An investigation of the correlations between subjective and objective measures of bowel inflammation in Spondyloarthritis

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ABSTRACT

**Introduction:** Spondyloarthritis (SpA) and Inflammatory Bowel Disease (IBD) are related conditions of unknown aetiology demonstrating both common clinical features and common genetic and immunological pathomechanisms. Patients with SpA frequently exhibit intestinal inflammation and many develop significant gastrointestinal symptoms. Conversely, many patients with IBD develop an inflammatory arthritis. It has been proposed that an increase in intestinal permeability is an important mechanism in the aetiology of both conditions. However, to date the association between symptoms, intestinal pathology and altered gut permeability has been poorly elucidated.

**Methods:** Patients who fulfilled the Assessments in Spondyloarthritis Internation Society (ASAS) criteria for axial spondyloarthritis were recruited to the study. Gastrointestinal symptoms were assessed using the Dudley Inflammatory bowel symptom Disease Questionnaire (DISQ). Intestinal permeability was measured using the three sugars test, which measures the differential urine recovery of ingested sucralose, L-rhamnose, and lactulose. Small-intestinal lesions were assessed with wireless capsule endoscopy (WCE). An indirect measure of intestinal inflammation (faecal calprotectin) was also used. Drug therapy – including the use and dose of NSAIDs was recorded. Patients with SpA were assessed clinically including a measure of disease activity - the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and CRP.

**Results:** In total, 35 patients and 15 healthy controls completed the DISQ and underwent a sucralose, lactulose and L-rhamnose absorption test to assess intestinal permeability. Ten patients underwent WCE. The majority of patients were taking NSAIDs (25/35). DISQ scores were significantly increased in patients compared to controls (p<0.0001) and were significantly correlated with BASDAI scores (p<0.001). Intestinal permeability was not significantly different between patients and controls, and was not associated with DISQ or BASDAI scores. Faecal calprotectin results were high in some patients, but the correlation with DISQ scores was not significant (p=0.169) although a trend was apparent. WCE showed mild to severe ulcerations/erosions to be remarkably common (8 out of 9 complete studies). Macroscopic lesions of the duodenum appeared to be associated with bowel symptoms, while some severe lesions in the jejunum and ileum
were often asymptomatic. **Conclusions:** We conclude that both gastrointestinal symptoms and intestinal lesions are common in SpA patients. The use of non-steroidal anti-inflammatory drugs appears to be associated with intestinal symptoms in SpA patients, but ileocolonic ulceration is commonly asymptomatic. The DISQ appears to be a good screening tool for identifying patients with bowel symptoms which are more common with upper GI involvement and especially with lesions in the duodenum. Faecal calprotectin and WCE identified a high proportion of SpA patients as having asymptomatic lesions of the jejunum and ileum, common sites of inflammation in IBD.
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<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AS</td>
<td>Ankylosing spondylitis</td>
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<tr>
<td>ASAS</td>
<td>The Assessment of SpondyloArthritis international Society</td>
</tr>
<tr>
<td>ASQoL</td>
<td>Ankylosing spondylitis Quality of Life</td>
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<tr>
<td>BASDAI</td>
<td>Bath Ankylosing Spondylitis Disease Activity Index</td>
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<tr>
<td>BASFI</td>
<td>Bath Ankylosing Spondylitis Functional Index</td>
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<tr>
<td>BASMI</td>
<td>Bath Ankylosing Spondylitis Metrology Index</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn’s disease</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>DISQ</td>
<td>Dudley Inflammatory bowel disease Symptom Questionnaire</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>IBDU</td>
<td>Inflammatory bowel disease unclassified</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>ReA</td>
<td>Reactive arthritis</td>
</tr>
<tr>
<td>SAGE</td>
<td>SpondyloArthritis Genetics and the Environment</td>
</tr>
<tr>
<td>SpA</td>
<td>Spondyloarthritis</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tissue necrosis factor alpha</td>
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<tr>
<td>UC</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>uSpA</td>
<td>Undifferentiated spondyloarthritis</td>
</tr>
<tr>
<td>WCE</td>
<td>Wireless capsule endoscopy</td>
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<tr>
<td>µg</td>
<td>Microgram</td>
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<tr>
<td>TGFβ</td>
<td>Transforming growth factor beta</td>
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*Table 1 List of abbreviations*
Chapter 1 – Introduction

Spondyloarthritis (SpA) and Inflammatory Bowel Disease (IBD) are related conditions of unknown aetiology. SpA refers to a group of interrelated diseases, including ankylosing spondylitis (AS), reactive arthritis (ReA) and arthritis associated with IBD, among others. The inflammatory bowel diseases include Crohn’s disease (CD) and ulcerative colitis (UC).

In recent years, evidence from clinical, histopathological, immunological, and genetic research has strengthened the apparent associations between SpA and IBD and has led to the development of a unified hypothesis referred to as the Gut–Joint axis (4,5). Between 6 and 13% of patients with SpA will develop concurrent inflammatory bowel disease (4-7). Conversely, between 10 and 35% of patients with IBD will develop spondyloarthritis, with arthritis often preceding the gut manifestation (8).

Ileocolonoscopy has revealed gut inflammation in the absence of obvious symptoms to be remarkably common in patients with SpA. Over half have microscopic changes seen on intestinal biopsy and many show macroscopic lesions (9-13). Wireless capsule endoscopy has shown promise in detecting macroscopic lesions in the small intestine of SpA patients (14), while faecal calprotectin (a non-invasive marker of intestinal inflammation) is often increased in patients with SpA. Intestinal permeability, or gut leakiness, is increased both in patients with IBD and in patients with SpA. It is possible that intestinal permeability plays a role in the pathogenesis of these conditions (15).

It has been thought that the inflammation and lesions in SpA patients are asymptomatic (9) but work by Stebbings et al has found that patients with AS have increased mean scores on a bowel symptom questionnaire (The Dudley Inflammatory bowel disease Symptom Questionnaire; DISQ) (16) and Sundström et al found gastrointestinal symptoms to be common in AS patients (17). While is not yet clear if the symptoms described by Stebbings et al and Sundström et al are a consequence of the histological inflammation or
macroscopic lesions found in previous studies, gut symptoms may indeed be more common in patients with inflammation (13).

Further supporting the link between SpA and IBD is the wealth of evidence from large-scale genome-wide association studies that have begun to elucidate their genetic relationship. The evidence from these studies has helped our understanding of the pathogenesis of SpA and IBD, so the genetic associations will be summarised in the context of pathological overlap.

Chapter 2 – Hypothesis and objectives

Patients with SpA experience symptoms consistent with inflammatory bowel disease as well as increased levels of faecal calprotectin (an indirect measure of intestinal inflammation); macroscopic and histological features of inflammation in the ileo-colonic mucosa; and increased intestinal permeability. It remains to be discovered how these findings are related to one another.

The primary hypothesis of this study is that gastrointestinal symptoms in patients with SpA may be associated with intestinal lesions or underlying intestinal permeability changes. Therefore, the objectives of the project are to investigate gastrointestinal symptoms in patients with spondyloarthritis using the DISQ, a validated instrument for assessing bowel symptoms in SpA and IBD. DISQ scores will then be compared to:

1. Macroscopic appearance of the small intestine, which will be examined with wireless capsule endoscopy.
2. Intestinal permeability, which may be a key aetiological factor linking SpA and IBD. This will be measured with a three-sugar absorption test.
3. Faecal calprotectin results, an indirect measure of intestinal inflammation
4. Spondyloarthritis disease activity, measured with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI).
Chapter 3 – Background and review of the literature

To address the link between gastrointestinal symptoms and underlying pathology in patients with spondyloarthritis, a few topics must be addressed. In the following section, a background summary of SpA and IBD will be given, followed by clinical evidence highlighting their overlap. The review will continue with genetic and physiological evidence, particularly highlighting the suspected role of intestinal permeability in disease pathogenesis.

3.1: Spondyloarthritis summary
3.1.1: Spondyloarthritis family
Spondyloarthritis (SpA) is a term used to describe a group of interrelated conditions: Ankylosing spondylitis (AS); reactive arthritis (ReA); psoriatic arthritis (PsA); spondyloarthritis associated with the inflammatory bowel disease; undifferentiated SpA (uSpA); and a subgroup of juvenile idiopathic arthritis (1, 3). The spondyloarthritides share many clinical characteristics and a strong common genetic predisposition largely determined by the association with the Human Leukocyte Antigen (HLA)-B27. Common clinical features can be divided into three groups: axial (spinal) arthritis, peripheral arthritis, and extra-articular features.

In the axial spine, inflammatory back pain and stiffness are a result of sacroiliitis and spondylitis. The peripheral arthritis typically affects the lower limbs in an asymmetrical, pauciarticular pattern, frequently associated with enthesitis.

Extra-articular features include inflammatory bowel disease (IBD), anterior uveitis and psoriasis. Different subtypes of spondyloarthritis are often found within the same family (3, 18).

3.1.2: Ankylosing spondylitis
Ankylosing spondylitis is considered the archetypical spondyloarthritis (3). Onset of symptoms, typically inflammatory back pain, is usually in the
second or third decade of life. Inflammatory back pain is characterised by a dull and persistent ache usually present in one or both buttocks or hips. Alternating buttock pain is suggestive of AS, but within a few months the pain will usually become bilateral and persistent. The pain is usually worst in the early morning, and can wake the patient from sleep. While rest does not alleviate the pain, exercise may do so (19), as does treatment with non-steroidal anti-inflammatory drugs (NSAID) (20). Classification is based upon the modified New York criteria, and requires radiologically visible evidence of structural change in the sacro-iliac (SI) joint, such as joint erosion and subchondral-bone sclerosis (2, 3). The changes seen on plain radiography occur late in the disease process (3).

3.1.3: Axial and peripheral spondyloarthritis
As an alternative to dividing SpA into subtypes, patients with spondyloarthritis may be grouped into those with predominantly axial disease, and those with predominantly peripheral disease (1). Classification of axial spondyloarthritis by the Assessment of SpondyloArthritis (ASAS) classification (Figure 1) allows earlier detection of disease than the New York criteria. Magnetic Resonance Imaging (MRI), part of ASAS axial SpA criteria, is a sensitive tool in the early stages of inflammation (21). In one large study, The German Spondyloarthritis Inception Cohort, little clinical difference was noted between patients with non-radiographic axial SpA and those who met the modified New York criteria for AS (19). Disease can be classified as peripheral SpA based on ASAS criteria (Figure 2).
Figure 1: ASAS classification criteria for axial SpA. Modified from Rudwaleit et al 2009 (1)

* - Sacroiliitis on imaging refers to either:
  - Definite radiographic sacroiliitis (grade 2 bilaterally or grade 3-4 unilaterally; according to New York criteria 1984 (2, 3))
  - OR MRI evidence of active (acute) inflammation of sacroiliac joints

** - SpA features are defined as:
  - Inflammatory back pain
  - Arthritis
  - Enthesitis (heel)
  - Uveitis
  - Dactylitis
  - Psoriasis
  - Crohn's disease or ulcerative colitis
  - Good response to NSAIDs
  - Family history of SpA
  - HLA-B27
  - Elevated CRP

Figure 2: ASAS criteria for peripheral SpA. Modified from Rudwaleit et al (22)

Peripheral Spondyloarthritis
Requires one of: arthritis, enthesitis or dactylitis

Plus one of:
Uveitis
Psoriasis
IBD
Preceding infection
HLA-B27
Sacroiliitis on imaging

Plus two of:
Arthritis
Enthesitis
Dactylitis
Inflammatory back pain ever
Family history of spondyloarthritis
3.1.4: Spondyloarthritis epidemiology

The prevalence and incidence of the spondyloarthritides differ between countries and are largely dependent on the prevalence of HLA-B27 in a given population. Prevalence in Caucasian populations is broadly similar – in France the prevalence has been estimated as 0.30-0.47% (23, 24), 1.73% in Germany (25) and 0.3 – 1.3% in the USA (26). AS, as defined by the modified NY criteria, has a prevalence of approximately 0.14-0.55% in these populations (25-27), and of 0.2-1% in Western countries in general (28). No estimate of prevalence or incidence of SpA has been undertaken to date in the New Zealand population.

