Doppler, TOE = transoesophageal echocardiogram, TP = true positive, FP = false positive, FN = false negative, TN = true negative, CI = confidence interval.

15. Summary Forest plot of Grade A and B studies

TCD

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Summary with TOE	192	7	20	228	0.91 [0.86, 0.94]	0.97 [0.94, 0.99]		
Summary without TOE	230	0	48	198	0.83 [0.78, 0.87]	1.00 [0.98, 1.00]		



TTE

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Summary with TOE	246	0	57	234	0.81 [0.76, 0.85]	1.00 [0.98, 1.00]		
Summary without TOE	242	0	36	198	0.87 [0.83, 0.91]	1.00 [0.98, 1.00]		

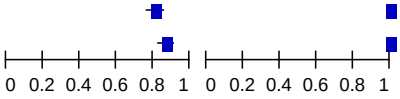


Figure 15: Summary forest plot of the sensitivity and specificity of each grade A study and each grade B study, between TTE and TCD. Grade A = A study that included both TTE and TCD as an imaging method, with TOE used as a gold standard in the same patients. Grade B = A study that only uses TTE and TCD as an imaging method in the same patients. Abbreviations: TTE = transthoracic echocardiogram, TCD = transcranial Doppler, TOE = transoesophageal echocardiogram, TP = true positive, FP = false positive, FN = false negative, TN = true negative, CI = confidence interval..

16. ROC curve for A and B Grade studies

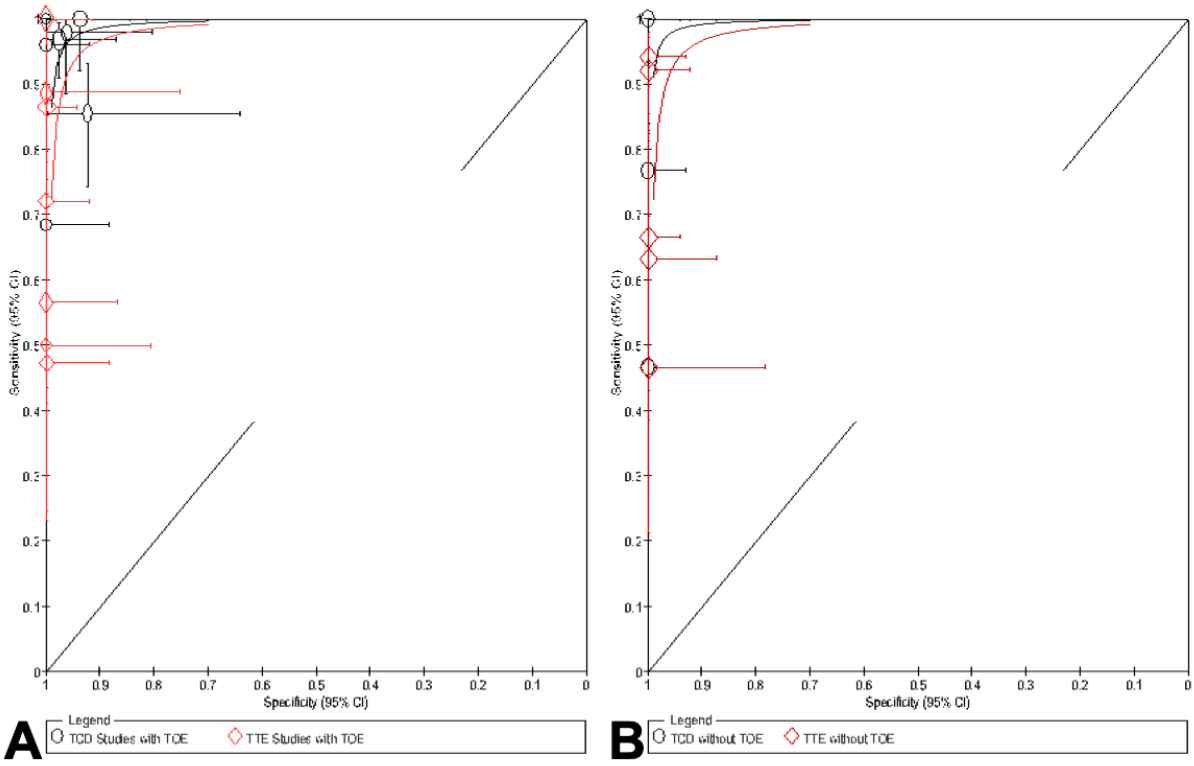


Figure 16: ROC curve for studies graded as A and B. Sensitivity is on the Y axis, specificity is on the X axis. Grade A = A study that included both TTE and TCD as an imaging method, with TOE used as a gold standard in the same patients. Grade B = A study that only uses TTE and TCD as an imaging method in the same patients. Abbreviations: ROC = receiver operating characteristic, TTE = transthoracic echocardiogram, TCD = transcranial Doppler, TOE = transoesophageal echocardiogram

Results of Grade B Studies

The forest plot for grade B studies that compared TTE and TCD without TOE as a reference standard can be seen in Figure 17. Since there was no reference standard, specificity was high (1.00) for both techniques across all studies. Additionally, the absence of a gold standard reference technique meant it was harder to decipher FN or FP results. If a technique diagnosed a patient as positive for PFO it was taken as a TP, and if the opposing technique failed to detect the PFO it was taken as a FN. With that in mind, neither technique had any false positives, but TCD gave the highest number of false negatives compared to TTE (10% vs. 7.5%). When the weighted sensitivity and specificity for the grade B studies was calculated, specificity was high for both TCD and TTE and there was no difference between the two techniques. Overall specificity for TCD was 1.00 (95% CI 0.98-1.00) and for TTE 1.00 (95% CI 0.98-1.00) (Fig. 15). However, sensitivity was lower overall in the TCD (0.83, 95% CI 0.78-0.87) studies compared to the TTE (0.87, 95% CI 0.83-0.91) studies (Fig. 15). The ROC curve was similar for both methods but favoured TCD overall (Fig. 16B).

17. Forest plot of the Grade B studies

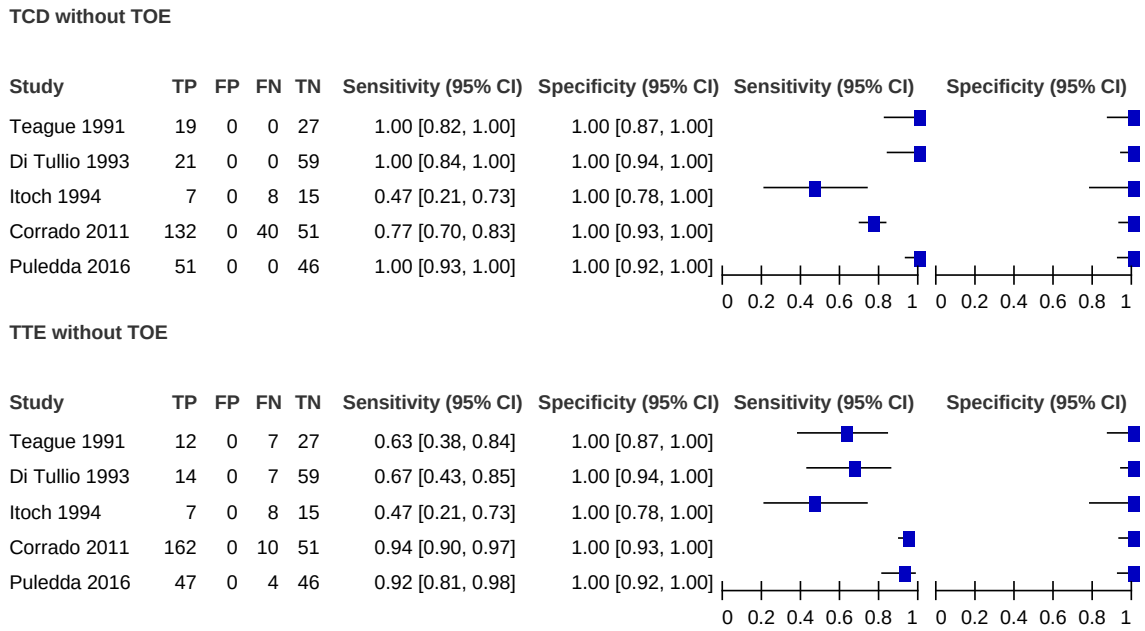


Figure 17: Forest plot of the sensitivity and specificity of each individual grade B study. Grade B = A study that only uses TTE and TCD as an imaging method in the same patients. Abbreviations: TTE = transthoracic echocardiogram, TCD = transcranial Doppler, TOE = transoesophageal echocardiogram, TP = true positive, FP = false positive, FN = false negative, TN = true negative, CI = confidence interval.

Comparison Between Grade A and Grade B Studies

There were no differences observed for specificity for either technique, irrespective of whether TOE confirmation was used (Fig. 15). For TCD, there was a trend towards higher sensitivity when TOE was used for comparison: without TOE (0.83, 95% CI 0.78-0.87) compared with TOE (0.91, 95% CI 0.86-0.94). In comparison, TTE saw a decline in sensitivity: without TOE (0.87, 95% CI 0.83-0.91) compared with TOE (0.81, 95% CI 0.76-0.85) (Fig. 15).

Discussion

This meta-analysis has shown that TCD is superior to TTE for detecting right-to-left shunts in patients who have experienced stroke or migraine when TOE is used as a gold standard. The specificity remains high for both techniques, irrespective of whether TOE is used as a gold standard. This indicates that TCD should be considered the first imaging test of choice for patients where a paradoxical embolism through a PFO is suspected. However, when no gold standard was used the sensitivity of TCD was significantly lower, and closer to that of TTE.

TCD is Superior to TTE for the ruling in of a Patent Foramen Ovale

TTE with harmonic imaging and agitated saline contrast is frequently used to screen for PFO as part of routine clinical care.[13] Despite its consistent use, studies involving TTE for PFO detection have been inconsistent in regards to the sensitivity of TTE when compared to the gold standard TOE.[48,128] There are multiple reasons that can be attributed to this inconsistency, such as the inability to perform the Valsalva manoeuvre during TOE,[79] or poor imaging quality using TTE.[92] An alternative diagnostic method, TCD, challenges the sensitivity and specificity of TTE and TOE, is minimally invasive, and can be used by the bedside similarly to TTE.[74] However, TCD also comes with pitfalls such as the inability to decipher between an intracardiac shunt and an intrapulmonary shunt, which is often reflected in TCD's specificity.[93] In the present meta-analysis and systematic review, studies comparing TTE and TCD against a gold standard reference technique were compared to studies that did not use a gold standard technique, the sensitivity of TTE dropped (0.87 - 0.81) (Fig. 15). Opposingly, the sensitivity of TCD increased (0.83 - 0.91) (Fig. 15). Prior to the addition of the gold standard, the sensitivities of TTE and TCD were relatively similar (TTE: 0.87, TCD: 0.83), and the confidence intervals overlapped (95% CI 0.83-0.91 for TTE, 0.78-0.87 for TCD) (Fig. 15). However, when studies involving TOE were included, the confidence intervals widened, bridging the gap of sensitivity between TTE and TCD (95% CI 0.76-0.85 for TTE, 0.86-0.94

for TCD). The improvement in sensitivity for TCD with TOE reflects a reduction in the number of false negatives, as sensitivity is the number of true positives divided by the sum of the true positives and false negatives. This was true in the present study, where there were 48 false negatives when TCD was used without TOE, and only 20 when TOE was used (Fig. 15). However, there were 7 false positives with TCD that were not present on TTE.

