Table 18 Incidence of heterozygous and homozygous mutations for all pooled samples

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Common Homozygote</th>
<th>Heterozygote</th>
<th>Rare Homozygote</th>
<th>Failed sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD2</td>
<td>R702W</td>
<td>27</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NOD2</td>
<td>G908R</td>
<td>29</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NOD2</td>
<td>1007fs</td>
<td>29</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OCTN1</td>
<td></td>
<td>10</td>
<td>15</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>IL23R</td>
<td></td>
<td>28</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>DLG5</td>
<td></td>
<td>26</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ATG16L1</td>
<td></td>
<td>9</td>
<td>13</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>TLR4</td>
<td></td>
<td>27</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>NOD1</td>
<td></td>
<td>20</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 19 Observed and expected incidence of single nucleotide polymorphisms (continued in Table 20)

<table>
<thead>
<tr>
<th>dbSNP number</th>
<th>SNP name</th>
<th>expected common homozygote (wild type)</th>
<th>observed wild type</th>
<th>expected heterozygote</th>
<th>observed heterozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2066844</td>
<td>R702W</td>
<td>86.22%</td>
<td>87.10%</td>
<td>13.26%</td>
<td>3.23%</td>
</tr>
<tr>
<td>rs2066845</td>
<td>G908R</td>
<td>92.99%</td>
<td>93.55%</td>
<td>6.89%</td>
<td>3.23%</td>
</tr>
<tr>
<td>rs5743293</td>
<td>1007fs</td>
<td>95.30%</td>
<td>93.55%</td>
<td>4.65%</td>
<td>6.45%</td>
</tr>
<tr>
<td>rs1050152</td>
<td>OCTN1</td>
<td>26.23%</td>
<td>32.26%</td>
<td>49.97%</td>
<td>48.39%</td>
</tr>
<tr>
<td>rs11209026</td>
<td>IL23R</td>
<td>95.18%</td>
<td>90.32%</td>
<td>4.76%</td>
<td>6.45%</td>
</tr>
<tr>
<td>rs1248696</td>
<td>DLG5</td>
<td>81.86%</td>
<td>83.87%</td>
<td>17.23%</td>
<td>9.68%</td>
</tr>
<tr>
<td>rs2241880</td>
<td>ATG16L1</td>
<td>32.65%</td>
<td>29.03%</td>
<td>48.98%</td>
<td>41.94%</td>
</tr>
<tr>
<td>rs4986790</td>
<td>TLR4</td>
<td>83.66%</td>
<td>87.10%</td>
<td>15.62%</td>
<td>6.45%</td>
</tr>
<tr>
<td>rs6958571</td>
<td>NOD1</td>
<td>64.78%</td>
<td>64.52%</td>
<td>31.41%</td>
<td>29.03%</td>
</tr>
</tbody>
</table>
Table 20 Observed and expected incidence of single nucleotide polymorphisms (part II)

<table>
<thead>
<tr>
<th>dbSNP number</th>
<th>SNP name</th>
<th>Expected rare homozygote</th>
<th>observed rare homozygote</th>
<th>Chi square (2 degrees of freedom)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2066844</td>
<td>R702W</td>
<td>0.51%</td>
<td>0.00%</td>
<td>7.011</td>
<td>0.03</td>
</tr>
<tr>
<td>rs2066845</td>
<td>G908R</td>
<td>0.13%</td>
<td>0.00%</td>
<td>2.526</td>
<td>0.28</td>
</tr>
<tr>
<td>rs5743293</td>
<td>1007fs</td>
<td>0.06%</td>
<td>0.00%</td>
<td>0.096</td>
<td>0.1</td>
</tr>
<tr>
<td>rs1050152</td>
<td>OCTN1</td>
<td>23.80%</td>
<td>0.00%</td>
<td>1.238</td>
<td>0.54</td>
</tr>
<tr>
<td>rs11209026</td>
<td>IL23R</td>
<td>0.06%</td>
<td>0.00%</td>
<td>0.145</td>
<td>0.93</td>
</tr>
<tr>
<td>rs1248696</td>
<td>DLG5</td>
<td>0.91%</td>
<td>0.00%</td>
<td>2.794</td>
<td>0.25</td>
</tr>
<tr>
<td>rs2241880</td>
<td>ATG16L1</td>
<td>18.37%</td>
<td>0.00%</td>
<td>2.206</td>
<td>0.33</td>
</tr>
<tr>
<td>rs4986790</td>
<td>TLR4</td>
<td>0.73%</td>
<td>0.00%</td>
<td>4.352</td>
<td>0.11</td>
</tr>
<tr>
<td>rs6958571</td>
<td>NOD1</td>
<td>3.81%</td>
<td>0.00%</td>
<td>0.164</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Table 21 Incidences of Genetic Mutations in MAP Positive Samples

<table>
<thead>
<tr>
<th></th>
<th>Major homozygotes (wild type)</th>
<th>Heterozygotes</th>
<th>Rare homozygotes</th>
<th>Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCTN1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IL23R</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>DLG5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R702W</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G908R</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ATG16L1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TLR4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1007fs</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NOD1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
DISCUSSION

In this analysis of MAP amongst patients with inflammatory bowel disease compared to controls, there was no association between the presence of MAP and patients with inflammatory bowel disease, or with the presence of inflammation in samples. The rates of positivity for MAP were notably lower than those in previously published studies. Many studies to date show a positive association between the presence of MAP and inflammatory bowel disease. There are two main possibilities for this difference compared to previous studies. The first is that the techniques used were more specific, and there were less false positives. Alternatively, it may be that the technique used in this study was less sensitive, and understated the true abundance of MAP.