3.2: Inflammatory bowel disease

3.2.1: Inflammatory bowel disease classification and symptoms

Inflammatory Bowel Disease encompasses two idiopathic conditions: Crohn’s disease (CD) and ulcerative colitis (UC). Patients may be diagnosed with inflammatory bowel disease unclassified (IBDU, otherwise known as indeterminate colitis) when it is not possible to differentiate between CD or UC (29, 30).

IBD causes severe detrimental effects on quality of life, places a heavy burden on health resources (31, 32) and exerts a small but measurable reduction in life expectancy (33). Common systemic symptoms in IBD include fever, night sweats, weight loss, fatigue, growth retardation, primary amenorrhoea and anorexia (34). The prevalence of depression and anxiety are higher in IBD patients than the general population, with psychological illness associated with higher disease activity (35).

The symptoms produced by IBD depend on the area of the gastrointestinal tract involved, and therefore will differ for UC and CD. Patients may be incontinent or have severe urgency. Diarrhoea can be nocturnal and may contain mucus or blood. Constipation can occur, particularly in patients with
distal UC. CD patients may experience nausea and vomiting as well as abdominal pain and cramps. Pain and rectal bleeding may be present at defaecation (34).

3.2.2: Crohn’s disease

Crohn’s disease is most commonly diagnosed in patients between the ages of 10 and 30 (36) and usually persists in a chronically remitting and relapsing pattern for life (37). It can affect the gastrointestinal (GI) tract anywhere from mouth (38) to anus (34), but most commonly affects the ileum and colon (33), areas of high bacterial load (39). Within the GI tract, Crohn’s disease produces lesions that are asymmetric, transmural and occasionally granulomatous. Their discontinuous nature can produce skip lesions, where normal mucosa separates areas of disease. The affected areas of gut may display a cobblestone appearance and deep ulcers and fissures may be visible (29).

Intestinal complications in Crohn’s disease include:

- Fistulae and perianal disease (33, 34)
- Intraabdominal abscesses (33, 34)
- Inflammatory strictures (33, 34)
- Haemorrhage possible – more commonly from ileal ulceration than colitis (33, 34)
- Increased risk of intestinal cancers in patients with extensive and severe Crohn’s disease (40-42)

Crohn’s disease is associated with a number of extra-intestinal manifestations (43), especially in the joints, eyes, skin and biliary tract (33, 43). The arthritides commonly found in IBD patients will be covered later.

Crohn’s disease more commonly affects women than men, but an increasing incidence in males over the past decade may signal a change in gender prevalence (34). Estimates of prevalence for Crohn’s disease vary according to ethnicity and geographic area. In North America, prevalence estimates range from 26-199 cases per 100,000 people; the disease has been estimated to affect 144 per 100,000 people in Europe (36). Crohn’s disease may be particularly common in NZ, with an estimated prevalence in Canterbury of 155 per
100,000 persons in 2005 (44). The incidence rate in NZ and Australia is estimated to be 16 per 100,000 person-years, compared to <1 in Asia and South America and 1-3 in southern Europe and South Africa (34).

3.2.3: Ulcerative colitis summary
The mean age of diagnosis for ulcerative colitis is generally 5 to 10 years older than the age for CD (36, 45) with a disease course that follows a chronic pattern of remission and relapse (46). Inflammation in ulcerative colitis is usually found in a continuous, superficial, diffuse lesion extending proximally from the rectum. Disease does not extend beyond the large intestine, although mild inflammation may be found in the terminal ileum in ‘backwash ileitis’.

Complications of UC within the intestine include (33, 34):
- Haemorrhage – from ulcers. Rectal bleeding is suggestive of UC over CD
- Colon cancer risk
- Toxic megacolon (possible in CD, but less common)

UC affects men and women in approximately equal numbers (34), with estimated incidence rates ranging from 1.5 to 20.3 per 100,000 person-years in Europe and 2.2 to 14.3 per 100,000 person-years in North America. Ulcerative colitis has an age standardised incidence rate of 7.6 per 100,000 and prevalence of 145 per 100,000 persons in Canterbury, NZ (44).

3.3: Inflammatory bowel disease in spondyloarthritis

3.3.1: Introduction to intestinal involvement in SpA
There is now a wealth of evidence confirming the presence of inflammation in the gut of patients with SpA. Colonoscopy studies in SpA patients have shown common inflammation at the microscopic and macroscopic levels in patients without obvious gut symptoms (9, 13). Measuring faecal calprotectin in stool samples gives an indirect measure of intestinal inflammation and is
elevated in patients with SpA (47, 48). Finally, wireless capsule endoscopy, a relatively new procedure that allows visualisation of the entire small intestine has shown promise in detecting lesions in the gut of patients with SpA (14, 49).

3.3.2: Gut inflammation in spondyloarthritis

There appears to be a spectrum of gut involvement in SpA. Overt inflammatory bowel disease affects 6-13% of patients with SpA (4-7, 11), while a proportion of patients with SpA demonstrate no gut involvement. Between these extremes lie the patients who have subclinical inflammation. Subclinical lesions include histological inflammation, inflammation measured by surrogate markers and macroscopic inflammation seen on endoscopy.

Many SpA patients who do not have confirmed IBD still have symptoms relating to the gut. The Dudley Inflammatory Bowel Disease Questionnaire (DISQ), which is used in this study, has detected increased gastrointestinal symptoms scores in SpA patients (16). Sundström et al (17) has also shown gastrointestinal symptoms to be common in SpA. Exactly how the symptoms are related to underlying pathology is still unclear, but they do correlate with both direct and indirect measures of intestinal inflammation. The strongest relationship between symptoms and inflammation exists with the lesions of Crohn’s disease-like appearance (9, 12, 13). Courville et al (50) reviewed 29 patients in whom ileitis had been confirmed. None of those with ileitis in the absence of symptoms developed Crohn’s disease, whereas two thirds of those patients with gastrointestinal symptoms and ileitis progressed to Crohn’s disease. Symptomatic ileitis, therefore, seems predictive of a worse prognosis.

The DISQ was developed as a brief self-administered alternative to the more extensive Inflammatory Bowel Disease Questionnaire (IBDQ). The IBDQ is a disease-specific quality of life index. It has been validated as a good measure of therapeutic efficacy in patients with IBD (51) in several different cultural and linguistic areas (52). The DISQ is an easy to administer and cost-effective method of examining gastrointestinal symptoms in a clinical setting. In patients with SpA, high scores in this questionnaire are associated with higher faecal calprotectin results (53), suggesting a link with intestinal inflammation.
Non-steroidal anti-inflammatory drugs are the cornerstone treatment in spondyloarthritis (54). These drugs provide symptomatic relief in the short term (55), and limit radiographic progression, regardless of symptoms, when taken on a daily basis (56). Lesions to the gastrointestinal tract are a common side effect of these drugs. Of particular interest in this study is NSAID enteropathy, which overlaps with Crohn’s disease in areas of pathophysiology and appearance. NSAID enteropathy is usually subclinical, and involves increased intestinal permeability, inflammation, erosions and ulceration. More severe clinical outcomes have been reported, including anaemia, perforation, diverticulitis and death (57). While it is known that NSAIDs cause frequent gastrointestinal lesions in the upper GI tract (58), damage to the GI tract distal to the duodenum accounted for almost 40% of the serious gastrointestinal events that developed in a cohort of naproxen users (59). As well as inducing enteropathy, the use of NSAIDs is associated with relapse in IBD patients (60, 61). Because NSAIDs are so commonly used in SpA, NSAID enteropathy will be reviewed along-side SpA and IBD in the coming sections.

3.3.3: Ileocolonoscopic data in spondyloarthritis patients - histology

Biopsies of tissue from the colon and terminal ileum have shown that ulceration and inflammatory lesions are very common in patients with SpA. Reports of histological inflammation in SpA vary, but the prevalence is probably between 50 and 60% in AS (9, 13, 62, 63) with similar levels in SpA as whole (10, 11, 13), although reports of prevalence as high as 80% in AS (12) and 100% in undifferentiated SpA (64) have been published. Lesions are often grouped into acute or chronic by microscopic appearance. Chronic lesions form the majority of SpA histological abnormalities (9, 13, 63). A subset of these are described as having “ileal localization and the patchy, discontinuous distribution of the lesions and the microscopic features” reminiscent of Crohn’s disease (9). These Crohn’s-like lesions have a worse prognosis than the other lesions of chronic appearance, which in turn have a worse prognosis than lesions of acute appearance. There is evidence that in some cases
intestinal lesions and arthritis flare in parallel (9). Sulphasalazine treatment may be able to suppress the intestinal inflammation with a corresponding remission in SpA (10).

3.3.4: Ileocolonoscopic data in spondyloarthritis patients – macroscopic appearance
Ileocolonoscopy has revealed that the macroscopic appearance of the gut mucosa in SpA patients is frequently abnormal. Again, reported rates vary; it is probable that 29.2 to 50% of patients with SpA have macroscopic abnormalities (9-12, 63, 65), although one study reports almost two thirds of USpA patients had lesions visible during endoscopy (64). Hascelik et al found patients with macroscopic inflammation may have more axial disease, with significantly increased BASDAI, decreased chest expansion and longer-lasting morning stiffness. Patients with inflammation had worse quality of life (12).

NSAID treatment alone is unlikely to be the primary cause of colonic inflammation in SpA (10). None of the ileocolonoscopic studies have demonstrated a significant relationship between intestinal lesions and NSAID dose. Rheumatoid arthritis patients taking similar NSAID dosages to SpA patients develop intestinal lesions much less frequently (9). These findings support the hypothesis that inflammation in the intestines of patients with SpA is related to IBD, rather than NSAID treatment.

3.3.5: Faecal calprotectin
Calprotectin, a 36500 Dalton protein (66), is present in the cytoplasm of neutrophils and monocytes (67). The protein can be detected in the faeces. Early work with faecal calprotectin found increased levels in patients with UC, CD and gastrointestinal carcinoma (68).

Faecal neutrophil excretion, as measured by $^{111}$In labelled leucocytes, does not appear to increase in chronic active gastritis (69). With $^{111}$In labelled leukocyte levels correlating well with faecal calprotectin levels, it is widely accepted therefore that gastric pathology does not contribute significantly to elevations
in faecal calprotectin (70, 71). Faecal calprotectin is sensitive for detecting inflammation in both the small and large intestines (72).

Meta-analysis has shown the test to be an effective screening tool in secondary health care. In adults with suspected IBD, an abnormal faecal calprotectin can change a pre-test probability of 32% to a post-test probability of 91% of having the disease (73). The test is also effective for ruling out a functional disease (such as irritable bowel syndrome) as a cause of symptoms (74). Faecal calprotectin concentrations are increased in AS patients (47, 48, 75) and their healthy first-degree relatives (48). In the relatives, raised faecal calprotectin was associated with CT changes in the sacroiliac joint (48).

One study of healthy volunteers found that faecal calprotectin results increased from a baseline median of 11µg/g to 82µg/g after a 2-week treatment of NSAIDs. 75% of volunteers were above the upper limit of normal at the end of the 2 weeks (76). Tibble et al demonstrated that a group of arthritic patients (predominantly rheumatoid arthritis and osteoarthritis) that had chronically taken NSAIDs frequently had increased faecal calprotectin. 44% of these patients had abnormally high faecal calprotectin results. Of this 44%, one fifth had levels comparable to patients with IBD (70). Age and sex do not appear to influence faecal calprotectin results (70, 76).

3.3.6: Wireless capsule endoscopy

Wireless capsule endoscopy (also variously called WCE, video capsule endoscopy, VCE, or pill endoscopy) is a minimally invasive procedure that allows visualisation of the small intestine. Patients swallow a capsule about the size of a large vitamin pill (26mm by 11mm). Peristalsis carries the capsule through the gut, and it will eventually be passed naturally in the stool. Within the capsule is a camera that takes 2 photos per second, an LED light source, batteries, a transmitter and an antenna (77). The photos are wirelessly sent from the capsule to a recording device that the patient carries in a sling. While the battery of the capsule lasts for up to 12 hours, the study is usually terminated after 8 hours. This is partially for convenience, but also because the caecum is usually reached by this stage. After the 8 hours of recording, the
recording device can be plugged into a computer. A software package converts the individual photos into a video of the gastrointestinal tract.