In the A grade studies (those with TOE confirmation), the forest plot demonstrates an increase in sensitivity over time for TTE. This is reflective of the evolution of the technique throughout the 1990s into the late 2000s as imaging improved and harmonic imaging was introduced.[129] This increase in sensitivity does not seem to be as prominent in TCD, although the TCD device has also seen significant improvements in detection over time.[71] The ROC curves are also both very strong, indicating that despite inconsistencies, both methods bring strong diagnostic capabilities for PFO detection. It has helped that over the years the target population for PFO screening has been clinically refined. Although it is still under debate, the association between CS and PFO is understood now more than it was in the 1990s which has helped decipher the target population for PFO screening. Therefore, the prevalence of PFO in patients undergoing a bubble study is higher now than in the 1990s. This is somewhat demonstrated in the present systematic review and meta-analysis, as the two papers with the highest prevalence of PFO detection (83% and 77%) were conducted in 2010[69] and 2011,[125] and the four studies with the lowest prevalence of PFO detection (26%, 35%, 36%, 39%) were conducted in 1993,[81,123] and 1997,[53] with the exception of Souteyrand et al.,[122] which was conducted in 2006 (prevalence of 35%).

Current Perspective in the Literature Comparing TTE and TCD

There are several meta-analyses that have looked at the diagnostic ability of TTE and/or TCD against the gold standard TOE.[78,79,93] While they are all recent, none of them are limited to studies that applied both techniques to the same cohort of patients. The generalised outlook seemed to present TTE as the less sensitive, but more specific technique across the three studies, and this is consistent when the present study is included in the comparison (Table 10).

Table 10: Comparison of Meta-analyses that investigate TTE and/or TCD against TOE

	TTE SENSITIVITY (%, 95% CI)	TTE SPECIFICITY (%, 95% CI)	TCD SENSITIVITY (%, 95% CI)	TCD SPECIFICITY (%, 95% CI)
REN ET AL.,[78] 2013	88 (79-94)	97 (92-99)		
MOJADIDI ET AL.,[79] 2014			97 (94-98)	93 (86-97)
KATSANOS ET AL.,[93] 2016	45 (31-60)	100 (97-100)	96 (93-98)	92 (86-96)
PRESENT STUDY 2020	81 (76-85)	100 (98-100)	91 (86-94)	97 (94-99)

Legend 10: Weighted sensitivity and specificity for the present study were taken from the results of the A grade studies (studies that compared TTE and TCD against TOE). Abbreviations: TTE = transthoracic echocardiography, TCD = transcranial Doppler, TOE = transoesophageal echocardiography, CI = confidence interval. Note: only the present study included the same patients in both TCD and TTE comparisons.

Katsanos et al.,[93] evaluated the sensitivity and specificity of both TTE and TCD against the reference standard TOE. However, the included studies did not require the application of TTE and TCD in the same patients, it merely includes all papers that compared TTE to TOE, or TCD to TOE in the same meta-analysis. Katsanos et al., also uncovered a very poor sensitivity for TTE (45%), which was attributed to poor imaging quality[93] and acknowledged that imaging quality has improved over time for TTE. However, the two studies that gave the lowest level of sensitivity for TTE were conducted in 2003 and 2006, which is after the introduction of harmonic imaging.[130] They eventually concluded that TTE was the best option for ruling in a PFO as it has a higher likelihood ratio compared to TCD and is therefore more likely to diagnose a PFO as a true positive, but for the general diagnostic yield, TCD outweighed TTE.[93] Ren et al., found TTE to have reasonable specificity (88%),[78] especially when compared to Katsanos, or the present study. While 88% was the highest level of sensitivity out of the three meta-analyses that compared TTE to TOE, it is still far from perfect and Ren et al. put this down to age, initial disease, PFO size, and acoustic window.[78] They argued that older age prevented patients from performing an adequate Valsalva manoeuvre, and increased the amount of age related fat, decreasing the sensitivity of TTE.[78] Mojadidi et al., found TCD to be the most specific (97%) of the three studies that compared the technique.[79] They found that

increasing microbubble threshold from 1 microbubble to 10 microbubbles increased the specificity (89 – 100%) without compromising sensitivity (98 – 97%) for positive PFO detection.[79] They put this down to shunt type, with a cut-off used to decrease the number of FP results created by insignificant shunts (intrapulmonary shunts).[79] However, this is controversial as small shunt size does not necessarily rule out a shunt through a smaller PFO.[131]

TTE and TCD are relatively similar in some ways, such as they are both used by the bedside, are non-invasive, and run at a reasonably low cost.[74,104]The TCD device is easy to operate and interpret and is highly sensitive, as well as being portable for a diagnosis in the ward.[132] TTE also has several diagnostic advantages as it is able to rule out other cardiac sources of emboli (such as an pulmonary arteriovenous fistula, left ventricular thrombus, left ventricular dysfunction, dilated atria, or valve disease), by imaging the heart directly.[87] This is somewhat reflected in the results of this meta-analysis. For example, studies without TOE used as a reference standard showed a higher level of sensitivity using TTE compared to TCD (87% and 83% respectively). This may be indicative of TTEs ability to recognise and diagnose the location of the shunt (i.e. which shunts are occurring through a PFO, rather than through an intrapulmonary shunt).[78] On the other hand, if a timing technique such as the rule of 9 is not applied TCD classes any microbubble passing through the MCA as a general RLS, rather than a positive PFO.[75] The rule of 9 is when a PFO is deemed to be present when at least nine microbubbles pass through to the left heart and appear in the MCA within 9 seconds of injection, and helps the clinician differentiate between shunt types using TCD.[89]

Implications of the Results in Current Literature

The accurate diagnosis of a PFO is important for young patients experiencing migraines, or who have had a cryptogenic stroke. If left undiagnosed, a PFO can allow the passage of venous emboli into the arterial system, which can cause a trail of cerebral destruction.[13] The importance of an accurate diagnosis allows the consideration and planning of secondary prevention, whether that involves percutaneous closure of the PFO using an Amplatzer device,[133] or the administration of anticoagulants to decrease the likelihood of a cerebral event reoccurring.[13]

Limitations of the Diagnostic Techniques and Current Gold Standard

It still remains unclear which method should be regarded as the 'true' gold standard. Although this study has referred to TOE as the gold standard, it may be an imperfect one.[48] This is because sedation is often used during TOE which can lead to false negative results if the patient cannot perform an adequate Valsalva manoeuvre.[17] A gold standard approach that requires the presence of a PFO in two of the three methods of detection has been suggested and indeed used in a study by González-Alujas et al., which may be a suitable approach. This 'two out of three diagnosis' would alleviate the uncertainty TOE holds as a gold standard and would improve the likelihood that the correct diagnosis is being made. For example, it may involve a two-step primary bedside method of PFO detection using both TCD and/or TTE, prior to the patient having a TOE. If both of the TTE and TCD are positive, the patient is diagnosed with PFO prior to the TOE, which can then be conducted with the primary intention of determining the PFO for suitability for repair. If only one is positive the TOE may be used for a confirmatory diagnosis as well as assessing the suitability for repair. However, TTE still holds an advantage as it can rule out other sources of emboli that may have contributed to the presenting condition. And thus, it may be argued that TTE should be the first method used, and if positive, direct referral to TOE, but if not positive further assessment by TCD may be beneficial (Fig. 18). For example, if the sensitivities of TCD and TTE calculated in the present study were applied to a hypothetical 100 person cohort with an 80% prevalence of PFO, TCD would detect 73/80 true positive patients, while TTE would detect 65/80 true positive patients. The use of TCD as an additional method of diagnosis would detect 7 more true positive cases that TTE otherwise would have missed, highlighting the benefit of adding this extra diagnostic step.

18. Proposed diagnostic pathway for PFO diagnosis

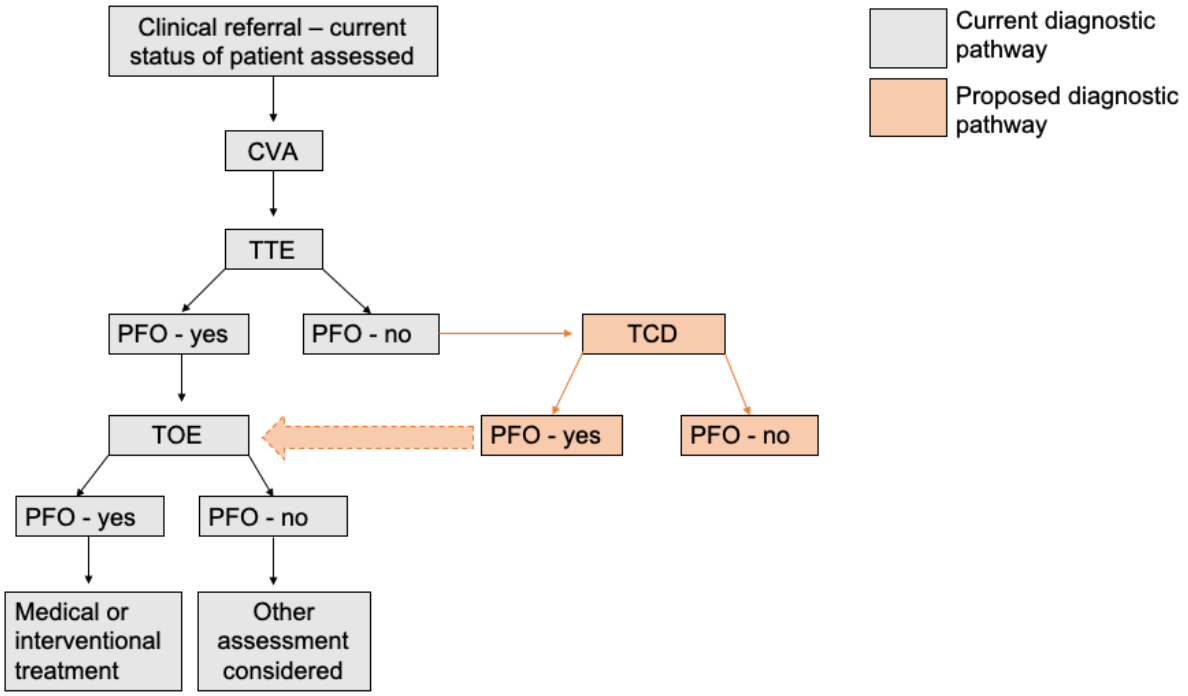


Figure 18. Patients will be assessed following a cerebral event, factors such as age, co-morbidities, RoPE score, etc will help clinicians decide the next steps. Patients with a likely cryptogenic stroke will be referred for a routine TTE, and bubble study which would usually indicate the presence or absence of a PFO. The proposed pathway suggests that in clinical situations, a TCD will be undertaken either concurrently, or following the TTE + agitated saline. Abbriviations: PFO = patent foramen ovale, CVA = cardiovascular assessment, TTE = transthoracic echocardiography, TOE = transoesophageal echocardiography