Pill endoscopy is useful for investigating the small intestine for occult causes of gastrointestinal bleeding; iron-deficiency anaemia secondary to small-intestinal pathology; and suspected or established Crohn’s disease (78, 79). In Crohn’s disease, the procedure may be used to make the diagnosis or to assess disease prognosis, activity, severity and extent (78, 80, 81).

There are, as yet, no accepted criteria for the diagnosis of Crohn’s disease using wireless capsule endoscopy. Mow et al has suggested the presence of more than 3 ulcerations (82). Others have considered villous oedema, erythema, vasculitis, cobblestone appearance, nodular lymphoid hyperplasia and lymphangiectasia to be to be early CD manifestations (83). Bleeding, exudate, pseudo-polyps and ulcer size may also be taken into account (84). The Lewis score separates the small intestine into three tertiles and collates a score based on villous oedema, ulcer number, longitudinal extent of damage, and stenosis severity (85).

Wireless capsule endoscopy can detect the significant pathology in the small intestine produced by non-steroidal anti-inflammatory drugs. WCE has revealed mucosal breaks (including ulcers and erosions of the mucosa) in 40% to 55% of healthy volunteers after two weeks of non-selective NSAID intake (76, 86). COX-2 selective agents are favourable in the short-term, with only 16% developing mucosal breaks after 2 weeks of treatment (86), but this difference is largely eliminated in the long term; patients taking COX-2 selective agents for longer than 3 months had similar pathology to patients taking non-selective NSAIDs for the same length of time (87). The lesions are similar in appearance to the lesions of small-intestinal Crohn’s disease. While Crohn’s disease patients have a significantly higher median number of ulcers than patients with NSAID enteropathy, there is a large degree of overlap. In lieu of a clinical background, doctors will commonly not be able to make a firm diagnosis (88).
It seems certain that chronic NSAID use causes mucosal ulceration in the small bowel. However, in patients with SpA, NSAID use is unlikely to be the sole contributor to the extensive lesions often noted on WCE. Capsule endoscopy was employed by Eliakim et al (14) to examine 20 patients with SpA: 16 patients had seronegative peripheral arthritis, 3 had isolated sacroiliitis and 1 had AS. Capsule endoscopy was compared with ileocolonoscopy. Importantly, no patient had used NSAID in the preceding 2 months. In total, 9 of the 20 patients showed abnormalities including areas of oedema, erythema, dilated lymphatics, mucosal breaks, erosions and linear and aphthous ulcerations. Findings reminiscent of Crohn’s disease were noted in 6 of the 9 patients and 4 patients had appearances described as “diagnostic” of CD (14) according to criteria used by Mow et al (82). Ileocolonoscopy found abnormalities in the colon of 4 out of 20 patients, and 1 in the ileum. A further 8 patients had upper gastrointestinal pathology on capsule endoscopy. Findings were indicative of gastritis, duodenitis, oesophagitis or oesophageal metaplasia.

Rimbas et al also used WCE to examine the small intestine of SpA patients. These researchers found that patients taking NSAID had significantly higher Lewis scores than those not taking NSAID. Mean scores in SpA patients not on NSAID were still notably high, showing again that there is probably an underlying intestinal pathology independent of nonsteroidal anti-inflammatory drugs (49).

WCE has been used to help diagnose IBD in patients with juvenile idiopathic arthritis (JIA, a subtype of SpA). 3 youths were given WCE when IBD was suspected. Only one of the three had recognisable gut symptoms, but all three children showed lesions in the small intestine that pointed to Crohn’s disease. Ileocolonoscopy with biopsy allowed the definitive diagnosis to be made (89).

3.4: Joint involvement in inflammatory bowel disease

Just as a range of features of inflammatory bowel disease is present in patients with SpA, evidence suggests that there is a spectrum of joint involvement in
patients with IBD. This spectrum includes a severe axial spondyloarthritis consistent with AS at one end, through subclinical sacroiliitis, to IBD without joint manifestations. Spondyloarthritis is common in patients with IBD, but the reported prevalence varies widely between studies. AS fulfilling the modified NY criteria can be found in 3-11% of patients with Crohn’s disease (8, 90-94) and between 1.1 and 6.4% of patients with UC (8, 90), although a prevalence as high as 12% has been reported (91). SpA is found in 10 to 35% of patients with IBD (93, 95, 96) with Crohn’s patients affected more often than UC patients (8, 93, 96). Peripheral joint involvement in IBD patients is manifested by enthesitis, synovitis, and migratory pauciarticular arthritis. In contrast to the axial manifestations, which are independent of IBD, the peripheral arthritic symptoms follow a parallel course of disease with inflammatory activity in the bowel (8, 95, 97).

Beyond the overt AS seen in IBD patients, many develop a subclinical sacroiliitis. Different modalities, with variable sensitivity, have been employed for investigating the SI joint for sacroiliitis. As such, prevalence estimates of asymptomatic sacroiliitis range widely from 2 to 32% in patients with IBD (8, 93-95, 98). Sacroiliitis may be correlated with IBD disease progression (95), although this suggestion has proved controversial (98).

Inflammatory back pain is also common in IBD patients, with 10-30% of patients with IBD, including up to 52% of Crohn’s patients, thought to experience inflammatory back pain (8, 94, 95, 99). Sacroiliitis by CT or plain radiograph occurs in only a minority of these patients however (99), and there doesn’t seem to be a relationship with functional measures such as lumbar spine mobility (95).

3.5: Pathogenesis: The gut-joint axis

As outlined above, there is strong evidence for a clinical overlap between SpA and IBD. Further evidence for the overlap of these conditions is provided by the study of the underlying pathophysiology of the two conditions.
The gastrointestinal tract has a contiguous layer of epithelial cells that separates the luminal contents from the host tissues. If this barrier is more permeable than normal, bacteria or bacterial products from the normal microflora may cross from the lumen to the lamina propria and gain uncontrolled access to the intestinal immune system. In genetically susceptible people, this may trigger inflammatory disease at the local and systemic level. It is widely believed that this process is important in the aetiology of both IBD and SpA. The combination of intestinal permeability, the gut microflora’s huge antigenic potential, and the well-documented genetic predisposition in IBD and SpA will be discussed in the following sections. Because the lesions caused by NSAIDs are also associated with changes in intestinal permeability and the gut microflora (100), NSAID enteropathy will be discussed here with the SpA and IBD pathogenesis.
**1) Intestinal Permeability**

Inherited or acquired altered integrity of epithelial barrier.

**2) Native Microflora**

Bacteria or bacterial antigens translocate from intestinal lumen and are detected by dendritic cells.

**3) T-Cell Differentiation**

Dendritic cells promote differentiation of naïve T cells into pro-inflammatory cells, including T_{H}17 cells.

**4) Inflammatory Milieu**

Pro-inflammatory cytokines, including TNFα and interleukins 17 and 23 are released.

**5 and 6) Inflammatory Spread**

Inflammatory cytokines and T cells induce intestinal inflammation locally [5], or SpA via the bloodstream [6].

Figure 3: The gut-joint axis
3.5.1: The gastrointestinal barrier
Enterocytes form a barrier that serves the dual roles of separation of the luminal environment from the systemic circulation, and the absorption of water and nutrients from the gut lumen (15). Fat-soluble substances cross the enterocytes‘ phospholipid bilayers, diffusing down concentration gradients (101). Transport proteins stud the cells’ membranes, allowing the selective uptake of water-soluble products. Binding these enterocytes together are the intercellular junctions, forming a relatively impermeable layer (15). Tight junctions, complexes of more than 50 proteins (101) lie nearest to the lumen, with the adherens junction underneath (15). These connections form the paracellular pathway, a dynamic channel that has a constantly changing permeability to molecular traffic (15, 101).
Alteration of the epithelial barrier can lead to:
1) Leak flux diarrhoea, via diffusion of ions and water from the intestinal circulation to the intestinal lumen; and
2) Increased uptake of antigens, sometimes leading to intestinal and systemic inflammation (102).

3.5.2: Intestinal permeability
A variety of methods, discussed below, have been used to assess intestinal permeability and changes in permeability are noted in both IBD and SpA (103-110). Both diseases are associated with alteration in the tight junctions and adherens junctions between enterocytes: The E-cadherin/catenin complex is an important component of the adherens junctions of epithelial cells (111), and may have a role in intestinal permeability alterations (112). The complex is upregulated in the gut inflammation of SpA patients (113) and IBD patients (114). Patients with Crohn’s disease have tight junction strand discontinuities evident on electron microscopy (115) and their tight junctions are more sensitive to luminal stimuli, even in non-diseased mucosa (116).

Measurement of the permeability of the intestine has been achieved in both humans and in animal models. Usually a subject will drink a mixture of test substances and levels will subsequently be detected in the subject’s urine. Using different substances, especially different oligosaccharides, allows for
permeability in different areas of the gastrointestinal tract to be measured. Lactulose, mannitol and L-rhamnose are broken down by the microflora of the large intestine. This makes these three sugars effective measures of small intestinal permeability. Sucralose and $^{51}$Cr-labelled ethylenediaminetetraacetic acid (Cr-EDTA) are stable throughout the gastrointestinal tract, and spend most of their transit time in the large intestine. This means they may provide information on colonic permeability (15). In humans, permeability does not alter significantly with gender or age (105, 109, 117).

Lactulose, a disaccharide, is usually combined with a monosaccharide such as mannitol or L-rhamnose. The monosaccharides are capable of diffusion through the abundant small channels at the tips of intestinal villi and so their absorption is proportional to the small intestinal surface area. Diseases that decrease small intestinal surface area, like coeliac disease, decrease the absorption of the monosaccharides. Lactulose, being larger than the monosaccharides, passes through paracellular channels at the bases of the intestinal villi. Small intestinal permeability is usually expressed as a lactulose:mannitol or lactulose:rhamnose ratio. This can be thought of as a measure of small intestinal epithelial permeability per unit of surface area (15).

Intestinal permeability is increased in patients with Crohn’s disease. Evidence for the correlation between permeability and clinical disease activity is contradictory (103, 104), but an increased permeability in patients in remission does seem to predict relapse (104, 106).

Intestinal permeability seems to be a primary lesion in IBD. Supporting this are a few key findings:

- Intestinal permeability may precede Crohn’s disease onset in humans (118), as it does in animal models (discussed below);
- Many first degree relatives also have permeability abnormalities (105, 119).
- Patients have tight junctions that are more sensitive to luminal stimuli, even in non-diseased mucosa (116)
• Using a zonulin inhibitor to prevent an increase in intestinal permeability in mice that are susceptible to Crohn’s disease ameliorates the disease (120)

NSAIDs are known to cause increased gastrointestinal permeability. Whether this effect is systemic or local is unknown. Bjarnason et al (121) found the increased permeability to be related to a drug’s ability to inhibit cyclooxygenase and concluded that NSAIDs acted predominantly systemically to influence intestinal permeability. Inhibition of cyclooxygenase decreases prostaglandin production, resulting in a prolonged permeability increase (122). Reuter et al (100) showed that local effects were more likely to induce the permeability changes. The NSAID nabumetone inhibits cyclooxygenase but is non-acidic and does not cause an increase in intestinal permeability (123). This shows that the action of cyclooxygenase inhibition is of less importance than local effects. Interestingly, nabumetone causes less gastrointestinal perforations, ulcers and bleeds, as well as fewer overall gastrointestinal symptoms than other NSAIDs (124). The local effects are a result of NSAIDs decoupling oxidative phosphorylation in enterocyte mitochondria. This causes a decrease of intracellular ATP and leakage of mitochondrial calcium with subsequent tight junction dysfunction (125).

Intestinal permeability measurement in patients with SpA has produced varying and sometimes contradictory results. In SpA and AS, increased permeability has been found using the $^{51}$Cr-EDTA resorption test (107-110), low weight PEG-400 (126) and the lactulose:mannitol ratio (127-129). The lactulose:mannitol ratio was not significantly increased in one study of patients with AS (130). Another study in SpA patients found no increase in the $^{51}$Cr-EDTA resorption test compared to NSAID-taking controls (131). A single study has examined SpA patients in Otago. Mean and median L:M ratios were higher than normal in a group of 9 patients, but 8 of these patients were taking NSAIDs at the time (129).

There is no clear association between intestinal permeability and colonoscopic findings in SpA patients (131), although histological lesions of chronic appearance are associated with increased intestinal permeability (110). While
NSAIDs complicate analysis of these studies by increasing intestinal permeability, there are studies that show that spondyloarthritis is associated with an altered barrier function beyond a level that can be explained by these drugs. SpA patients taking NSAID often have higher permeability results than NSAID-taking controls (126), and patients with SpA not taking NSAIDs often have increased intestinal permeability (108, 110). It is also of note that first degree relatives that are not taking NSAIDs also have increased intestinal permeability (109, 127).

3.5.3: The human microflora
At birth, the gastrointestinal tract is free of microorganisms. The gut is populated over the course of successional events by many billion bacteria (132). By the time we are adults, the number of bacterial cells in the human body is estimated to outnumber human cells by 10 to one, with more than 70% of these bacteria living in the colon (133). The gastrointestinal tract increases in bacterial density from the stomach and duodenum, with approximately 10-1000 colony-forming units (cfu) per millilitre, to the jejunum and ileum with $10^4 - 10^7$ cfu/ml, culminating at the colon, which homes an incredibly dense and diverse bacterial population, with approximately $10^{12}$ bacterial cells per ml, the highest cell density recorded in any ecosystem (133, 134). Strict anaerobes in the gut are more than two to three orders of magnitude more common than facultative anaerobes and aerobes (133).

3.5.4: Bacteria and the pathogenesis of SpA, IBD, and NSAID enteropathy
Rodent models have shown that bacteria are a key factor in the development of SpA, IBD, and NSAID enteropathy. The intestinal permeability that plays an important part in disease pathogenesis appears to be intricately linked to the gut microflora. This link is supported by evidence that microorganisms are capable of altering the gut barrier, and that artificially bypassing the epithelial barrier provokes a local and systemic inflammatory reaction. It is of note that disease pathogenesis can be largely uncoupled by keeping subjects in a germ-free environment, or by preventing intestinal permeability. Taken
together, this shows the connected role of intestinal permeability and the gut microflora in the pathogenesis of SpA, IBD, and NSAID enteropathy.

Certain common intestinal bacteria appear to be linked to disease pathogenesis. *Bacteroides* species seem particularly important in development of the chronic intestinal inflammation in rodent models of SpA (135, 136). In humans, many bacterial species or genera may be associated with SpA and IBD, including *Bacteroides vulgatus*, *Klebsiella* species, *Shigella* species, *Campylobacter* species and *Yersinia enteroxolitica* (137). Various bacteria, including *Klebsiella pneumoniae*, *Streptococcus viridans*, *Clostridium perfringens*, *Vibrio cholerae*, and certain *Escherichia coli* strains, can synthesize toxins that cause an increased intestinal permeability by altering the tight junction barrier (101, 138, 139). On the other hand, certain probiotics (138, 140-143) can effect an apparent decrease in intestinal permeability which could suggest a protective potential.

Yamada *et al* injected peptido-glycan-polysaccharide into the distal colonic wall of genetically susceptible rats. Bypassing the epithelial barrier caused an acute inflammatory reaction after 3 days, followed by a second reaction at 3-4 weeks. The second phase of inflammation caused arthritis, granulomatous colitis, and mucosal ulcers. This research showed that a breach of the intestinal barrier can result in inflammation at the local and systemic levels in a genetically susceptible host (144).

Transgenic HLA-B27, β₂ microglobulin positive rats develop an inflammatory disease with striking similarities to the spondyloarthritides. The inflammatory disease involves the axial and peripheral joints, the gastrointestinal tract, the male genital tract, the heart, the nails and the skin (145) with higher disease susceptibility related to higher HLA-B27 expression (146). Rats raised in a germ-free environment do not develop the arthritis and gastrointestinal inflammation, although still develop the typical skin and nail lesions and the testicular inflammation and infertility that are found in non-germ-free rats (147). Rats that also lack β₂ microglobulin do not develop any of the disease phenotypes in a germ-free environment (148). Inbred B10.BR (H-2k) mice are at a higher risk of developing ankylosing enthesopathy, a disease
with similarities to human AS (149). These mice do not develop disease in a
germ-free environment (150).

The pathogenesis of NSAID enteropathy, as with SpA and IBD, is dependent
on gut bacteria. NSAID enteropathy does not develop in the germ-free rat
(151). Reuter et al (100) compared the effect of nitrofenac and diclofenac in
rats. These are two NSAIDs with similar effects on prostaglandin synthesis
but differ in that nitrofenac does not undergo entero-hepatic recirculation.
Diclofenac ingestion was followed by a large increase in gram-negative
bacterial numbers as well as marked intestinal permeability and ulceration,
while nitrofenac, which had no effect of bacterial numbers, caused no
ulceration and only a small change in intestinal permeability. In humans, the
ileum has more bacteria than the jejunum (39) and appears to be more
frequently affected by NSAID-induced ulceration than the jejunum (152). This
parallel between areas of bacterial colonisation and intestinal lesion
susceptibility may also be seen in IBD (33).

3.5.5: Immunological overlap between spondyloarthritis and
inflammatory bowel disease

The human gastrointestinal tract maintains immune homeostasis through a
delicate balancing act. The body displays tolerance towards the native
microflora, a process dominated by FoxP3+ regulatory T cells (T_{reg}) and the
immune-suppressive cytokines IL-10 and transforming growth factor β (TGF-
β) (153, 154). On the other hand, the gastrointestinal system must be able to
eliminate pathogenic bacteria, and so is populated by dendritic cells and
macrophages, which can recruit pro-inflammatory T helper 1 (T_{H1}) cells and T
helper 17 (T_{H17}) cells, with their respective pro-inflammatory cytokines. This
section will examine the evidence that links pathogenic changes in these
immunologic factors to a common aetiology in SpA and IBD.

The tolerance that exists in the normal gastrointestinal tract is lost in active
inflammatory bowel disease (155), while AS patients show a reduced IL-10
response to autologous bacteria (156). Furthermore, the intestinal mucosa of
IBD patients and AS patients show increased numbers of T$_{reg}$ suggesting an incomplete attempt to suppress the inflammatory environment (157-160).

Dendritic cells present antigens from bacteria that breach the epithelial barrier. After migrating to mesenteric lymph nodes, dendritic cells produce interleukin-12 (IL-12) and 23 (IL-23) and promote differentiation of naïve T cells to T$_{H1}$ and T$_{H17}$ cells, among others. When IL-23 is presented to the IL-23 receptor of naïve T helper cells, it causes phosphorylation of Janus kinase 2 (JAK2) and tyrosine phosphorylation of IL23R. This then causes activation of signal transducer and activator of transcription 3 (STAT3), which migrates to the nucleus to induce activation of pro-inflammatory cytokines and anti-apoptotic proteins. This pathway is a central part of the differentiation of naïve T cells into T$_{H17}$ cells (161).

With inflammatory T lymphocytes primed, inflammation can spread to the joint, a process that relies upon both homing and adhesion mechanisms. T cells that mature in the gut lamina propria have the capacity to bind efficiently to both gut mucosa and the synovium of the joint (162), using an overlapping set of adhesion molecules (163). Salmi and Jalkanen (164) found that lymphocytes derived from the small intestine of IBD patients used multiple homing receptors to adhere to venules in the synovial membrane. May et al (165) found identical T lymphocyte lines in tissue from the colon and synovium in a patient with SpA, suggesting shared antigen recognition. Particles in the circulation frequently become lodged in anatomical areas where poorly vascular tissues are adjacent to highly vascular tissues. The joint contains many of these areas, and is therefore prone to deposition of antigens, which may serve to incite an inflammatory reaction (166).

T$_{H1}$ cells produce the pro-inflammatory cytokine interferon gamma (IFN-γ) (154) and have a key role in fighting infection of intracellular microorganisms (167). These cells appear to have a role in Crohn’s disease pathogenesis (161); a T$_{H1}$ response marks early Crohn’s disease in what resembles an acute infectious process, and the affected mucosa of patients with Crohn’s disease may have increased numbers of T$_{H1}$ cells (167). The cells are important in the systemic disease of HLA-B27 rats (168) and may be involved in human uSpA.
and ReA (169). Both AS and Crohn’s disease patients have altered T_{hi}1 responses to *E. coli* (170) and are both associated with polymorphisms in or near TNFSF15 (171, 172), which drives co-stimulation of T_{hi}1 and T_{hi}17 cells (167).

T_{hi}17 cells are normally present in healthy lamina propria, acting as important regulators of intestinal homeostasis, but under the influence of IL-23 have the capacity to become auto-inflammatory (173). The cells are characterised by secretion of the pro-inflammatory cytokine interleukin (IL)-17 and are important for clearing of certain extracellular pathogens, including *Klebsiella* spp. and fungi (174). Acting on the ubiquitous IL-17 receptor (175), IL-17 stimulates production of IL-1, IL-6 and TNF-α from fibroblasts, endothelial cells, macrophages and epithelial cells (176).

The IL23/IL17 axis is of central importance in SpA and IBD. T_{hi}17 lymphocyte numbers are increased in both groups (157, 174, 177) and a number of genetic polymorphisms involved in the axis have been found in SpA and IBD patients (178-188). Macrophages in Crohn’s disease and AS produce larger amounts of IL-23 than healthy controls when stimulated by bacteria or lipopolysaccharide (LPS) (189, 190). Furthermore, direct evidence of the importance of the IL-23/IL-17 axis comes from IL-17A inhibition in AS patients. An early proof-of-concept trial has shown promising results, displaying good safety and efficacy over 6 weeks (191). Similarly, inhibition of an IL23 subunit has been found to induce a clinical response in Crohn’s disease (192).

SpA and IBD are both associated with variants in the genes that encode the IL-23 cytokine (161, 185) and a subunit of its receptor (IL23R) (178-184). As stated above, STAT3 is a protein responsible for linking IL23R to transcription of inflammatory cytokines. Polymorphisms in the STAT3 gene are associated with AS and IBD (180, 186, 187). Further polymorphisms common to these diseases are involved in upregulating the IL-23/IL-17 axis, including linking pathogens to the T_{hi}17 response (185, 188, 193) and upregulating IL17 (171, 172, 194) and IL-23 production (185, 195, 196). Evidence that implicates HLA
class I mishandling in the IL-23/IL-17 axis further links these cytokines to a common disease pathogenesis, and will be summarised below.

Tissue necrosis factor alpha (TNFα) is an important pro-inflammatory cytokine in both diseases. Raised levels of TNFα are found in the faeces, blood, and mucosa of IBD patients (197). AS patients, especially those with enthesitis, have raised levels of TNFα in the plasma (198) and TNFα mRNA is increased in AS patients’ SI joints (199). Inhibition of TNF by an array of new ‘biological’ agents has proved very successful in treatment of IBD (197) and SpA (200). TNFα itself feeds back into the IL23/IL17 axis, amplifying the differentiation of naïve T cells into T\(^{H17}\) lymphocytes (201). Polymorphisms in the gene that encodes the TNF receptor have been found associated with AS (202) and IBD (202-204). Interestingly, TNFα is able to increase intestinal permeability (205), showing another role where this pro-inflammatory cytokine can feed back into the pathogenesis of both diseases. This feedback may represent the link between the bacterial microflora and increased intestinal permeability.

Alternative activation of the IL23/IL17 axis may occur from misprocessing of HLA class I molecules. The Major Histocompatibility Complex (MHC) on chromosome 6 contains around 220 genes mostly with immune regulatory functions. The MHC is also known as the Human Leukocyte Antigen (HLA) in humans. This 3.6 megabase region contributes about half of the total AS risk (206). Within the MHC are the genes of the Human Leukocyte Antigen (HLA) classes I, II and III. HLA class I molecules usually present defective, degenerated, or virus-derived intracellular peptides on cell surfaces, while class II molecules generally present extracellular peptides (207). The pathogenesis of SpA and IBD appear to be linked to HLA class I molecules, with genetic associations found with HLA-B27 and endoplasmic reticulum aminopeptidases (ERAP).