Bias within the A and B grade studies

The QUADAS 14-item checklist indicated that, in the majority of the studies, there was limited bias. One study that indicated a high level of bias was Corrado et al.[125] The paper itself provided limited information and so the author was approached and thus provided us with raw results which allowed for the inclusion of this study into the meta-analysis.[125] Although it scored poorly for bias – this is a result of the nature of the way the paper was presented to us, and not on the methodological quality it possesses. One source of bias may be patient selection. However, this meta-analysis was conducted with the aim to compare the diagnostic abilities of TTE and TCD for the presence of a PFO, not to evaluate the prevalence of PFO in the population. Therefore, in regard to patient selection, selection bias is not present if the study group only contains patients referred on to a clinician following a cerebral event or migraine. To ensure that this was consistent, if studies used a control group to represent the general population, this group was removed (if possible) and if not possible, the study was excluded. For example, the B grade study by Itoh et al., involved two control groups in the study to

represent a healthy cohort (n=11) and a stroke cohort with obvious aetiologies (n=11), which were soon removed during extraction. This study indicated which patients were positive with TTE/TCD so this could be done without skewing results.[124] Often patients were only referred on to a second technique after a positive first technique.[134] If this was the case the study was excluded. If these particular studies had been included, a PFO that may have been initially misdiagnosed as negative would never be challenged by a secondary technique. This would result in an overestimation of specificity for the initial reference technique.

Study Limitations

This study had several limitations. Some of these studies had a sub-par methodological quality, which is why some studies were classed as grade A, and some as grade B. Additionally, many studies had some degree of selection bias with the study design, which allowed some methods to screen more patients than others. The authors of some studies which may have been eligible for inclusion were approached for further investigation, but if they did not respond the study was excluded. Some papers may have been suitable in terms of comparing TTE and TCD but had to be excluded as the cohort was not in stroke or migraine patients. Additionally, publication bias was a limitation to this study, as only published work was able to be reviewed. The higher prevalence of PFO in this study may not translate over to the prevalence of PFO in the general population. However, as aforementioned, the studies included were conducted to investigate the diagnostic capabilities of TTE and TCD, not observe overall presence, so patients with suspected PFO would be put forward for assessment. With that in mind a higher prevalence of PFO can be somewhat expected as the mechanisms behind PFO are now more understood than ever, refining the patient population getting referred on for a bubble study. Furthermore, although the techniques were all described in detail for the majority of the studies, we were still unable to determine how similar the application of methods between studies was, meaning the standardisation of the techniques was a further limitation to the present study.

Conclusion

This systematic review and meta-analysis showed that TCD was the more sensitive method, but the less specific method compared to TTE when TOE was used as a gold standard reference technique. When there was no gold standard used, both techniques showed a lower level of sensitivity. To our knowledge, this is the first meta-analysis conducted to look at the use of both bedside techniques (TTE and TCD) in the same patients presenting with CS or migraine.

This if of importance as there is discussion within the literature regarding the current gold standard TOE, and how it may not be the optimal method in terms of sensitivity or specificity. By comparing the two bedside techniques in the same cohort the best comparison can be made. The study was conducted by three reviewers and was assessed for all forms of bias. As more evidence comparing TTE and TCD emerges in the literature, the evaluation of TTE and TCD can be further discussed and clearer conclusions can be made, which will only have a positive clinical outcome for future patients diagnosed with a PFO.

Chapter Four: Discussion

Summary of Results

This thesis has discussed the implications of the link between patent foramen ovale (PFO) and ischaemic stroke, investigated ways in which PFO can be diagnosed in case studies, and systematically reviewed current literature to find the optimal diagnostic method. In the case studies, all three of the patients were diagnosed with a PFO, two of which were deemed to be an incidental finding. Benefits and pitfalls were revealed for both transthoracic echocardiography (TTE) and transcranial Doppler (TCD) during the case studies, making the judgement of the optimal technique even harder. While TCD was able to diagnose one particularly at-risk case with a shower curtain sized shunt, it lacked the ability to optimise the middle cerebral artery (MCA) in a patient where there was an absence of temporal bone window. TTE was able to positively diagnose all three shunts as intracardiac shunts, and directly image the septum, as well as other areas of the heart. However, at times TTE struggled with imaging due to inflated lungs when a standardised method of the Valsalva manoeuvre was conducted. The meta-analysis found that TCD was the optimal test for the ruling in of a PFO, but only when transoesophageal echocardiography (TOE) was used as a gold standard. Otherwise, the optimal test was not obvious. A shift in sensitivities was seen for both techniques when TOE was included as a gold standard.

The diagnostic abilities of TTE versus TCD is still under debate

Discrepancies within the literature continue to fuel the ongoing debate regarding the optimal method for PFO detection for patients who have experienced cryptogenic stroke (CS). Despite these discrepancies, both TTE and TCD are considered strong diagnostic tools. Several recent large scale studies have used either TTE[51,135] or TCD[65,91,127] as reliable diagnostic methods to detect the presence of a right-to-left shunt (RLS) in patients presenting with CS or migraine. Various studies have compared the techniques side by side with and without comparison to a reference standard, and the common trends tend to be; higher sensitivity of TCD,[34,122,127] and higher specificity of TTE.[34,69,12269,122] Most of the time this was attributed to TCD's inability to differentiate between shunt types, and TTE struggling with poorer image quality and smaller shunt detection.[34]

TTE has several distinct advantages over TCD in regards to imaging the heart directly. TCD has lower specificity compared to TTE, which may be due to extracardiac shunts causing false positive results.[93] Additionally, because TCD does not image the heart (and therefore the contrast) directly, it will not be able to tell if a poorly timed Valsalva manoeuvre was performed – which may result in a RLS of blood, but without contrast. If this occurs, TCD will not be able to image any microbubbles, indicating the absence of a PFO leading to a false negative diagnosis.[48] Furthermore, TTE, unlike TCD, is able to image larger shunts without a saline contrast or a Valsalva manoeuvre by using either 2D imaging alone to assess the interatrial septum, or using color Doppler to visualise the movement of oxygenated blood from the right atrium (RA) across the interatrial septum into the left atrium (LA).[68] This approach to PFO detection is not very specific and the technique improves significantly when contrast and the Valsalva manoeuvre is used.[68]

More generally, TTE is also able to broadly evaluate the functioning of the heart to assess for a range of conditions that may cause stroke or other dysfunction (Table. 11).[136,137] This demonstrates the versatility of TTE as a technique when imaging heart. TCD also has several other uses in clinical practice, but not as many that evaluate conditions or measures directly attributed to the cause of stroke (Table 11).[105,138–140]

Table 11: Diagnostic abilities of TTE and TCD for conditions other than PFO

TTE	TCD
- Left atrial myxoma - Valvular vegetation - Left atrial appendage - Left ventricular thrombus - Left ventricular aneurysm - Dilated cardiomyopathy - Aortic stenosis - Mitral stenosis - Mitral valve prolapse - Mitral annular calcification - Ejection fraction - Wall motion abnormalities	Conditions directly involved in stroke: - Intracranial steno-occlusive disease - Subarachnoid haemorrhage (vasospasm) - Extracranial disease - Atherosclerosis of cerebral blood vessels - Hyperaemia - Sickle cell disease May have some involvement in stroke: - Intercranial pressure - CO^2 reactivity and autoregulation - Brain death

Legend 11: Table describes other conditions that TTE and TCD can clinically evaluate other than PFO presence, highlighting the alternative uses of each technique for detecting cause of stroke or general abnormalities. Abbreviations: TTE = transthoracic echocardiography, TCD = transcranial Doppler, PFO = patent foramen ovale.

Several older studies have highlighted a lower sensitivity of TTE.[81,82,124] Most attributed this to low image quality, and therefore the inability to diagnose smaller shunts or shunts during the Valsalva manoeuvre. However, with the introduction of harmonic imaging the image quality has improved and with it the sensitivity of the test.[48] This was apparent in my meta-analysis. If the pooled sensitivities for grade A studies were calculated separately for studies prior to 2000 and studies after 2000, studies prior gave a range of sensitivity from 47% to 72%[53,81,82] while studies after gave a range of sensitivity from 57% to 100%[34,48,69,122]. This shows an improvement in the application and ability of TTE, and puts forward the question: does the current ability of the technique need to be reassessed with studies that must include harmonic imaging?

As well as the inability to image the heart directly, TCD has other pitfalls. If the timing of microbubbles appearing in the MCA is not measured, TCD is unable to determine if a shunt is intracardiac or intrapulmonary. Additionally, the lack of a sufficient temporal window may impair the ability for TCD to insonate the MCA. This was the case in case study 3. This is not an uncommon problem, and has been shown to affect 10-20% of stroke patients.[91] Hu et al., found that woman were also more likely than men to have an insufficient temporal window, with twice as many older (>60 years) females having an insufficient window compared to men in the study.[141] A study by Duan et al., demonstrated that 9% of people who were under investigation for PFO presence had an insufficient temporal bone window, and were subsequently excluded from the study.[90] This is a major pitfall of TCD as a diagnostic technique. Alternatives to the temporal window such as the transorbital approach,[90] suboccipital approach,[142] or submandibular approach[114] are possible. They image separate cerebral arteries at different depths, and often require the power of the transducer to be reduced. The Italian SISIFO study successfully used the trans-occipital approach at a depth of 70-90mm as an alternative to participants without a suitable temporal bone window.[23] However, similar to TTE, some patients do not have an adequate acoustic window for imaging, despite best efforts to optimise the image. Adequate training and continued experience using TCD is required to minimise technical errors and optimised utility of TCD.

Impact of Shunt Size

There is inconstancy within the literature regarding PFO size and stroke recurrence. While some studies say that larger shunt size is often correlated with a ‘high risk’ PFO (more likely

to cause recurrence),[143] others have found no correlation between shunt size and stroke recurrence.[144] The Risk of Paradoxical Embolism (RoPE) score is a newly developed score that helps the user determine the causal relation of the PFO to the CS.[31] It uses risk factors such as age, smoking status, history of hypertension, stroke, or diabetes, as well as if a cortical infarct was present on imaging to rank the patient from 1 – 10.[95] A high RoPE score indicates a high chance of the PFO being causal to the CS, and vice versa. However, a high RoPE score is matched with a lower risk of recurrence, indicating that since the patient is in reasonable health (and therefore ranked highly on the RoPE score) they are less likely to have a recurrent event, compared to those with a lower RoPE score.[94] While the size of a PFO does not influence the RoPE score, several papers have made an association between smaller shunt size and stroke recurrence. In a very recent study, Turc et al., found that those with a smaller shunt were more likely to have recurrent events in CS patients compared to those with a larger shunt (1.3 vs 0.6 incidence rate of recurrent stroke per 100 person years, respectively).[143] Thaler et al., also found that smaller shunt size is associated with recurrent stroke.[95] Although these findings are relatively novel, and somewhat unexpected, the true mechanism behind recurrent stroke and high RoPE score is yet to be uncovered.[95] It may be possible, that once the mechanism is more understood, the size of the PFO and consequential shunt could be included in the RoPE score as it may indicate the contribution of PFO to the CS, as well as the likelihood of it causing recurrence. For example, Wessler et al., found that a large RLS diagnosed with TCD was correlated with high RoPE score, indicating that there is an alignment of some sort between the two.[145] More generally, the lack of knowledge regarding the effect of shunt size on future recurrence stresses the importance of each diagnostic technique being able to accurately detect and grade a PFO, even if it may be small, and highlights an advantage of TCD and a pitfall of TTE, especially as a first choice of imaging modality. TCD, which has a much more sensitive 5-point grading scale compared to TTE, may be more suited to assessing for RLS on the frontline. Its ability to detect a singular microbubble travelling through the MCA puts it at a distinct advantage compared to TTE for diagnosing smaller shunts in particular.

The sensitivity of TTE also seems to be positively associated to PFO size.[78] This is likely to be due to the image quality of TTE impairing its ability to detect smaller shunts.[17] The sensitivity of TTE appears to increase with shunt size in some of the papers in the meta-analysis that used TOE confirmation.[34,69,81] This may have contributed to the lower pooled sensitivity of the studies with a higher prevalence of smaller shunts than larger shunts. Zito et al., found that TTE was able to detect just over half the shunts found by TOE, however, of the shunts that

TTE did detect, 92% of them were classed as medium or large shunts.[34] Additionally, Maffe et al., found that while TTE only managed to detect 63% of the medium sized shunts, the method was able to detect 96% of the large shunts.[69] Di Tullio et al, found this increase in sensitivity to be true in both TTE and TCD, with the increase seen in patients with CS rather than stroke of determined cause.[81] This indicates that the larger shunts were more easily detected compared to the smaller shunts that may have been an incidental finding in those with a stroke of recognised origin/cause.[81] While sensitivity is still an ongoing issue for TTE, studies have investigated the use of a 5 microbubble cut off to help improve the specificity of TTE, and have shown promising results (increase of specificity from 57% to 89%), especially when used with abdominal compression.[51]

Is the Current Gold Standard Adequate?

The use of TOE as a gold standard method is widely applied in the literature.[88] In the present thesis, the meta-analysis showed a shift of sensitivities in studies with TOE as the gold standard, and studies without a gold standard (Fig. 15). While the sensitivity of TCD improved, the sensitivity of TTE declined. To our knowledge, this meta-analysis is novel in the way it compared pooled studies that included both TCD and TTE with and without a gold standard. Reasons that may have attributed to this difference may be: TCD being the most sensitive method, TTE providing false negative results due poor imaging quality, or TOE providing false negative results and therefore misdiagnosing what may have been correctly diagnosed patients.

In the case series, none of the patients were referred on for a TOE, so TTE and TCD could not be compared against a gold standard. Cases 1 and 2 had a positive result with both TTE and TCD indicating a PFO was present; case 3 was unable to have the TCD due to an insufficient temporal window, but was deemed to have a PFO after a positive TTE. The current use of TOE as a gold standard is controversial[85] as it has a higher than average reporting of false negative results due to patients being unable to perform an adequate Valsalva while under sedation.[88] The case studies have demonstrated the ability of a mixed two-method approach of PFO detection that appears to be highly accurate. Several other studies have proposed alternative gold standard references or pathways of diagnosis. Caputi et al., suggests TCD and TTE should be the first methods of choice for PFO diagnosis,[88] which Komar et al., somewhat agrees with, suggesting that the patient should only go on for a TOE if assessment for closure is needed.[74] Stafford et al., proposes that TCD should be the first method of screening. If negative the

patient should have a TTE, but if positive the patient should go on to a TOE for assessment.[68] González-Alujas et al., took a different approach, and used the presence of a PFO to be definitive if there was concordance between any two techniques in their diagnosis.[48] While the present study is too small to be conclusive, and has not been able to assess the ability of TOE, the resulting pitfalls and advantages of TCD and TTE are similar to those in large scale studies. It is obvious that more information is needed to propose and establish an alternative gold standard that challenges TOE – whether this may be a single technique or a mix of several.

Consistency of the Valsalva Manoeuvre

The Valsalva manoeuvre increases the sensitivity of TTE, TCD and TOE by shunting microbubbles through a PFO, were there to be one.[52] However, studies tend to be inconsistent between their application of the manoeuvre – with some studies teaching patients the prompt prior to the assessment,[48] some using a calibrated device,[146] some using a coughing technique,[53] and some using abdominal compression.[51] All of these techniques work – but some are better than others. In the present thesis, 3 different techniques were applied to the case studies, all of which produced a different result. When a calibrated device was used in case 1, the sonographer could not image due to inflated lungs; in case 2 abdominal compression was applied which resulted in a very large RLS; and in case 3 the patient was asked to perform the Valsalva manoeuvre on their own accord (no prior training).

The use of either a trained Valsalva manoeuvre, or a backup technique (such as abdominal compression) after an unsuccessful Valsalva manoeuvre appeared to pay off in regards to the sensitivity of TTE in both A and B grade studies in the present meta-analysis. González et al.,[48] and Maffè et al.,[69] used either a secondary technique after a failed Valsalva manoeuvre, or trained their patients prior to the assessment, and had the highest levels of sensitivity for TTE in the A grade studies (100% and 89% respectively). Contrastingly, Di Tullio et al.,[81] and Nemec et al.,[82] both did no prior training and had no backup technique and showed the lowest levels of sensitivity for TTE (47% and 50%). A similar trend can be seen amongst the B grade studies – patients trained to perform the Valsalva manoeuvre prior to assessment had a sensitivity of 94% for TTE (Corrado et al.[125]) whereas patients with no prior training and normal prompted Valsalva manoeuvre gave a sensitivity of 47% for TTE (Itoh et al.[124]).

The use of abdominal compression to prompt a more successful Valsalva manoeuvre is an up and coming technique that has shown promising results when using TTE, TCD, and TOE.[51,54,147] It is particularly useful in sedated patients during a TOE,[147] when a normal Valsalva manoeuvre is unsuccessful,[148] or in patients that have abdominal obesity.[54] In case 2, the patient was classed as obese, and showed small shunting during a normal Valsalva manoeuvre on both TTE and TCD. However, when abdominal compression was applied, a large shunt was seen on both methods, indicating the severity of the shunt and therefore suggesting the need for treatment which was later carried out. This emphasises the importance of a proper Valsalva manoeuvre so the shunt can be graded with the highest level of accuracy and an educated decision can be made regarding treatment versus no treatment. More generally, it emphasises the importance of creating the largest shunt through a PFO in time with the saline injection, in order to optimise imaging.

Clinical Application

Stroke is a leading cause of death and disability worldwide, and cryptogenic strokes are believed to account for up to 30% of all ischemic strokes.[149] A CS is as a stroke that has an undetermined pathogenesis and its cause cannot be attributed to the usual sources of embolism that can cause an ischemic stroke.[150] The presence of a PFO appears to be associated with CS, and the diagnosis of a PFO may provide an answer to those patients with CS as to how embolism occurred.[22] When a patient is diagnosed with a PFO, clinicians must determine the contribution of the PFO to the presenting condition (was it an incidental finding or pathological), the risk of the PFO causing recurrent events, and the decision going forward (leave the PFO, administer oral drugs to hinder recurrence, or close the PFO). Factors that weigh in on these conclusions often involve shunt size, current medication, age of patient, existing health conditions (such as hypertension, obesity, previous history of ischemia), or the presence of ischemia on magnetic resonance imaging (MRI). There is a higher chance of CS patients presenting with a PFO than in the general population.[47,134,144] The mechanism behind this relationship is thought to be due to the PFO providing a route for venous, unfiltered blood to cross the interatrial septum into the arterial system where it can cause ischemia if an embolic source gets lodged in a cerebral artery.[13] Although a relationship exists between the two, the presence of a PFO is not necessarily causal for CS.[117] This relationship is still under investigation, so it is imperative that the techniques used to assess for a PFO provide the utmost level of clarity so the correct assessment and decision can be made going forward. As studies

produce novel findings about each diagnostic technique, and methods that help enhance them, the precision of the diagnoses improves. This present thesis has explored two diagnostic techniques (TTE and TCD), and highlights issues and benefits of each in real life case studies.

The contribution of PFO to stroke and other conditions

A patent foramen ovale does not only have a relationship with cryptogenic stroke, but has been linked to other conditions such as migraine[151] and decompression sickness.[9] The pathology of migraine is still under debate, but is thought to be due to a phenomenon called 'cortical spreading depression' which is characterised by a self-propagating wave of cortical excitation closely followed by temporary depression of neuronal activity leading to headache-like symptoms.[131] PFO size appears to have a correlation with migraine. Schwerzmann et al., found a PFO in 47% of migraine patients compared to 14% of healthy controls, and found that larger shunt size was correlated with presenting migraine.[131] Monte et al. found that the PFO diameter was smaller in patients with migraine compared to those with CS.[152] Additionally, it has been said that TTE is less sensitive when it comes to smaller PFOs, and may therefore be less accurate in detecting patients with migraine.[78] The grade A study by Zito et al., which comprised of 50% migraine patients, matches this finding as TCD demonstrated a high level of sensitivity (96%) compared to TTE (57%) when compared against the gold standard TOE.[34] While the general consensus seems to indicate some form of correlation between PFO and migraine,[12,24,98] a large scale trial by Mattle et al., found that the closure of PFO did not decrease the number of monthly migraines in a cohort of patients undergoing closure due to migraine.[36] The association between migraine and PFO warrants further exploration and while the present thesis does not explore migraine directly, it does add to the conversation regarding its association with PFO and the ways it can be detected.