Human Leukocyte Antigen (HLA)-B27 is strongly associated with the spondyloarthritides, conferring 16-40% of the total SpA risk (206). The HLA-B27 antigen, introduced above in an animal model of SpA, is found in over
90% of those with AS, with an estimated prevalence of only 8% in the healthy European population (208, 209). There are over 62 known HLA-B27 subtypes to date, each bearing different geographic distributions and conferring different levels of AS risk. HLA-B*2705 is known as the ‘parent subtype’ and is found in nearly every population worldwide. So far, only HLA-B*2705, B*2702, B*2704 and B*2707 have been proven to increase AS risk, while the subtypes B*2706 and B*2709 may be protective. The different subtypes may differ by only one or two amino acids, but these small changes can alter the peptide-binding properties of HLA-B27 (206, 210).

In patients with IBD alone, there is no association with HLA-B27, but those with IBD and concurrent AS have an HLA-B27 prevalence of 25-78%. Notably, multiple MHC loci other than HLA-B27 have been found to be associated with both IBD and AS independently (211-220).

Several hypotheses have been proposed to explain the role of HLA-B27 in the pathogenesis of SpA.

• The arthritogenic antigen hypothesis suggests that certain peptides are presented specifically by HLA-B27. After presentation, it is proposed, cytotoxic T lymphocytes would cross-react with auto-antigens. This would cause a subsequent cytotoxic T-cell driven inflammation in the joint and sites of auto-antigen presence. HLA-B27-specific cross-reaction has been found with the self-peptide vasoactive intestinal peptide receptor 1 (221) and with another derived from type VI collagen (222).

• Misfolding of the HLA-B27 molecule within the cell (223). Large amounts of misfolded heavy chain propagate within the cell, leading to endoplasmic reticulum stress and the unfolded protein response. This response involves NF-κB translocating to the nucleus of the affected cell, in turn stimulating pro-inflammatory cytokines and chemokines including TNF-α and interleukins 1 and 6. In the rat model of SpA, HLA-B27 misfolding can activate the IL-23/IL-17 axis (173). These authors found a striking T_{H17} expansion in the HLA-B27 rat colon –
this could potentially help link the HLA-B27 misfolding to the colitis seen in this animal model.

- HLA-B27 heavy chains can form stable dimers that act as ligands for natural killer (NK) cells. It has been found that expression of the dimers in SpA patients leads to proliferation of KIR3DL2 NK cells (224, 225). There is downstream stimulation of the survival, proliferation and IL-17 production of KIR3DL2 CD4+ T cells in SpA patients’ peripheral blood and synovial fluid (226). This provides evidence linking HLA-B27 heavy chain dimers to the IL23/IL-17 axis.

ERAP 1 and 2 are aminopeptidases that work in concert to trim peptides in the endoplasmic reticulum for HLA class I presentation (227). ERAP1 probably has an immunomodulatory role, as it is capable of promoting the shedding of the IL-6 receptor and the type 1 tumour necrosis factor receptor (228). ERAP1 variants are associated with AS risk (178), while ERAP2 is associated with CD risk (229). Evans and others have recently shown that ERAP1 polymorphisms only increase AS risk in HLA-B27 positive individuals, suggesting that HLA-B27 incites the disease process through mistakes in antigen processing (185).

3.6 Literature review summary
The evidence presented above suggests an underlying mechanism that links spondyloarthritis and inflammatory bowel disease. Increased intestinal permeability allows increased levels of bacterial antigens to cross the epithelial barrier, producing local inflammation. Immune cells, primed by these foreign antigens, release pro-inflammatory cytokines and home to the intestinal mucosa and the joint. The result is an inflammatory spectrum that may present as spondyloarthritis, inflammatory bowel disease, or a combination of both. The following research will examine the relationship between the gastrointestinal symptoms of spondyloarthritis patients, intestinal permeability, intestinal inflammation, and macroscopic appearance of the small intestine.
Chapter 4 – Methodology

4.1 General methodology

4.1.1 Ethical approval
Ethical approval was obtained from the Lower South Regional Ethics Committee, New Zealand (LRS/11/03/013). Written informed consent was obtained from all participants in accordance with the Treaty of Helsinki.

4.1.2 Recruitment methodology
Patients were identified from the SpondyloArthritis Genetics and the Environment (SAGE) database. This is a new database that involves patients with axial spondyloarthritis in several regions in New Zealand. All participants had attended the rheumatology outpatients department at the Dunedin Public Hospital. Patients all had a confirmed diagnosis of axial SpA as defined by ASAS criteria (1).

Controls were recruited from a database of volunteers who had previously taken part in research in the rheumatology department and from poster advertisements in the hospital.

4.1.3 Inclusion criteria
Inclusion criteria for patients were:

- Diagnosed axial spondyloarthritis, confirmed by fulfilling ASAS criteria
- Aged 18 years old or over
- Willing to participate, and able to give written informed consent.

Inclusion criteria for controls were:

- Aged 18 years old or over
- Willing to participate, and able to give written informed consent
4.1.4 Exclusion criteria

Exclusion criteria for patients were:
• Diagnosed inflammatory bowel disease
• Aged under 18 years old
• Unwilling or unable to give written informed consent
• Major psychiatric illness or chronic infection

Exclusion criteria for controls were:
• Diagnosed chronic disease, including inflammatory bowel disease or spondyloarthritis
• Aged under 18 years old
• Unwilling or unable to give written informed consent
• Major psychiatric illness or chronic infection

4.1.5 Consent and preparation

Both patients and controls were sent a letter of invitation. This was followed up by a telephone call, inviting to come in for the first meeting. Repeat telephone calls were made if necessary.

Individuals from both groups met with the investigator. Interviews were held in the Rheumatology Outpatients Department, Dunedin Hospital, and the outpatient department at Oamaru Hospital. Written consent was obtained.

For patients, clinical data were gathered from recent SAGE visits. These data included several questionnaires, physical parameters, faecal calprotectin testing, and blood tests. Patients performed intestinal permeability testing and filled out DISQ and BASDAI questionnaires on test day.
A group of patients were selected for wireless capsule endoscopy based on DISQ scores collected during permeability testing and SAGE visits.

Controls performed intestinal permeability testing and filled out the DISQ on test day.

Study methodology is summarised in a flow chart below (Figure 4).
4.2 Assessment of patients with SpA

4.2.1 Collection methodology
Clinical data for each patient had been collected in accordance with a protocol developed for the SAGE study. There was an average of 20 weeks between SAGE assessment and permeability testing. DISQ and BASDAI were collected during the permeability testing.
4.2.2 Assessment of bowel symptoms – The DISQ

Bowel symptoms were evaluated using the Dudley Inflammatory Bowel Symptom Questionnaire (DISQ). The DISQ has 15 questions that gather information on severity and frequency of bowel symptoms. Validated in IBD patients by Kwon et al (230), and as a modified version by Stebbings et al in patients with axial SpA (16). The DISQ’s 15 questions are answered 0-4, where 0 = none (never), 1 = mild (occasionally), 2 = moderate, 3 = severe, 4 = incapacitating. Questions are grouped into two health domains assessed over the previous week: bowel symptoms and systemic symptoms. The scores (0-4) are added up for each of the 15 questions for a total out of 60. In a study of 68 patients with axial SpA it was demonstrated that a score of 11 or higher represents bowel symptoms sufficient to affect quality of life in patients with IBD. In patients with diagnosed IBD, a score of less than 19 indicates remission (53). During analysis, the patient group could be divided into three subgroups based on DISQ scores. Those with scores of 0-10 were classed as “low”; those with scores of 11-18 were classed as moderate; and those with scores over 18 were classed as severe.

The DISQ can be found as an attached document in the appendix.

4.2.3: Assessment of disease activity in SpA - BASDAI

Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (231) which has demonstrated good reliability, validity and responsiveness (232). The 6 questions of the BASDAI can be analysed on a 10 centimetre visual analogue scale or on a 0 – 10 numerical rating scale (NRS). We have used an NRS, as preferred by ASAS (20). The BASDAI measures current arthritic disease activity and can be found in the appendix.

4.2.4: Assessment of disease duration

Disease duration was estimated for each patient using the retrospective data from the SAGE database. Most patients in the database have a date of
4.2.5: Assessment of systemic arthritic or intestinal inflammation - CRP
C-Reactive Protein (CRP) is a marker of inflammation that correlates with disease activity in AS (233) and may be associated with morning stiffness and/or night pain (234). Elevated levels of CRP in the plasma of patients with AS has been found to be associated with radiographic sacroiliitis and syndesmophytes (19). CRP is also useful as a marker of inflammation in IBD (235, 236) CRP testing was performed by Southern Community Laboratories. The normal range for CRP is 0-5mg/L.

4.2.6: Assessment of intestinal inflammation – faecal calprotectin
Measuring faecal calprotectin is a useful screening tool for suspected IBD (73), and is increased in AS patients and their relatives (48). NSAID use also causes increased faecal calprotectin levels (70, 76). It is a marker of small and large intestinal inflammation, but is insensitive to upper GI damage.

During SAGE clinics, patients are generally asked to collect a faecal sample for measurement of faecal calprotectin. Southern Community Laboratories performed the testing. The results of this test were added to the SAGE database and were subsequently accessed for the current research. We used the Southern Community Laboratory’s recommended upper limit of 50µg of faecal calprotectin per gram of stool to determine an abnormal result. Other studies examining IBD have found good diagnostic precision with cut-off values at 100µg/g (237) and 150µg/g (238).

4.2.7: Assessment of quality of life - ASQoL
Quality of life was measured in the SAGE clinics using the ASQoL, an 18-question questionnaire (239). The ASQoL has been validated in the New Zealand population (240). Patients mark ‘Yes’ or ‘No’ for each question. The
number of ‘Yes’ answers are added up to give a score out of 18 – the higher the score, the poorer the current quality of life. The ASQol is an attached document in the appendix.

4.2.8: Assessment of enthesitis: MASES
Enthesitis, inflammation at the site of insertion of tendons, ligaments, joint capsules or fascia to bone, is characteristic of SpA (241-243) and can range from asymptomatic to severe and disabling (243). The Maastricht Ankylosing Spondylitis Enthesitis Score (MASES) involves the examiner pressing 13 entheses. Every enthesite the patient reports at tender is given a score of 1. The total number of tender sites is added to give a score of 0 to 13 (20). Sites of enthesitis examination are shown on the MASES document, which is in the appendix.

4.2.9: Assessment of swollen and tender joints
Swollen joints and tender joints are examined by pressing firmly on forty-four sites on the patient. The number of swollen joints is added up and the process is repeated for tender joints to give two separate scores of 0 to 44 (20). The sites are shown on the ASAS 44 joint count document in the appendix.

4.2.10: Assessment of functional disability: BASFI
The Bath Ankylosing Spondylitis Functional Index (BASFI) gives a measure of the patient’s physical abilities (244). Patients answer 10 questions about daily tasks with a score of 0-10, with 10 showing a very high level of disability. The mean score from the 10 questions is calculated to give the patient’s BASFI score (20). The BASFI questionnaire is attached in the appendix.

4.2.11: Assessment of mobility: BASMI
The Bath Ankylosing Spondylitis Metrology Index (BASMI) is a quick, reproducible set of measurements and is sensitive to change across the disease spectrum. The index involves five clinical measurements that reflect axial mobility. Each measurement gets a score of 0 – 2; points are added together for a BASMI score of 0 – 10 (245).
The five measurements are (20):
1. Assessment of cervical rotation
2. Tragus to wall distance in cm
3. Lateral flexion assessed with the patient against the wall
4. The modified Schober test to measure anterior lumbar flexion
5. Abduction of the hips, judged by measurement of intermalleolar distance

The BASMI can be found as an attached document in the appendix.

4.2.12: Assessment of NSAID dose

Information about NSAID use was collected during the SAGE clinics. A score of 0-100 was calculated, using the ASAS recommendations (54).

\[
\text{Equivalent NSAID score} = \frac{(\text{Equivalent NSAID score}) \times (\text{days of intake during period of interest}) \times (\text{days per week})}{(\text{Period of interest in days})}
\]

Equivalent NSAID score is based on an index of 100 for diclofenac at a dose of 150mg per day. Lower daily doses score lower; a daily dose of peroxicam of 10mg, for example, would get an equivalent NSAID score of 50.

Patients were not asked to record their daily usage in this study. The days of usage per week were estimated, and time period of 30 days was used.