Peripheral Emboli

While the majority of the focus regarding paradoxical embolism lies with neurological disorder, the effects of emboli travelling downstream to the periphery has been somewhat ignored. Case 2 and case 3 in the present thesis both showed emboli travelling downstream using TTE (Case 3 – Fig. 12). While the PFO in both cases was classed as severe using TTE, case 3 showed notably less microbubbles in the left heart compared to case 2. The fact that peripherally travelling emboli were still seen in case 3 indicates that the percentage of bubbles travelling downstream may be higher than anticipated. A case study by Daly et al., investigated

a patient with upper limb ischemia that had a large PFO discovered by TCD, but not TTE.[153] While TCD cannot visualise emboli travelling down the aortic arch, it can be speculated that if a shower is present, emboli will also be travelling downstream. Emboli that travel peripherally can cause complications if the blood flow to an artery providing blood to an organ (such as the kidney) is restricted.[19,154] While the percentage of peripherally travelling emboli is still being discussed, Dao et al., found emboli travelling peripherally in 2.9% of patients undergoing assessment for PFO.[19] Further study needs to be conducted to estimate the percentage of peripherally travelling emboli, in order to make stronger associations with peripheral events. A greater understanding between PFO and peripheral embolism may also promote the use of TCD as a diagnostic tool when assessing for the cause of peripheral embolism.

Treatment and age

Ischemic stroke accounts for around 87% of all stroke types, the majority of which occur in persons over the age of 65.[8] However, in those under 65 years of age, up to 43% of ischemic strokes are classed as cryptogenic.[50] As aforementioned, PFO size increases with age.[13] Age plays a role in PFO diagnosis in terms of its causal effect, future risk assessment and what decision is made regarding treatment.[13,25,31,68] Due to the accumulation of other comorbidities that tend to come with older age, the mechanisms behind stroke at an older age do not translate well to younger individuals.[155] Younger patients (<55 years) that present with a PFO tend to be more at risk of recurrence RLS though a PFO compared to those who are older.[13] Although the risk of general stroke recurrence is still higher in older patients due to other existing factors. Age is an important factor that needs to be taken into consideration when deciding on treatment for PFO.

If the PFO is thought to be of concern for recurrence events, the decision to treat it using medical or interventional methods can be made. Medical treatment usually involves the administration of oral anticoagulants or antiplatelet agents, whereas interventional treatment usually involves the closure of the PFO using a device.[10] Devices used for the percutaneous closure of a PFO have evolved through the years,[156] and the Amplatzer septal occluder is now a common method of treatment for PFO. This method involves the physical closure of the PFO using an umbrella-like device put into place by a transeptal sheath via the femoral vein.[157] One particular study by Cifarelli et al., observed the recurrence rates of patients having undergone PFO closure after 3 years.[158] Patients under the age of 55 years showed a 1% recurrence rate

for an ischemic event, while those over the age of 55 years had a much higher recurrence rate of 16%.[158] Although both of these recurrence rates are lower than the rate without treatment (20.4%),[5] the older population is at a significant disadvantage. Older patients not only have a higher chance of device dislodgement,[18] but are also more likely to have secondary health conditions and may already be on anticoagulants, hindering their ability to be administered more due to abnormal bleeding.[159] While there have been several studies investigating the recurrent rates of a cerebral event in treated PFO patients following a small number of years,[160–162] few studies have looked at the recurrent rates over many years. Mono et al., looks at the long-term recurrent rates of the use of anticoagulants versus PFO closure and has given interesting results.[117] In the anticoagulant group (mean age 51 ± 13 years, n=158) there was a 20.3% recurrent rate, and 10.1% death rate after 8.1 years of follow up (± 4.7 years).[117] On the other hand, the medical closure group (mean age 50 ± 12 years n=150) had a recurrent rate of 10.7% and death rate of 4.7% over 9.2 years follow up (± 3 years).[117] This demonstrates that the effectiveness of transcatheter closure as a treatment option in the long run, compared to anticoagulants which appeared to have no long term benefit. Again, this cohort was under the age of 55 years, which indicates that this may be the best option for a younger patient (<55), rather than an older patient (>55). While this meta-analysis is not directly investigating treatment options for a PFO, the overarching importance of an accurate diagnosis in the first place is clear.

Do Neurologists and Cardiologists diagnose a Patent Foramen Ovale differently?

Cardiologists and neurologists tend to have different objectives when it comes to diagnosing a CS patient. Cardiologists are not only interested in the presence of a RLS, but the type of shunt, size of shunt, and how suitable the shunt is for closure, if needed. Neurologists may be more interested in the stroke and the determination of its cause. As a result, different specialists tend to assess the condition in different ways. For example, typically TCD is the first test used by neurologists and TTE by cardiologists, and their expertise is reflected in higher use of each test retrospectively. In the present meta-analysis, a study conducted predominantly by cardiologists had the lowest level of specificity for TCD (92%).[69] Although the TCD procedure itself was conducted separately by a neurologist, there was no mention of the timing of microbubble appearance in the MCA. In fact they mention using a 25 second window of recording following the Valsalva manoeuvre. This would allow for intrapulmonary shunts to be included and confused with PFOs. Indeed, they conclude that TCD "does not allow a cardiac control of

shunts, loading more with false positives[69]". This is likely to be what lead to the lower specificity of TCD through a higher rate of false positives.

In 2013 Pristipino et al., conducted a large scale working group study to help create a shared management scheme for CS patients with a PFO.[163] The study gave clarity around the diagnostic workout, described different scenarios that would indicate probable pathogenesis, outlined the different characteristics that a PFO could have and what they implied, and recommended medical and interventional therapies based on recurrence.[163] However this was established the same year as the RoPE score by Kent et al., which essentially pooled the collective evidence presented by Pristipino, and made a tool which could indicate the causal effect of the PFO in terms of the CS and the likelihood of recurrence.[31] The success of each paper and its suggestions can be reflected in the number of citations for each paper, with Kent et al., being cited six times more than Pristipino et al.

Strengths and Limitation of this Thesis

This study showed several strengths in the fact that the sample size, although small, demonstrated a variety of presenting issues with either technique. This enabled each technique to be properly criticized and added to the ongoing conversation around optimal method of detection. Additionally, the conduction of the meta-analysis was a huge strength as it was novel, and was able to provide some additional strength to my thesis when patient numbers were diminished. This study had several, large limitations. Firstly, the process around sourcing ethical approval took almost 4 months. Originally it was thought that approval through OUHEC was needed, but we later found that we needed to escalate the ethical application to HDEC. The decision to apply for an amendment to contact previous eligible patients was made (which was accepted). However, at this particular moment COVID-19 arrived in New Zealand, and the hospital was shut down. At this stage I had only recruited the 3 participants presented in this thesis. Lock down ended mid-way through May, bearing in mind my thesis was due in June. There was no further opportunity to recruit patients, and the lack of patients (anticipated to be a 40-60 person study) has had a severe impact on the statistical significance this thesis holds. Additionally, the reliability analysis was cut short by COVID-19 as I was unable to access the hospital and the TCD machine, so unfortunately only 2/5 participants were able to have all three reliability tests conducted, hindering the results. One particular limitation of this study was my inability to observe cerebral blood flow using TCD through an alternative

window. At the time of screening case 3, I had only ever practiced insonating the MCA, though the temporal window, and did not want to try a separate window for the first time on a patient. In hindsight, I should have practiced several other alternatives to the temporal window in case of its absence.

Future Direction

A lot of work is needed in this area to gain more clarity around the optimal method for PFO diagnosis. It is obvious that TCD is a promising diagnostic tool for a bedside diagnosis, but more information regarding its strengths and limitations is needed. This study investigated many aspects which could improve the diagnostic certainty of TTE and TCD, as well as TOE indirectly. More research into avenues such as the use of abdominal compression to provoke a RLS, the imaging of the aortic arch, and the injection of agitated saline into the femoral vein would be advantageous. To truly get a representation of the effects of these techniques a large scale study is needed that involves the use of TCD, TTE, and TOE. Whether or not TOE should be used as a gold standard is controversial, but it certainly brings more to a diagnosis than if it were absent. The application of the newly established RoPE score would be beneficial, as well as a follow up to indicate its accuracy of indicating the likelihood of recurrence. Treatment follow up in all of these patients, alongside a non-treatment group would help shed light on what types of treatment were better for what age, and how the RoPE score indicates this. More insight is needed regarding PFO size, as well as the effect of newly introduced imaging modalities in papers of different decades. Overall, a firm stance is known about PFO diagnosis, but there is certainly more to be known, which would be hugely beneficial to those diagnosed with PFO.

Conclusion

The present thesis has explored the potential techniques for PFO diagnosis in patients with ischemic stroke, cryptogenic stroke, or migraine. More clarity is needed as to which technique is optimal to detect a PFO, to determine its causal effect with CS, and to identify the longer term prognostic impact for the patient. We compared the two bedside techniques TTE and TCD in patients in the Southern District Health Board that presented with stroke, or stroke like neurological symptoms. Both techniques demonstrated good diagnostic ability, but neither was ideal. TTE was able to grade all three shunts, and indicate the most at risk shunt. However, it was less able when a calibrated device inflated one patient's lungs. TCD gave great diagnostic

accuracy for 2/3 patients but was not able to produce a diagnosis on the third due to an insufficient temporal bone window. Two of the participants had the PFO classed as an incidental finding, which was attributed to no stroke like infarcts on later imaging, and a high chance of neurological symptoms being due to a mechanism unrelated to PFO. We also investigated the use of the two techniques in the current literature and found interesting results in terms of sensitivity when a gold standard reference was introduced. The current status of TTE and TCD in the literature aligns with the findings of the case study in regards to some of the pitfalls and advantages of each technique. The meta-analysis also found that several measures such as a consistent Valsalva manoeuvre, the year the study was conducted, or type of clinician performing the technique influenced the resulting diagnostic ability of the consequential tests. Overall, this thesis explored the use and current status of TTE and TCD as diagnostic methods for PFO detection. The treatment of a PFO can be lifesaving, so ~~the~~ accurate diagnosis and assessment is crucial.

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Appendix

Protocol

Detection Methods for a Patent Foramen Ovale: A Comparative Cardiac Imaging Study

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Protocol 6.0

9th September 2019

Hanna Van der Giessen

Supervisors:
Gillian Whalley
Sean Coffey
Luke Wilson

For UOHEC (Health) Ethics Approval

Table of Contents

1. SUMMARY

1.1. Study Title

Detection Methods for a Patent Foramen Ovale: A Comparative Cardiac Imaging Study.

1.2. Overview

A patent foramen ovale (PFO), is a small hole in the heart that allows blood to pass from the right to left atrium. Young patients (<65 years) that have had a cryptogenic stroke are routinely screened using echocardiography for the absence/presence of a PFO. The gold standard for PFO detection is a transoesophageal echocardiogram (TOE), however it is an invasive procedure. A transthoracic echocardiogram (TTE), is less invasive and therefore performed initially. Transcranial Doppler (TCD) is an alternative method of detection, which is minimally invasive and may improve on the sensitivity of the TTE. Typically, TTE and TOE are performed within cardiology departments where these patients are referred, but TCD is not. This study will investigate and compare these three detection methods on a population of young, cryptogenic stroke patients.

1.3. Lay Abstract

A PFO is a type of hole in the heart that can be treated with a relatively low risk procedure. Under certain conditions, a PFO opens and allows blood clots to cross through the hole, which can cause a stroke. A stroke that does not have an identifiable cause is called a cryptogenic stroke, and patients that have a stroke of this nature are referred to the cardiology team to assess if the heart was involved in the origin of the clot. This can be completed with direct imaging the heart, or indirectly by imaging a blood vessel of the brain with ultrasound. This study will investigate the best way to identify the presence of a PFO in patients with a cryptogenic stroke by comparing these techniques.

1.4. Scientific Abstract

Patients with a cryptogenic stroke have an unidentified cause of emboli. These patients are often referred for an echocardiogram as a common cause of cryptogenic stroke is a PFO. A PFO can cause right-to-left cardiac shunting, allowing venous blood to traverse the interatrial septum, entering the arterial blood stream. The venous blood has microplaques and microemboli present that are typically filtered out by the lungs but, in the presence of a PFO, these can travel to the brain or other sensitive areas of the body without filtration. When this happens, these microemboli, or indeed larger emboli, may cause a stroke or systemic embolus. Echocardiography methods such as a TTE, TOE, or TCD paired with agitated saline, with and without provocation with the Valsalva manoeuvre, can be used to detect if the patient has a

PFO. Although TOE is arguably the gold standard for PFO diagnosis, it is invasive and often requires sedation which may impair the patient's ability to perform the Valsalva manoeuvre. TTE is the first cardiac imaging investigation performed in the management of stroke, as it is non-invasive, can be performed at the bedside, and provides additional information beyond detection of PFO, such as identification of other features that are high risk for embolism, e.g. mural thrombus. Those who screen positive for a PFO may have a TOE to confirm findings, accurately identify the size, and help plan management. In some patients in whom a PFO is still considered likely, but it isn't clearly identified with TTE, a TOE may be performed even if the TTE is negative. Literature regarding the sensitivity and specificity of TTE and TCD is contradictory, and there are limited studies directly comparing these methods in a sample population. This study will compare the diagnostic utility of TTE and TCD for PFO diagnosis in a population of young, cryptogenic stroke patients.

1.5. Objectives

This project aims to optimise the initial process of PFO identification in patients with a cryptogenic stroke. Specifically, the clinical sensitivity and specificity of the TTE will be compared to TCD in PFO diagnosis and in those who are positive to either method with the gold standard TOE. It will also evaluate different areas where agitated saline can be imaged (interatrial septum, aortic arch, and middle cerebral artery). This project will provide important information about the optimal approach to PFO detection and assessment of future embolic risk by adding to the evidence regarding PFO diagnosis.

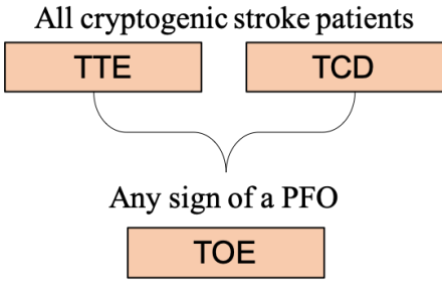

Figure 1: The primary objective from this study is to optimise PFO detection. Because of controversy in literature regarding the optimal detection method (TTE vs TCD), the study will use and compare both methods on all patients. If a PFO appears to be present, TOE will be used as the gold standard to reassess the PFO, as well as the sensitivity and specificity of both detection techniques.

1.6. Investigators

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1.7. Population

As part of their regular clinical management patients with a cryptogenic stroke or systemic embolus are referred to the Dunedin Hospital cardiology team for the evaluation of a cardiac source of embolus, which includes a bubble study with agitated saline for PFO screening. These patients will be invited to participate in this research study. If the patient consents to this research study, in addition to the normal echocardiographic assessment the patient will also have their anterior and middle cerebral circulation assessed with TCD during the bubble study for PFO screening. The study cohort is expected to be a minimum of 40-60 patients.

1.8. Duration

September 2019 – June 2020

2. RATIONALE

2.1. Background Information

During foetal development, the foetus is provided with oxygen rich blood by the mother[164]. The oxygenated blood bypasses the developing lungs through a small hole called the foramen ovale, located in the septum between the right atrium (RA) and left atrium (LA) which normally closes soon after birth, allowing the blood to assume normal circulation through the lungs[165]. A patent foramen ovale (PFO) is a remnant of the foetal circulation where the foramen ovale does not close completely following birth, affecting ~25% of the population[46]. Even in healthy individuals, the venous circulatory system contains micro-

plaques and small thrombi which are normally cleared by the lungs[20]. But in the presence of an inter-cavity connection (such as a PFO), a cardiac shunt where blood moves from the right to left atrial can lead to micro-plaques entering the arterial circulation with the potential to lead to an embolic stroke if they reach the brain[19]. Studies vary in regard to the percentage of strokes caused by an emboli reaching the brain via a PFO, but figures tend to indicate that the percentage lies between 2 – 11% of stroke patients[46,166]. Studies also show that cryptogenic stroke patients are significantly more likely to have a PFO compared to previously diagnosed stroke patients[81].

Patients having experienced a cryptogenic stroke are typically referred for further investigation. There are three common ways to detect a PFO. A transthoracic echocardiogram (TTE) paired with agitated saline uses ultrasound to observe microbubbles in the LA or left ventricle (LV), or directly traversing through the septum itself, indirectly indicating the presence of a PFO[4]. A transoesophageal echocardiogram (TOE) uses the same technique, but uses an ultrasound probe in the oesophagus, which provides higher resolution imaging of the septum (including direct visualisation of the PFO) and evaluation of the suitability for repair[81]. Transcranial Doppler (TCD) also uses agitated saline, but examines arteries closer to the brain such as the middle cerebral artery (MCA) which is supplied blood from the aorta, providing an indirect method of PFO detection[79]. During a TCD both visual and audio outputs are provided and a Spencer Shunt grade (1 – 5) is used to rank the size of the PFO. In echocardiography, the number of bubbles traversing the septum are noted, but the defect is not graded per se. Currently, when patients are being observed using any of these methods, they are asked to perform the Valsalva manoeuvre. This involves the patient forcing exhalation which causes a change in intrathoracic pressure; this action is used to elevate the pressure in the right atrium relative to the left atrium and therefore force the PFO to open and allow blood and agitated saline to pass through the PFO if one is present[78].

In New Zealand, current clinical approaches typically involve patient referral to an echocardiography laboratory where TTE and TOE can be performed, and TCD is rarely part of that diagnostic pathway. TCD has been promoted as having better sensitivity and specificity than TTE but is not commonly used in clinical practice[46,74]. It is common for TOE to be used when there is a high clinical suspicion of a PFO.

Although many studies have focused on the role of a PFO in cerebral embolic events, it should be acknowledged that emboli that cross a PFO can also cause other systemic embolic events (such as in the kidneys and GI tract)[19]. Importantly, TCD will only indirectly provide a risk assessment for such emboli (as the emboli do not travel to the brain), but current echocardiography methods may detect the potential for non-cerebral embolic events. The addition of the aortic arch imaging will allow us to assess the risk of non-cerebral emboli as the direction of travel of the bubbles (into the neck vessels, or into the descending aorta) will be visualised directly.

The detection of a PFO is important because closing the defect, using the minimally invasive option of transcatheter closure has been proven to reduce the risk of further strokes[27,

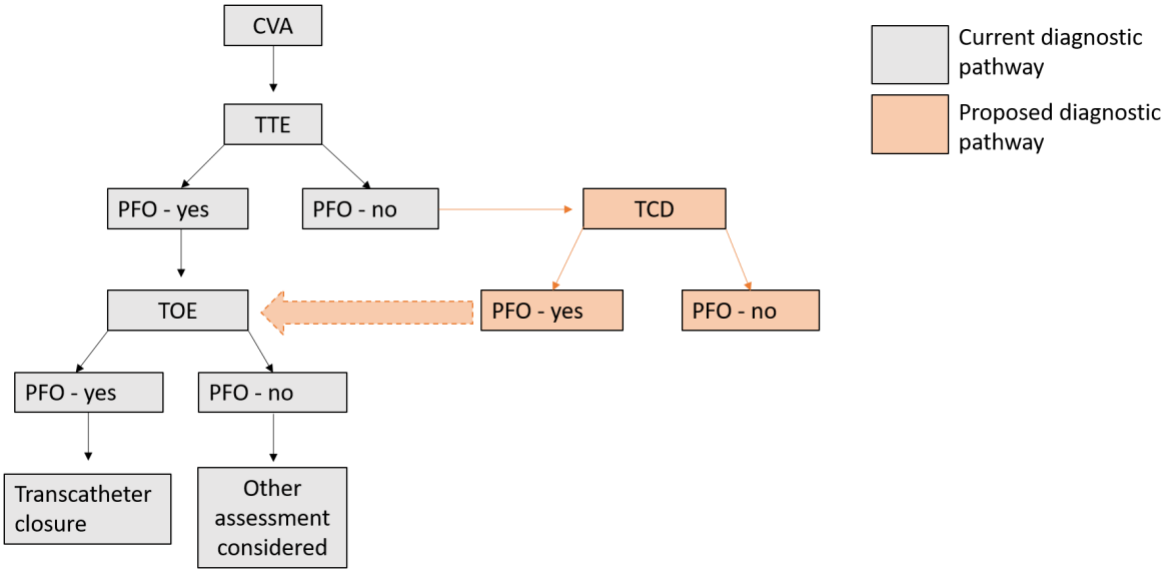

Figure 2: Proposed future clinical pathway, depending on the results of this study. Patients with a cryptogenic stroke will be referred for a routine TTE, and bubble study which would usually indicate the presence or absence of a PFO. The proposed pathway suggests that in clinical situations, a TCD will be undertaken either cocurrently, or following the TTE + agitated saline. Due to literature questioning the specificity and sensitivity of TTE compared with TCD, the addition of TCD screening may provide patients with a more accurate diagnosis.