4.2.13: Assessment of TNF-α antagonist dose

TNF-α antagonist usage was noted during SAGE clinics. All patients receiving the drug were receiving the same dosage (40mg adalimumab fortnightly, delivered subcutaneously). No patients had stopped using adalimumab since commencement. Patients were thus recorded as users or non-users.

4.2.14: Clinical data

Patient notes were reviewed if further information was needed. This was done to check the results of blood tests and to gain information on drug
therapy. Iron status was of particular importance for patients that had wireless capsule endoscopy performed.

4.3: Intestinal permeability testing

A kit was given to each volunteer (patients and healthy controls). For patients, the kit included:

- Instruction sheets and information sheets
- A 24-hour urine collection bottle, prepared with 1 gram boric acid as a preservative
- The Dudley Inflammatory bowel Symptom Questionnaire (DISQ)
- The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) questionnaire
- The three sugars, as described by Anderson et al (246):
  - 5 grams sucralose (Tate & Lyle ANZ Pty Ltd, Auckland, New Zealand)
  - 1 gram L-rhamnose (Sigma Aldrich, Castle Hill, Australia)
  - 5 grams lactulose (Solvay Pharmaceuticals, Brussels, Belgium)

Control volunteers were not given a BASDAI, but their kits were otherwise identical.

Patients and controls abstained from non-steroidal anti-inflammatory drugs (NSAID) and alcohol for 5 days before the test. Both of these agents are known to increase intestinal permeability (121, 247).

Volunteers (patients and controls) fasted from midnight before starting the test until the end of the collection. They were free to drink as much water as they wanted. On the morning of the test, volunteers mixed the sucralose into 60 millilitres of water, and mixed the rhamnose and lactulose together into 120 ml of water (246). They then drank the two mixtures in succession. For the next 5 hours, all urine was collected in the collection bottles. On the same day, all volunteers filled out the DISQ. Patients also completed the BASDAI. After finishing collection, the volunteers came in for their second visit to deliver the sample. This meeting was usually on the afternoon of the test, but
occasionally this was not convenient. If this was the case, volunteers froze their sample at -18°C until they could bring it in.

Once the sample was returned, the total urine volume was recorded and two 12 ml samples were taken from the collection bottle. These were frozen at -80 degrees Celsius in the Dunedin Hospital. One sample from each volunteer was later sent on dry ice to Monash University, Melbourne, Australia for analysis.

Analysis of the samples was performed by high-performance liquid chromatography (HPLC) (246, 248). The respective percentages of lactulose, rhamnose and sucralose excreted were calculated. A ratio of lactulose:rhamnose excretion was then calculated.

4.4: Examination of the macroscopic appearance of the small intestine – Wireless capsule endoscopy

4.4.1: Selection and recruitment
10 patients were selected from DISQ scores provided during the permeability test and SAGE clinics. All ten were sent information about wireless capsule endoscopy. A telephone call was made to obtain oral consent and to arrange a time for the procedure. 2 packets of Klean-Prep (Norgine, Uxbridge, U.K), an iso-osmotic bowel-cleansing agent containing Polyethylene Glycol (PEG), were sent to each volunteer with instructions on how to prepare for the procedure.

4.4.2: Preparation
Patients were asked not to eat from midday the day before the procedure. They could drink clear fluids. At 4pm on the day before the endoscopy, they drank the 2 litres of Klean-Prep, aiming to finish it in two hours.
4.4.3: The procedure

Each patient came in to the Gastroenterology Unit at the Dunedin Public Hospital at 8 a.m. on his or her procedure day. Written informed consent was obtained. 8 adhesive leads were placed on the volunteer’s abdomen. The leads were plugged into a data recorder that hung from the volunteer’s shoulder. The volunteers took one tablet of metoclopromide HCl 10mg (Pacific Pharmaceuticals, NZ) to accelerate gastric emptying. They then swallowed the Pillcam (Given Imaging Ltd, Yoqneam, Israel) with simethicone 100mg (Nice Pak Products (NZ) Ltd., NZ) dissolved in water (249). Simethicone is used to reduce bubble formation and increase visibility.

The patient was free to leave the hospital after swallowing the camera. They were asked to not undergo strenuous exercise. For two hours, they were to fast completely. After the two hours they were allowed clear fluids, followed by a light meal after another 2 hours. They were asked to return to the Gastroenterology Unit 8 hours after the capsule was ingested. This is the procedure length used by the Gastro unit for reasons of convenience. While the battery life may last up to 12 hours, the caecum is usually reached after 8 hours. The data recorder and leads were removed, and the patient was discharged. The capsule is disposable and passes naturally in the stool.

4.4.4: Video analysis

Photos taken by the camera are stored on the data recorder. Once the data recorder is plugged into a PC, software puts these photos together to create a video. An experienced Gastroenterologist read the videos and applied diagnostic criteria developed by Mow et al (82). He was blinded to DISQ scores, faecal calprotectin results, and clinical data.
4.5: Statistical analysis

Dr. José A. García from the Department of Preventive and Social Medicine, University of Otago performed the statistical analysis. The statistical program R was used to run t-tests, Spearman’s tests, Kruskal-Wallis tests, Wilcoxon tests and Fisher’s exact tests, where applicable (250). A p value of <0.05 was considered significant. Data were presented as means or medians, as appropriate.

Chapter 5 – Results

5.1: Patient demographic data

Patients were recruited from the Department of Rheumatology at the Dunedin Public Hospital. Between June 2011 and September 2011, a total of 64 potential patient volunteers were identified. Eight were excluded from the study because they had already been diagnosed with inflammatory bowel disease. Twenty-one patients were unable to be contacted or were unwilling or unable to participate. 35 patients met inclusion criteria and agreed to participate.

From 20 suitable controls, three abstained and two could not be contacted. Fifteen volunteers met inclusion criteria and agreed to participate in the intestinal permeability study as controls.

The patient group and control group were similar in age and sex distribution. The mean age for the patient group is 43.7 years (range 20-80); for the control group, the mean age was 39.6 years (range 21-66). Median ages were 42 for the patient group and 41 for the control group. Wilcoxon rank sum test with continuity correction showed no significant difference between the two groups (p=0.4085). Pearson’s Chi-squared test with Yate’s continuity correction showed the gender distributions to not be significantly different (p-value=0.8988). The patient group had 22 males and 13 females; the control
group had 9 males and 6 females. Demographic information and patient clinical data are shown in detail in Table 2.

5.2: Bowel symptoms/DISQ

5.2.1: DISQ scores
Mean DISQ scores were 11.54 (SD=8.67) and 3.40 (SD=2.87) for patient and control groups respectively. These means were significantly different (Welch Two Sample t-test; p-value<0.0001). This indicates higher gastrointestinal symptoms in the patient group.

5.2.2: DISQ scores and spondyloarthritis symptoms
The mean BASDAI and mean ASQoL were 4.0 (SD=2.14) and 6.73 (SD=5.57) respectively. The BASDAI was positively correlated to DISQ scores (Spearman’s rho = 0.566; p-value < 0.001). No significant correlation was found between ASQoL and DISQ, although this approached significance (Spearman’s rho = 0.321; p-value=0.06).

5.2.3: DISQ scores and clinical parameters
There was no significant association between DISQ scores and any of the following: faecal calprotectin, CRP, disease duration. These data are given in Table 3.

5.2.4: DISQ scores and faecal calprotectin
There was a non-significant positive trend found between DISQ scores and faecal calprotectin results (Spearman’s rho = 0.278; p-value=0.1691). We separated DISQ scores into “low”, less than 11; “moderate”, 11-18; and severe, greater than 18. This showed a trend towards greater bowel symptom scores being associated with greater faecal calprotectin scores, but this was also not significant (Kruskal-Wallis rank sum test p-value = 0.1735). The relationship between DISQ and faecal calprotectin results is shown in Tables 3 and 4 and Figure 15.
5.2.5: DISQ and NSAID usage

Participant numbers in the study were too small to justify comparing NSAID dosage using the ASAS recommendations for reporting NSAID intake (54). We instead used a binary system to create a group of patients who were taking any dose of NSAIDs at the time of the study, and compared them to the group of patients not taking any NSAIDs.

Patients on NSAIDs had higher median and mean DISQ scores than patients not currently taking NSAIDs, but the differences were not significant (Wilcoxon rank sum test p-value = 0.1812). DISQ scores were then separated into low, moderate, and severe groups. This showed that patients with moderate or severe scores were more likely to be NSAID users. A patient with a severe DISQ score was approximately 3 times as likely to be currently on NSAIDs as a patient with a low score. None of these associations met significance however. The non-significant association between NSAID dosage and DISQ is shown in Tables 5, 6 and 7.

5.2.6: DISQ and TNF-α inhibitor use

Use of TNFα inhibitors was uncommon in the study group, and not significantly associated with DISQ scores (p-value 0.3323 with Wilcoxon rank sum test.) The median DISQ score of those on the TNFα inhibitors was notably lower than those who were those on the drugs. These data are shown in Table 8.

5.3: Permeability testing

5.3.1: Permeability results

Permeability testing showed detectable levels of lactulose in the urine samples of 6/50 participants; rhamnose in all participants; and sucralose in 34/50 participants (Table 9). Permeability testing showed no significant difference in sucralose permeability between patient and control groups (Wilcoxon rank sum test: p-value=1), as shown in Table 10. With low
numbers of patients with detectable levels of lactulose, a correlation between lactulose:rhamnose ratios and DISQ scores was not found.

5.3.2: Smoking and permeability results
Patients who self-identified as current or previous smokers were grouped together and compared to ‘never-smokers’. Smoking did not appear to contribute to the permeability. Only one of the 6 patients with detectable levels lactulose in their urine was ever a smoker. There was no significant difference between the sucralose permeability of smokers and the sucralose permeability of previous and current smokers (Wilcoxon rank sum test p-value=0.4471), Table 11.

5.3.3: NSAID use and permeability results
There was no significant difference in sucralose permeability between the NSAID user group and the NSAID non-user group (Wilcoxon rank sum test p-value=0.8289), (Table 12). No volunteers (patients or healthy controls) had used NSAID in the 5 days before permeability testing.

5.3.4: Sucrelose permeability and DISQ/BASDAI
There was no significant correlation between sucrelose permeability and DISQ scores (Spearman’s rank correlation rho=0.154; p-value = 0.4091) or sucralose permeability and BASDAI scores (Spearman’s rank correlation rho=0.1137; p-value = 0.5425).

5.4: Wireless capsule endoscopy (WCE)

5.4.1: WCE patient demographic data
10 patients were recruited from the permeability test patient group. Seven patients were chosen because they had severe DISQ scores. One patient was chosen because he had a low DISQ score with a concurrently high faecal calprotectin; one patient because he had a low DISQ score and a mild faecal calprotectin; and one patient with a moderate DISQ score, who had
previously reported a severe DISQ score. Demographic data are shown in Figure 16.

5.4.2: WCE patient reports

Wireless capsule endoscopy revealed small intestinal lesions in most patients that underwent examination. The association between symptoms and macroscopic ulceration/erosion was complex, with one asymptomatic patient showing extensive ulceration, and two patients with severe symptoms showing only mild intestinal damage. These results are summarised as individual case reports, as follows.