2.2. Rationale

It is estimated that approximately 20-25% of the New Zealand population are currently living with a PFO. At the moment, the primary method of PFO detection is TTE. While TTE has reasonable diagnostic accuracy, some studies have shown that the TCD method may provide even more diagnostic certainty[46,53,74,93]. There have been studies comparing the two methods, but not many comparing the two in the same patients, with the third method as a basis for specificity (TOE). By using the same two techniques on each patient, a consensus regarding the specificity and sensitivity of each technique will be more attainable, and the use of TOE in the stages closer to potential PFO closure will give an indication if either of the methods is providing the user with false or misleading diagnostic information.

PFO detection has focused on two aspects: visualisation of non-transpulmonary microbubbles traversing from the right to the left side of the heart (using TTE or TOE), and detection of bubbles reaching the brain (TCD). These approaches ignore the fact that some bubbles, and therefore emboli, may actually travel to the descending aorta and lead to peripheral embolic events. To our knowledge, no research has evaluated the presence of agitated saline within the aortic arch, where bubbles would then travel into other parts of the body. This is of importance because hypothetically, although most of the shunted venous blood would travel to the brain, some blood will reach other parts of the body and cause complications (such as in the kidney). A more in depth understanding regarding the number of bubbles that

may be travelling in this direction will add to the literature about how a PFO can affect alternative parts of the body. It may also provide insight into the origin of other embolic events that affect the periphery, and perhaps indicate that aortic arch screening for a PFO may be a viable diagnostic option.

This project also carries significance to both scientific literature and clinical practice. Firstly, the study will add to the evidence regarding PFO diagnosis, which may simplify and improve diagnostic procedures in the future. Secondly, improved detection of cryptogenic stroke patients may lead to improved management of such patients. Additionally, it may highlight another area where PFO detection could be useful.

2.3. Risks and Benefits

2.3.1. Risks

A minor potential risk is discomfort – some people find the pressure of the ultrasound transducer troublesome, but is usually minor. There is a small amount of transient pain associated with the intravenous cannula used with the agitated saline injection and participants will also have to give up their time. Every effort will be made to minimize any discomfort and be as efficient as possible.

2.3.2. Benefits

The primary benefit from this study is that it will increase our understanding of how to manage patients with cryptogenic strokes. This study will add to the literature regarding the optimization of PFO detection methods. Some direct patient benefit may occur to participants in the study as some may have a more accurate diagnosis of PFO by the introduction of TCD. This will influence the decision whether or not they get the PFO closed, which may in turn prevent a secondary stroke or transient ischemic attack (TIA) in the future. By creating and finding more clarity, perhaps the detection of PFOs in the future will be done with more ease and certainty than in the present day.

3. OBJECTIVES

3.1. Primary

- To provide further insight into the best method for primary PFO detection in cryptogenic stroke patients by comparing TTE and TCD sensitivity and specificity, and in those who are positive with TOE as the gold standard of diagnosis. This will provide patients with a more specific, and sensitive diagnostic procedure and help practitioners reach the right decision regarding percutaneous PFO closure.

3.2. Secondary

- Additionally, this study will also image the course of saline bubbles through the aortic arch, which will give insight into the additional complications a PFO could cause in other organs in the body. It also may prove to be a superior method of PFO visualisation and/or detection too.

4. STUDY DESIGN

This is a prospective study that will involve participants referred to the Echo team for a bubble study to assess potential intracardiac shunt. The patients will be consented before they come to the echo lab. Most patients will have the TTE and TCD in the same sitting if a PFO is suspected. However, some patients (not included in Figure 3) will just have a routine TTE first, and then if a suspicion subsequently arises regarding a PFO, the patients will be referred back to this particular study and a TTE + TCD bubble study will be conducted. Patients that have the bubble study initially (as outlined in Figure 3) will ideally have the TTE and TCD on the same day, and they will be conducted at about the same time of day (the TCD will be after the TTE). Recorded results from both the TTE and the TCD will be recorded and saved for analysis at a later date.

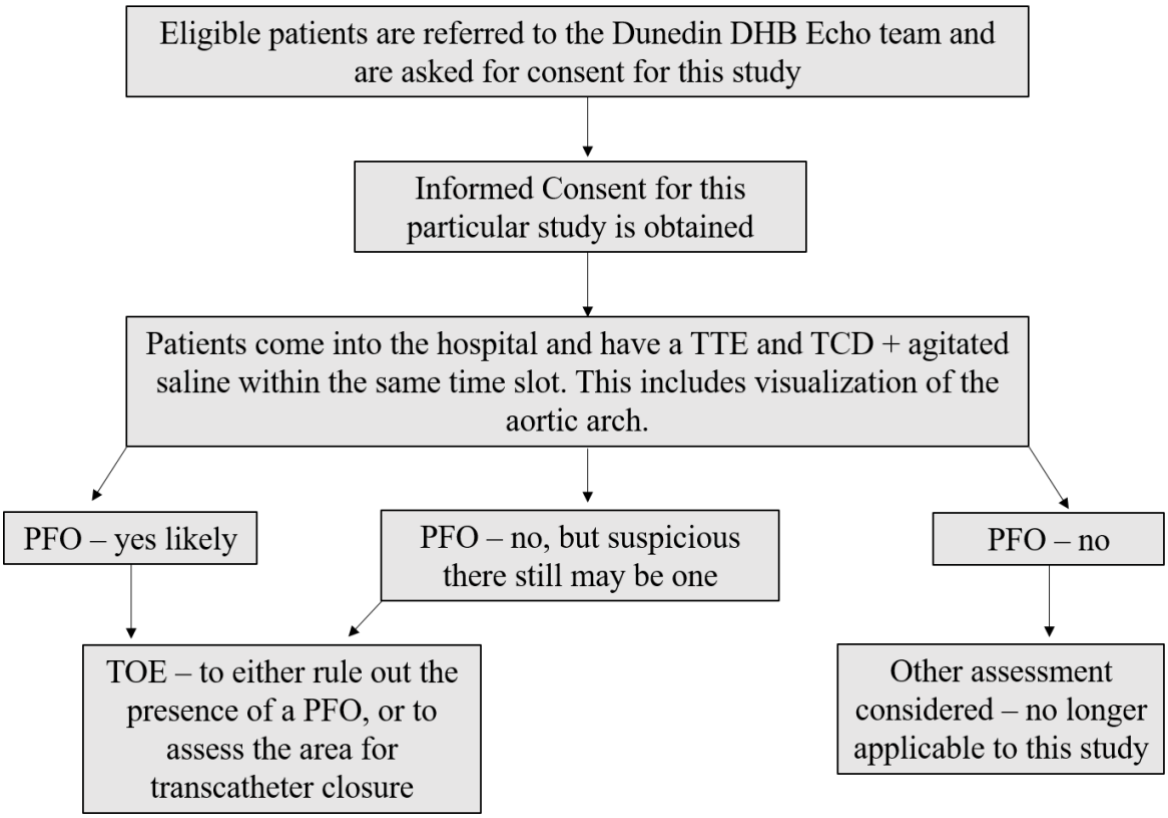

Figure 3: The study design as anticipated.

5. PARTICIPANTS

5.1. Inclusion Criteria

a) Patients that have been referred to the echocardiography service for a routine TTE after a cryptogenic stroke or systemic embolus.
b) Over the age of 18
c) Willing and able to provide informed consent.
d) Willing and able to comply with the study procedures.

5.2. Exclusion Criteria

a) Have another identified potential cause of cardiac emboli other than PFO in their routine TTE (e.g. left ventricular thrombus, myxoma, vegetation on valve).
b) Patients who are unable to perform the Valsalva manoeuvre.
c) Pregnancy.

5.3. Recruitment and Enrolment Process

Patients will be approached for the recruitment into this study when they are initially referred to the echo team for a TTE following a cryptogenic stroke or systemic embolism. This may include inpatients in the wards. Young stroke patients will usually have a bubble study during the same appointment as a structural TTE, so during recruitment, it will be made clear to the patients that the additional TCD will ideally be done on the same day as the TTE, to avoid patients having to return to the hospital. Most patients will have their TTE during their hospital admission.

There will be no monetary compensation for this study, as it is a small addition to their usual clinical testing.

Once patients have been asked if they would like to participate in this study, they will be given an appropriate period of time where they can then consider the offer, so they feel no time pressure or obligations. If the patients agree to participate, they will sign the consent form.

5.4. Visits

The clinically indicated TTE and the research TCD bubble study will ideally be conducted on the same day, but, if the participant wishes, can be performed separately. Further management, which is likely to include a TOE, is at the discretion of the treating clinical team.

6. PROCEDURES

6.1. Echocardiogram procedures

- Echocardiography will be performed by a sonographer using standard echocardiographic machines (Vivid S6, E9 or E95, GE Ultrasound or SC2000Prime, Siemens Ultrasound). Images will be recorded according to the recommendations of the American Society of Echocardiography. Recorded images will be transferred for analysis using the TomTec Image-Arena (TomTec, Unterschleissheim, Germany). Views for left ventricular and left atrial assessment will include the apical 4-chamber, apical 2-chamber, apical long axis and short-axis views. Images of the right ventricle and right atrium will be recorded in the apical 4-chamber view.
- Saline will be injected into the arm using a three-way tap, and two syringes of saline. In the three way tap the saline will be mixed with a little bit of drawn up blood to agitate the saline for optimal imaging. The volume of saline injected, as well as blood drawn up into the syringe for the saline agitation will be kept constant over all participants.
- Following the injection of the agitated saline, the heart will be continuously imaged and subsequently recorded as the patient performs the Valsalva manoeuvre.
- The Valsalva manoeuvre consists of the patient forcing exhalation for a period of time on a closed airway to create an increase in pressure within the chest, which if present, causes blood to shunt through the PFO. A successful Valsalva will be based on the visualisation of atrial motion (right to left) using TTE. If the patient is unable to perform the Valsalva manoeuvre manually, a mouthpiece attached to a mercury manometer will be used to encourage patients to exhale to their best ability.
- A PFO would be positively identified with one of the following by TTE: 1 to 9 bubbles (grade I), 10 to 20 bubbles (grade II), and 20 bubbles (grade III) appearing in the left atrium.
- The TOE is performed if clinically requested. While the results of the TTE and TCD are likely to be known to the performing clinician, we will subsequently anonymise the images are blinded adjudication.