Patient 1 in the capsule endoscopy group was a 49-year-old female. Her DISQ score of 20 showed severe gastrointestinal symptoms and her faecal calprotectin was high, at 242 micrograms/gram of faeces. Her CRP was 8mg/L, which is above the normal range and with a BASDAI of 4.4, indicating active disease (20). She was being treated with 75mg of diclofenac slow release twice daily with omeprazole cover for more than four years prior to the procedure. Wireless capsule endoscopy revealed inflammation of the small intestine increasing in severity from proximal to distal. Erosions and small ulcers were evident proximally, with large, deep ulcerations distally (Figure 6). Findings are consistent with those seen in inflammatory bowel disease (Mow’s criteria), although on appearance alone, NSAID-induced enteropathy remains a possible cause (82).
Figure 6: Patient 1 WCE. Images A-E show several large ulcerations surrounded by erythema in the distal small intestine.
Patient 2, a 40-year-old male, also had a high DISQ score (DISQ=22/60). His faecal calprotectin result was normal, at 42 µg/g. He had been taking naproxen 500mg twice daily for about 3 months before the procedure, and had been on equivalent doses of different NSAIDs previously. His CRP was 2mg/L, and had active SpA, evident with a BASDAI of 5.2. Capsule endoscopy found evidence of duodenitis and lymphoid follicle hyperplasia in the terminal ileum. The study was judged as having no evidence of small intestinal disease distal to the duodenum. Dark fluid obscured the view of the camera in places. Four images from this patient’s procedure are shown in Figure 7.
Figure 7: Patient 2 WCE. Images A and B show haemorrhagic duodenitis. Images C and D show lymphoid follicle hyperplasia in the terminal ileum.
Patient 3 was a 20-year-old male with no significant bowel symptoms (DISQ=5). Despite the low DISQ, his faecal calprotectin result was extremely high at >500 micrograms per gram and his CRP was 29. His BASDAI of 5.5 showed active disease. Wireless capsule endoscopy revealed antral gastritis and multiple ulcers in the small intestine. Some of these ulcers were completely circumferential (Figure 8). Mean cell haemoglobin was slightly low. He had not taken an NSAID for more than 2 years prior to WCE, which allowed NSAID enteropathy to be excluded as the cause of the lesions. A diagnosis of Crohn’s disease was made as appearances on WCE met the Mow criteria (82).
Figure 8: Patient 3 WCE. Image A shows an erosion in the mid jejunum. Images B and C show large ulcers in the distal ileum. Image D shows lymphoid hyperplasia in the terminal ileum. Image E shows an almost circumferential ulcer in the terminal ileum.
Patient 4 was a 41-year-old male. He had a high DISQ of 27 with a faecal calprotectin of 165. CRP was only slightly raised, at 5 mg/L and had been taking 20mg of tenoxicam daily for 12 months, and naproxen 500mg twice daily previously. His BASDAI, at 7.8, indicated severe disease activity. A small number of erosions and aphthous ulcers were seen throughout the small intestine (Figure 9). Appearances would be indicative of Crohn’s disease according to the Mow criteria, although the current use of NSAIDs precluded a definite diagnosis. Patient 4 was the only patient given wireless capsule endoscopy in whom lactulose was detected in the urine during permeability testing. His calculated lactulose:rhamnose ratio was 0.286, which could indicate increased paracellular permeability.
Figure 9: Patient 4 WCE. Small erosions are seen in pictures A-D.
Patient 5 was a 45-year-old male. His DISQ was low, at 6, and his BASDAI of 1.7 indicated inactive disease. The faecal calprotectin result was only just above the normal range, but his CRP was high at 15. Transferrin saturation, serum iron, serum ferritin, MCV, and MCH were all low. He had been taking 15mg meloxicam with omeprazole daily for two years. His capsule failed to leave the stomach, meaning no result could be determined.

Patient 6, a 47-year-old male, recorded a DISQ of 11 at permeability testing. One month previously his DISQ score had been 35. Faecal calprotectin was 196 and his BASDAI of 2.3 suggested inactive disease activity. WCE revealed haemorrhagic gastritis and multiple ulcers, becoming more evident distally (Figure 10). He was taking tenoxicam 20mg daily for 2 months before the procedure, but abstained from medications for 5 days prior to WCE. He had been taking meloxicam 15mg daily previously. Appearances on WCE met the Mow criteria for IBD, but in the setting of NSAID dosage, the diagnosis of NSAID enteropathy cannot be excluded.
Figure 10: Patient 6 WCE. Image A shows the stomach rugae. Image B shows a duodenal ulcer. Images C-F show erosions and ulcers in the small intestine.
Patient 7 was a 51-year-old male with a DISQ of 26, indicating severe bowel symptoms and a BASDAI of 4.2, indicating active SpA. Faecal calprotectin was high at 318 but he had a low CRP of 1 mg/L. This patient was being treated with a TNFα inhibitor (adalimumab), which he had been receiving for 21 months at a dosage of 40mg delivered subcutaneously every 2 weeks. He was also taking tenoxicam 20mg daily for 13 months. WCE demonstrated multiple erosions and small to large aphthous ulcerations from the mid-small intestine progressing distally, shown in Figure 11.
Figure 11: Patient 7 WCE. Images A-C show erosions throughout the small intestine. An ulcer can be seen in image D. Images E-F show more erosions.
Patient 8 was a 50-year-old male. His DISQ score was 19. He had been taking naproxen 500mg twice daily for 2 months, although he complained of bowel symptoms for many years even when his treatment consisted of only intermittent ibuprofen. He had a family history of IBD. His BASDAI of 5.8 indicated active disease. His CRP was very high at 38mg/L and he had a faecal calprotectin of 211. WCE showed several small aphthous ulcers throughout the small intestine, with numbers increasing distally (Figure 12). Full blood count was normal.
Figure 12: Patient B WCE. Image A shows an area denudation. Erosions can be seen in images B and C, with erosions evident in image D.
Patient 9 was a 33 year old male with a recent diagnosis of AS. His DISQ was 3, indicating little or no bowel symptoms. CRP was low at 2mg/L, and faecal calprotectin was 125. His BASDAI of 1 showed inactive disease. He had been taking tenoxicam 20mg intermittently as needed for 5 months. Wireless capsule endoscopy provided no evidence for small intestinal disease, showing only two tiny erosions in the distal small intestine, and lymphoid hyperplasia in the terminal ileum (Figure 13).

Patient 10 was a 51-year-old male with severe bowel symptoms and a DISQ of 36. His self-reported disease activity score was very high (BASDAI=8.6). His CRP was raised at 18mg/L, although his faecal calprotectin result was 77, only just above the normal range. WCE showed several erosions and ulcerations in the duodenal cap with small amounts of altered blood. The rest of the small intestine was disease-free (Figure 14). Despite the bleeding, there was no evidence of anaemia on full blood count. His NSAID dose was naproxen 550 mg daily without omeprazole cover which he had taken for more than 12 months.
Figure 13: Patient 9 WCE. Image A shows lymphoid hyperplasia in the terminal ileum; image B shows a single small erosion in the distal small intestine.

Figure 14: Patient 10 WCE. Image A shows petechiae in the duodenum, probably representative of haemorrhagic duodenitis. Blood is evident in image B, but particulate matter obscures the view. Image C possibly shows blood, but with a large amount of particulate matter.
### Table 2: Demographic data: Patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Number enrolled</td>
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<td>15</td>
</tr>
<tr>
<td><strong>Age (SD)</strong></td>
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<td>Mean = 39.6 (16.0)</td>
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<td>Median = 41</td>
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<tr>
<td><strong>Gender (% male)</strong></td>
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<td>60%</td>
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</tr>
<tr>
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<td>0/15</td>
</tr>
<tr>
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<td><strong>Mean DISQ (Min-Max)</strong></td>
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<tr>
<td><strong>Median faecal calprotectin (Min-Max)</strong></td>
<td>79.5 µg/g (6-500)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean BASDAI (Min-Max)</strong></td>
<td>4.0 (0.2-8.6)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean ASQoL (Min-Max)</strong></td>
<td>6.73 (0-18)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean BASFI (Min-Max)</strong></td>
<td>3.94 (0-8.8)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean BASMI (Min-Max)</strong></td>
<td>2.13 (0-6)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Peripheral joint count (tender) (Min-Max)</strong></td>
<td>1.5 (0-7)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Peripheral joint count (swollen) (Min-Max)</strong></td>
<td>1.29 (0-12)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>MASES (Min-Max)</strong></td>
<td>3.03 (0-9)</td>
<td>NA</td>
</tr>
</tbody>
</table>

NSAID: Non-steroidal anti-inflammatory drug; TNFα: tissue necrosis factor alpha; sDISQ: Dudley Inflammatory bowel disease Symptom Questionnaire; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASQoL: Ankylosing Spondylitis Quality of Life; BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; CRP: C-reactive protein
Table 3: Laboratory data and disease duration in patients with SpA compared to DISQ results

<table>
<thead>
<tr>
<th></th>
<th>Mean Result</th>
<th>Correlation with DISQ</th>
<th>Correlation p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calprotectin</td>
<td>143.3 µg/g</td>
<td>Rho = 0.278</td>
<td>0.1691</td>
</tr>
<tr>
<td>(Median = 79.5 µg/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>9.74 mg/L</td>
<td>Rho = 0.103</td>
<td>0.5556</td>
</tr>
<tr>
<td>Duration</td>
<td>108 months</td>
<td>Rho = 0.143</td>
<td>0.4134</td>
</tr>
</tbody>
</table>

Table 4: Faecal calprotectin and Low, Moderate, and Severe DISQ groups

<table>
<thead>
<tr>
<th></th>
<th>Number in group</th>
<th>Mean calprotectin</th>
<th>Median calprotectin</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISQ &lt; 11</td>
<td>19</td>
<td>122.833</td>
<td>61.5</td>
<td>179.666</td>
</tr>
<tr>
<td>DISQ 11-18</td>
<td>9</td>
<td>149.75</td>
<td>143.5</td>
<td>138.871</td>
</tr>
<tr>
<td>DISQ &gt;18</td>
<td>7</td>
<td>175.833</td>
<td>188</td>
<td>103.559</td>
</tr>
</tbody>
</table>

Figure 15: DISQ scores and faecal calprotectin

Non-significant correlation between faecal calprotectin results and DISQ scores. Median faecal calprotectin levels are given for low, moderate, and severe DISQ groups, with upper and lower quartiles.
Table 5: NSAID use and DISQ scores

<table>
<thead>
<tr>
<th></th>
<th>Fraction</th>
<th>Mean DISQ</th>
<th>Median DISQ</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current NSAID use</strong></td>
<td>25/35</td>
<td>12.48</td>
<td>11</td>
<td>8.661</td>
</tr>
<tr>
<td><strong>Not currently using NSAID</strong></td>
<td>10/35</td>
<td>9.2</td>
<td>6</td>
<td>8.690</td>
</tr>
</tbody>
</table>

Table 6: NSAID use and Low, Moderate, and Severe DISQ groups.

<table>
<thead>
<tr>
<th>DISQ</th>
<th>Current NSAID user</th>
<th>Not current NSAID user</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 11</td>
<td>12</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>11-18</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>&gt;18</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>25</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 7: Odds estimate of NSAID use for DISQ groups

<table>
<thead>
<tr>
<th>DISQ</th>
<th>Odds ratio estimate of current NSAID use (95% C.I.)</th>
<th>Fisher exact p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 11</td>
<td>1.00 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>11-18</td>
<td>1.926 (0.327 – 17.392)</td>
<td>0.670</td>
</tr>
<tr>
<td>&gt;18</td>
<td>3.080 (0.382 – 91.892)</td>
<td>0.375</td>
</tr>
</tbody>
</table>

Table 8: TNFα use and DISQ scores

<table>
<thead>
<tr>
<th></th>
<th>Fraction</th>
<th>Mean DISQ</th>
<th>Median DISQ</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current TNFα Inhibitor use</strong></td>
<td>5/35</td>
<td>9</td>
<td>6</td>
<td>9.7772</td>
</tr>
<tr>
<td><strong>Not currently using TNFα Inhibitor</strong></td>
<td>30/35</td>
<td>11.967</td>
<td>9.5</td>
<td>8.584</td>
</tr>
</tbody>
</table>
### Table 9: Intestinal permeability: Lactulose, L-rhamnose, sucralose

<table>
<thead>
<tr>
<th></th>
<th>Lactulose detected</th>
<th>L-rhamnose detected</th>
<th>Sucralose detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient group</td>
<td>5/35</td>
<td>35/35</td>
<td>23/35</td>
</tr>
<tr>
<td>Control group</td>
<td>1/15</td>
<td>15/15</td>
<td>11/15</td>
</tr>
</tbody>
</table>

### Table 10: Sucralose permeability: Patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Mean sucralose recovery</th>
<th>Median sucralose recovery</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient group</td>
<td>0.4%</td>
<td>0.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Control group</td>
<td>0.7%</td>
<td>0.2%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

### Table 11: Smoking and sucralose permeability

<table>
<thead>
<tr>
<th></th>
<th>Mean sucralose recovery</th>
<th>Median sucralose recovery</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never smoker</td>
<td>0.3%</td>
<td>0.2%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Previous or current smoker</td>
<td>0.5%</td>
<td>0.3%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