Echocardiographic Variables to be measured (in addition to assessment of septum)	
M-mode and 2D dimensions	Right ventricular end-diastolic dimension Left ventricle (LV): end-diastolic dimension, LV end-systolic dimension, interventricular septal thickness, LV posterior wall thickness, fractional shortening Aortic root, Left atrial diameter
2D area	Left atrial (Apical 4 chamber view) Right atrial (Apical 4 chamber view)

2D volumes	Left ventricular Simpson's biplane Left ventricular Simpson's single plane Left atrial Simpson's biplane Left atrial Simpson's single plane Right ventricular volumes
Doppler	Left ventricular outflow tract peak velocity Mitral E:A ratio Mitral deceleration time
Tissue Doppler	Medial mitral annulus (e', a', s') Lateral mitral annulus (e', a', s') Lateral tricuspid annulus (e', a', s')

3D=three-dimensional, E=early filling wave of trans-mitral flow, A=late filling wave of trans-mitral flow, e'=tissue velocity during early filling within the mitral annulus (MA), a'=tissue velocity during late filling within the MA, s'=tissue velocity during ventricular systole within the MA, E/e'=ratio of E wave to e', S=systolic, D=diastolic, A=atrial contraction.

6.2. Transcranial Doppler Procedure

- Standard 2-D, M-Mode and Doppler measurement will be conducted using the TCD device and will image the middle, and posterior cerebral artery (MCA & PCA)
- The M-mode on the TCD device will be used to assess the PFO and subsequent shunt in patients. The TCD machine will record both visual and audio recordings following the saline injection, and Valsalva.
- The TCD device will be setup and ready to use prior to the patient arriving into the room. After providing the patient with a lay explanation of the process, around 20ml of ultrasound gel will be placed onto the side of the patient's head, where the temporal window is. Any air bubbles will be smoothed out.
- The headpiece will be placed on the patients head and the top and back strap will be tightened to ensure the device is secure as well as the patient being comfortable.

- Once secure around 50ml of gel will be placed onto each of the probes, and they will be attached onto the headpiece and moved into place using the adjustors ready

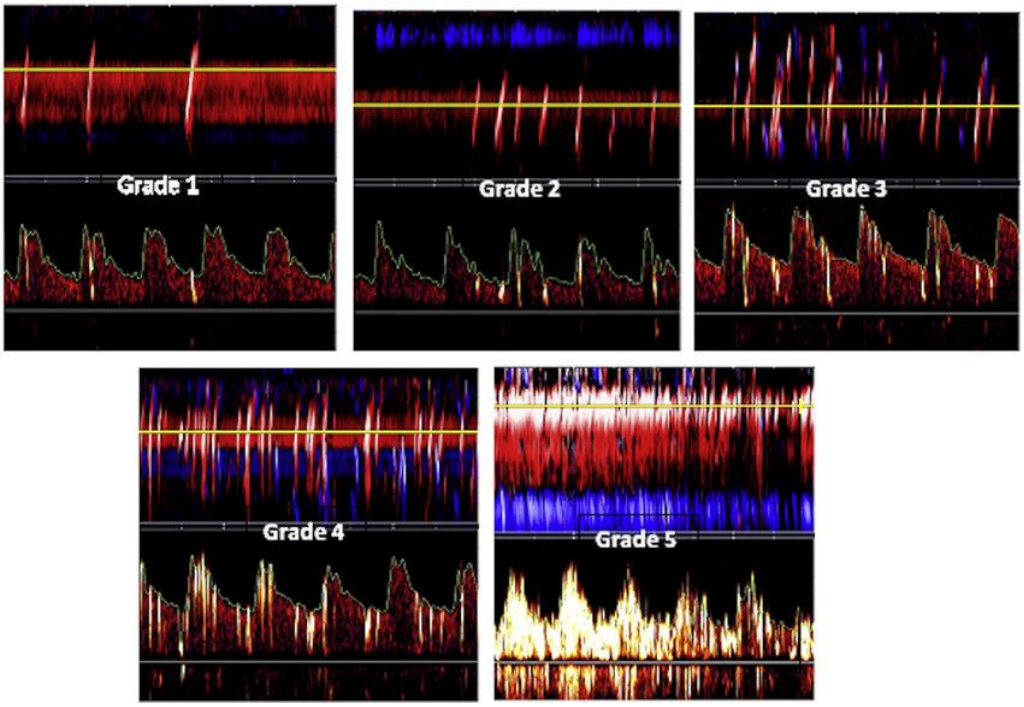

Figure 4: Transcranial Doppler screenshots of Spencer shunt grades 1-5. It can be seen that the presence of bubbles in the cerebral arteries is obvious; besides the visual output on the screen, a loud signal is heard from the audio output with each bubble crossing the patent foramen ovale. Grade 0, no microemboli detected; grade 1, 1-10 microemboli; grade 2, 11-30 microemboli; grade 3, 31-100 microemboli; grade 4, 101-300 microemboli; grade 5, >300 microemboli. (Source: Cardioembolic Stroke: Everything has changed. DOI:10.1136/svn-2018-000143)

6.3. Safety

Although all of the patients in this study will have previously had a stroke or systemic embolism, this particular investigation involves minimal risk. Depending on how the stroke or systemic embolism has affected the patient (i.e. impaired right side partial paralysis) actions such as getting on an off the bed may cause some discomfort or risk, in this case, measures must be taken to ensure patient safety, such as making sure the bed is low, and secure (brake is on). There is a slight risk of irritation with the venous cannula, but this will be inserted by the clinical team as part of their usual care. Similarly, there are very minimal risks associated with agitated saline injection but this is also part of the patient's clinical care. Any accidental injuries may be covered by ACC in New Zealand. In the very unlikely event that any participants requires acute care, staff will follow emergency procedures for their location. In the hospital, this is likely to be emergency department care, or calling a resuscitation team by calling 777.

In the event of an unanticipated event of significance, such as an injury during examination, study personnel are required to report the event as per their individual location details and also to the principal investigator (Dr Whalley) as soon as practical.

7. EVALUATION

There will be three phases of patient evaluation;

I. Initial TTE to diagnose or exclude any structural deficits.
II. This will in general be followed up in the same appointment by a TTE with agitated saline to investigate the presence or absence of a PFO
III. A TCD bubble study will be performed as a study procedure to investigate the presence or absence of a PFO, including views of the aortic arch during the agitated saline injection.
IV. As part of routine clinical care, a TOE is likely to be performed in those who;
    a. are positive for a PFO with either or both TTE and TCD, or
    b. are negative for PFO on both TTE or TCD but the cardiologist or treating clinician has high clinical suspicion for presence of a PFO suspects

    AND are potential candidates for PFO closure.

There will also be seven phases of analysis;

I. Echo staff will observe the TTE for structural deficits (clinical procedure)
II. Echo staff will observe the TTE bubble study for the presence of a PFO in the form of a right-to-left shunt (clinical procedure - investigators will be blinded to these findings up until after assessment of the TCD findings).
III. Investigators will perform and observe the TCD recordings for both visual, and audible signs of a PFO (study procedure).
IV. Results of TTE and TCD with agitated saline will be directly compared.
V. Alternative images from the TTE (i.e. aortic arch views) will be analysed
VI. The results from the TTE and TCD comparison, will be compared to the gold standard TOE once the patient has been tested.

Primary endpoint:

The sensitivity and specificity of TTE and TCD to detect a PFO. The gold standard definition of a PFO will be a PFO seen on TTE or TOE or TCD. A PFO will be deemed absent if both the TTE and TCD are negative and there is no further suspicion that a PFO is present, and/or the TOE is negative.

Secondary endpoints:

The sensitivity and specificity of TTE aortic view compared to normal TTE (without aortic view) to detect PFO. The gold standard definition of a PFO will be a PFO seen on TTE or TOE or TCD, a PFO will be deemed absent if both the TTE and TCD are negative, and/or the TOE is negative.

8. DATA RETENTION AND STATISTICS

8.1. Sample size

Recruitment into the study will be dependent on the number of cryptogenic stroke patients referred on for a TTE for PFO evaluation. The accrual rate is expected to be 1-3 participants per week, based on previous records, so the final sample size will be 40-60. If patients are referred on for a TOE, patient participation, and therefore the "follow-up" rate is expected to be high due to the patients prioritising their health.

8.2. Records of Data

Each participant will be provided with a unique study number this number will be used to identify them in the database. The research team will keep records of screening, consent forms, medical history. All de-identified records will be kept in locked areas in the University with no access to the public.

8.3. Statistics

The specificity and sensitivity of the TTE and TCD will be compared.

Test under investigation*	Gold standard of PFO (Either TTE or TCD, and/or TOE)**	
	PFO present	PFO negative
PFO present	A	B
PFO negative	C	D

* This is either TCD, TTE, TTE with aortic views, or TTE using different venous access
** This is a pragmatic gold standard definition based on usual clinical practice. Ideally all patients would receive a TOE to rule out a PFO, but this is in line with usual clinical practice.

$$sensitivity = \frac{\mathrm{A}}{\mathrm{A} + \mathrm{C}}$$

$$specificity = \frac{\mathrm{D}}{\mathrm{D} + \mathrm{B}}$$

9. ETHICS

9.1. Informed Consent

Informed consent will be obtained prior to any data being collected. All participants will be provided with a participant information sheet that outlines the study rationale and

expectations of them as participants. After having read this, and having an opportunity to have any questions answered, and the potential participants are free to decide if they wish to proceed with the study. If they wish to participate in the study they are required to sign a consent form and can proceed to participation.

Participant information sheets and consent forms will be HDEC-approved, and the participant will be required to read and review the document or have the document read to him or her. One of the investigators will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with anyone they choose or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented, and the original consent form will be filed in research record.

Additionally, as the head is regarded as Tapu i.e. sacred, in certain cultures, consent will be granted by the participant following the complete understanding and agreement of the procedure which will take place.

9.2. Confidentiality

All study records and data collected during and after the study will be stored in a secure area at the study institution. Data will be held in a single database centrally. Each participant will be given a unique study identification and all information will be in an electronic database linked to this number and there will be no way for you to be identified from this database. There is a chance that we will use this database for international comparisons, which will include sharing of databases. All analyses will be undertaken using the unique participant identifier, not name.

No information about participants will be released without their express permission. And the only reason for doing so will be the participant's request or as a requirement for clinical follow-up. The latter will only occur with the participant's permission. When data is released (for example in publications) all patient identifiers will be omitted.

9.3. Publication

Scientific publication of results will be organized and determined by the study investigators. It is likely that primary study outcomes will be initially sent to a general cardiology journal, with more specialized findings sent to a cardiovascular imaging journal.

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