### Table 12: NSAID use and sucralose permeability

<table>
<thead>
<tr>
<th></th>
<th>Fraction</th>
<th>Mean sucralose recovery</th>
<th>Median sucralose recovery</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current NSAID use</td>
<td>25/35</td>
<td>0.4%</td>
<td>0.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Not currently using NSAID</td>
<td>10/35</td>
<td>0.3%</td>
<td>0.2%</td>
<td>0.3%</td>
</tr>
<tr>
<td>AGE</td>
<td>GENDER</td>
<td>DURATION</td>
<td>DISQ</td>
<td>BASDAI</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>----------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>Female</td>
<td>7</td>
<td>20</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>54</td>
<td>22</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>25</td>
<td>5</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>90</td>
<td>27</td>
<td>7.8</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>282</td>
<td>6</td>
<td>1.7</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>235</td>
<td>11</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>91</td>
<td>26</td>
<td>4.2</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>54</td>
<td>19</td>
<td>5.8</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>438</td>
<td>36</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*Figure 16: Wireless capsule endoscopy patient demographics.*

Age = Years of age  
Duration = Months between diagnosis and permeability testing  
DISQ = Dudley inflammatory bowel questionnaire  
BASDAI = Bath ankylosing spondylitis disease activity index  
CRP = C-reactive protein  
Calprotectin = Faecal calprotectin score, given in micrograms per gram of faeces  
ASQoL = Ankylosing spondylitis quality of life questionnaire
Chapter 6 – Discussion

This study examined gastrointestinal symptoms in patients with spondyloarthritis. The principal aims were to investigate the relationship between gastrointestinal symptoms in patients with SpA, and intestinal permeability, intestinal inflammation and macroscopic lesions of the gastrointestinal tract. Clinically significant gastrointestinal symptoms were common amongst the SpA patients we studied, consistent with previous research (16, 17). Intestinal pathology was common in the study group, with macroscopic lesions affecting the majority of patients who underwent wireless capsule endoscopy, and most patients demonstrating high faecal calprotectin results. Some patients had faecal calprotectin results at levels consistent with inflammatory bowel disease. There was no significant difference in intestinal permeability between patients and controls.

We studied a well-defined cohort of patients who all met the specific ASAS criteria for axial spondyloarthritis. 97% of these patients were HLA-B27 positive, which indicates that the group is likely to represent the true ankylosing spondylitis population. The group showed a range of disease activity and disease duration. Our study group had an age distribution similar previous research, although we had a slightly lower male:female ratio (6). This suggests that this study’s findings may be generalizable to other axial SpA or AS groups.

There was a significant positive correlation between gastrointestinal symptoms and self-reported SpA disease activity, measured by the BASDAI. This could represent parallel disease activity between intestinal inflammation and joint inflammation suggested in previous studies (8-10, 17, 95, 97). Alternatively, in self-reported questionnaires, an element of bias in responses has been suggested, with individual patients exhibiting systematic overestimation or underestimation of symptoms (251), leading to scoring either high or low in both questionnaires.
Another potential source of bias is related to overlapping concepts between the DISQ and BASDAI (both have questions relating to fatigue, for example). Recent analysis of these questionnaires by Stebbings et al (53) has suggested that removing these overlapping sections does not remove the correlation between the DISQ and BASDAI. Regular NSAID use may also be a potential factor influencing the association between the BASDAI and DISQ, since patients with low disease activity may use less NSAIDs than those with severe disease (252, 253), thus protecting them from NSAID-induced gastrointestinal pathology. However, a previous study has suggested that gastrointestinal symptoms are independent of NSAID use (17).

Patients with SpA commonly demonstrate asymptomatic gastrointestinal inflammation (9, 13, 47, 48). We found macroscopic lesions (ulcers and erosions) in most patients examined (8 of 9 completed studies), and 17/26 patients also showed faecal calprotectin levels above the normal range. Sixteen of the 35 patients in our study had “moderate” or “severe” gastrointestinal symptoms, as judged by the DISQ. Our data suggest that there may be an association between symptoms and levels of calprotectin in the faeces, but our sample size was too small to find a significant association. Ulcers and erosions tended to increase in size and severity from proximal to distal in the small intestine, possibly indicating a pathogenic association with bacterial populations (39). Gut bacteria are likely to be an important factor in both NSAID enteropathy and IBD (151, 254, 255), with both diseases showing increased inflammatory activity in areas of increased bacterial load (33, 152). Ulcers caused by NSAID enteropathy can be indistinguishable from ulcers caused by Crohn’s disease (88), which causes difficulties in the interpretation of these macroscopic findings.

Faecal calprotectin is a sensitive and non-invasive measure of inflammation in both the small and large intestines (72), and is increased in patients with SpA and their first degree relatives (75). NSAIDs increase faecal calprotectin results, but cannot account for the entire inflammation burden seen in this population (75). Our results support these previous findings, suggesting that NSAIDs frequently increase faecal calprotectin to above the threshold of normal (50µg/g), but do not increase the levels by themselves.
The relationship between DISQ scores and faecal calprotectin was not significant, but there appeared to be a positive trend between the two variables. If an association was confirmed, it would suggest that patients with high DISQ had evidence of intestinal inflammation. However, in this study the wide variation in faecal calprotectin results within each group meant that a larger sample size would be necessary to demonstrate such an association. There was a significant length of time between calprotectin testing and the DISQ recording in some patients, which is likely to have contributed to the lack of a significant correlation.

Amongst the patients studied, there were individuals who showed a disparity between faecal calprotectin results, DISQ scores and wireless capsule endoscopy findings. Patient 3 reported a low DISQ score of 5, indicating little or no gastrointestinal symptoms, but his faecal calprotectin was >500µg/g and multiple large ulcers were found throughout his small intestine. This suggests that lesions of severe appearance in the jejunum and ileum do not necessarily produce symptoms.

Measurement of the faecal calprotectin is a sensitive measure of jejunal and ileal inflammation (72), and this was confirmed by our findings on WCE. The DISQ, however, appears to be more sensitive in identifying patients with duodenal inflammation. Lesions in the duodenum do not increase faecal calprotectin results (70, 71), but our findings suggest that they can cause severe gastrointestinal symptoms. Patient 10 had a modest elevation of faecal calprotectin (77µg/g), but reported a high DISQ score (36). This patient had several erosions and ulceration in the duodenal cap with no disease distal to the duodenum. Patient 2, with a faecal calprotectin of 42µg/g, had a DISQ of 22. WCE revealed haemorrhagic duodenitis, while the rest of the small intestine was disease-free. Both patients were taking a full dose of naproxen daily without omeprazole. Although it is possible that IBD was the cause of the duodenal inflammation and symptoms in these patients, NSAID-induced damage seems more likely. Ultimately though, this small study cannot conclude with certainty the underlying cause of gastrointestinal symptoms.
A small sample size meant the precise relationship between medication use and gastrointestinal symptoms or inflammation could not be elucidated. Only 5 patients in the sample group were being treated with TNFα antagonists and no significant association with DISQ scores was elicited. TNF is a pro-inflammatory cytokine that plays an important role in IBD (197), so there is a plausible biological link between TNF and bowel symptoms. If TNF antagonists improved DISQ scores, this would lend weight to the hypothesis that DISQ is related to intestinal inflammation. In this small study, four of the patients taking TNF inhibitors had low DISQ scores (mean = 4.75; median = 5.5; range = 1 – 7) and were not taking NSAIDs; while one patient had a high DISQ score (26) and was taking an NSAID at a full daily dose. This patient had a higher BASDAI than the other four patients and had multiple intestinal erosions and ulcers. These observations could point to a causal relationship between NSAID dosage and gastrointestinal symptoms; or an association between gastrointestinal symptoms and spondyloarthritis symptoms; but the results are difficult to interpret due to the small sample size.

In summary, although it is well established that NSAID use can result in small intestinal ulceration, the use of these drugs alone is unlikely to account for the macroscopic ulceration noted in the patients in this study. Previous studies have shown that intestinal inflammation in SpA patients is more common than in other groups of patients using NSAIDs regularly (9). Inflammation is also common in first degree family members of SpA patients (48), supporting the hypothesis of inflammation independent of chronic NSAID use. Amongst the ten patients in our study that underwent WCE, one patient was found to have severe small intestinal ulceration, but had no recent history of NSAID use.

Previous studies have suggested that intestinal permeability is increased in patients with SpA and IBD compared to controls (103-106, 108-110), although some studies have not found any significant difference. It has been proposed that increased permeability allows bacterial antigens to breach the intestinal barrier, and to come into contact with immunocompetent cells of the lamina propria, as well as to enter the systemic circulation. By doing so it is postulated that this results in a loss of the normal immunological tolerance to
Commensal bacteria (256). In contrast with most previous studies, our study did not demonstrate increased intestinal permeability in SpA patients compared to controls. Permeability was not associated with gastrointestinal symptoms or spondyloarthritis disease activity, measured by the BASDAI.

Lactulose was only detectable in six of 35 samples, in contrast with previous published studies that detect lactulose in most samples. The reason for the lack of sensitivity of our test is not entirely clear, but is likely to be multifactorial.

- It is possible that bacterial contamination of samples resulted in lactulose being eliminated. This is relatively unlikely considering L-rhamnose was detected in all patients, and urine containers were prepared with boric acid as a preservative.
- It is unlikely that the length of time between sample collection and testing was a major contributor to lack of sensitivity, because lactulose was detected in samples that ranged from those among the earliest and latest collected. It is possible that the period of time decreased lactulose in all samples, leaving only a few above a sensitivity threshold.
- Technical error of the laboratory detection is unlikely as the institution is experienced in this field, having previously detected lactulose in similar studies.

Considering the L-rhamnose results were fairly consistent across all volunteers, and sucralose permeability was not significantly different between patients and controls, it remains a possibility that our results are a true representation of intestinal permeability, and that this study population does not have increased intestinal permeability. This is at odds with the findings of a number of previous studies (107-110, 126, 127, 129) but may be concordant with others (107, 130, 131). Previous studies that have produced significant findings have ranged in size from 17 to 73 patients with SpA, compared to 35 in our study. This may suggest that sample size was not the only factor. Overall, our permeability data are inconclusive, but do not add strength to the hypothesis that an increase in paracellular permeability is associated with SpA or intestinal inflammation.
Chapter 7 – Conclusions

Genetic, patho-physiological, and immunological research suggests aetiological links between spondyloarthritis and the inflammatory bowel diseases. Six to thirteen percent of patients with spondyloarthritis develop inflammatory bowel disease (4-7) and many more develop asymptomatic intestinal inflammation (9, 13). Patients with SpA often experience significant gastrointestinal symptoms, but the relationship to gut lesions is unclear.

In our study patients with SpA frequently experienced clinically significant gastrointestinal symptoms measured by the DISQ. Furthermore, patients with SpA demonstrated high levels of faecal calprotectin. Although there was no significant correlation between DISQ scores and calprotectin levels, a trend was noted. The DISQ appears sensitive for detecting the symptomatic lesions in the upper small intestine, but faecal calprotectin or WCE is more sensitive for detecting asymptomatic lesions seen more distally. Combining faecal calprotectin testing with the DISQ may allow for a sufficiently sensitive toolset for detecting NSAID-induced intestinal damage and true inflammatory lesions related to SpA (57).

Increased intestinal permeability in SpA has been a common finding previously and is thought to be an important aetiological factor linking this disease with IBD. Our study failed to find a significant difference in intestinal permeability between spondyloarthritis patients and healthy controls, but this was likely to be a result of a lack of sensitivity in the test. With no significant permeability results, our data do not support the hypothesis that gastrointestinal symptoms are associated with a change in epithelial barrier integrity.

The use of NSAIDs is known to increase intestinal permeability and to induce intestinal inflammation and ulceration. Proximal intestinal disease caused by these drugs may account for some of the symptom burden seen in our patient group. The pathogenesis of NSAID enteropathy shows many common factors with IBD, and differentiating primary pathology due to SpA/IBD from
secondary pathology due to NSAIDs could be a focus for further research. One potential area is researching the possible effect of omeprazole in improving DISQ scores or macroscopic lesions in SpA patients.

Research is planned to continue, with patients who underwent WCE in this study to be offered ileocolonoscopy. This may help to delineate the cases of NSAID enteropathy from Crohn’s disease.
Chapter 8 – References


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Chapter 9 – Appendices