No association was noted between the presence of inflammation in biopsy samples and the incidence of MAP. This is consistent with previous studies, which do not suggest a difference in the microbiota due to inflammation. Patients who were positive for MAP were more likely to be early in the course of their disease (or at diagnosis). This alludes to the possibility that MAP may play a pathogenic role in initiation of this disease, rather than perpetuation of inflammation, however this is hypothesis forming only.

The association between MAP and IBD has been reported in a number of previous studies; however the proportions of patients positive for MAP in both IBD groups and control groups have varied dramatically, and indeed in some studies no association has been found. Different studies however show different levels of association and some studies show no association at all. Reasons for this include different methodologies for detection of MAP, different methods of specimen collection, and different approaches to targeting specific anatomical areas or areas of inflammation. For example, in some studies the analysis was performed on faecal samples rather than mucosal biopsies.

A number of factors may have contributed to the findings in this study. The detection of the IS900 sequence may depend upon methodology of specimen collection. Mucosal biopsies are known to have a different profile to faecal samples. The transportation of samples in RNAlater and refrigeration of samples
could have also contributed. The methodology of DNA extraction can differ significantly and may be particularly important as MAP is both a fastidious organism, and also if sterility is not perfectly maintained the incidence may be reported as falsely high due to environmental contamination. Performance and analysis of the PCR sequence may also differ significantly.

The presence of different treatments may also be important. It is possible that 5ASA based agents, corticosteroids, immunomodulators and anti-TNFα agents may all exert alterations on the mucosal microbiota. In this group of patients, concurrent medications were heterogeneous however 5ASA agents and corticosteroids were the most common. Unfortunately data regarding antibiotic use was not captured in this study due to patients having poor recollection whether they were treated with antibiotics in the previous two years. Due to the small number of MAP positive samples, no association between particular agents can be deduced. In addition, the sample was heterogeneous due to the different ages of patients, different durations from diagnosis, and different phenotypes. It is certainly feasible that microbial profiles could be different in different disease phenotypes, for example ileal disease compared to colonic disease.

Evidence arguing against the role of MAP as aetiological in inflammatory bowel disease is found in studies assessing MAP in other disorders separate to inflammatory bowel disease. An association between the presence of MAP and patients with irritable bowel syndrome (IBS) has been noted by Scanu et al (2007). This finding is more difficult to tie into the model of MAP being a causative agent of inflammation, given that IBS is generally accepted as a non-inflammatory bowel condition related in part to visceral hypersensitivity. More recently there have been associations noted between irritable bowel syndrome and small intestinal bacterial overgrowth (SIBO).

After commencement of this study, the results from an Australian multicentre study examining the response of patients with IBD to an antimycobacterial regimen targeting MAP were published as discussed in the literature search. One key limitation of the study was that patients were not assessed for the presence or absence of MAP. As a consequence, it did not assess the possibility that MAP is pathogenic in only a subset of patients. In addition, the varying duration of disease activity of patients recruited in that study meant that treatment of MAP at the
onset of disease where it may act as an initiating agent is not possible. Sartor (2005) suggests that the fact that chronic immunosuppression does not worsen IBD disease activity, as might be expected if a mycobacterium was playing an active role (analogous to *M. tuberculosis* infection) is an argument against MAP playing a pathogenic role.285

**Genotyping for single nucleotide polymorphisms**

These data represent a pilot study for a multiplex comparison of single nucleotide polymorphism (SNP) genotyping for mutations associated with IBD to microbiological status. Extracted DNA was sent for multiplex analysis for nine different single nucleotide polymorphisms associated with inflammatory bowel disease. In this study of 31 patients, no statistically significant difference in the incidence of SNPs was noted between the heterogeneous patient group and expected results. As previously mentioned, the biological validity of using this heterogeneous group was significantly decreased. In addition, the sample size was very small and almost certainly underpowered to detect a difference with expected mutation rates. Given this, this study acts as a pilot study to investigate the logistics and feasibility of analysing samples using this technique. The SNP results for the four patients who were positive for MAP were also presented, though no further analysis was performed on this group.

The premise that particular mutations related to innate immunity might correlate with the presence of particular organisms is an attractive one, particularly as different bacterial sensing receptors sense different bacteria. For example, *NOD2* senses peptidoglycans and *TLR4* senses lipopolysaccharides. Toll-like receptors have a 'cross-talk' with the microbiota and may help regulate intestinal homeostasis.70 This hypothesis rests on the premise that particular organisms are playing a particular role in different patients. The alternative hypothesis is that patients with inflammatory bowel disease as a whole have defective innate immunity, irrespective of the particular mechanism or gene mutation, and that inflammation occurs as a consequence of exposure to either the normal commensal microbiota, or alternatively to bacterial dysbiosis within the gastrointestinal tract.
The increasing complexity of IBD genetics has meant that the initial NOD2 mutations described in 2001 have been joined by mutations of the IL23R, ATG16L1, and TLR4 genes amongst others. Along with the NOD2 gene, these genes are associated with innate immunity, raising the possibility that altered bacterial sensing by the gastrointestinal immune system is responsible for inflammation in IBD. The mechanism of bacterial sensing of the NOD2 proteins via the peptidoglycan muramyl dipeptide (MDP) is well described; the latter has been documented as having reduced function in patients with NOD2 mutations.

**Limitations and potential improvements**

A number of different factors may have impacted upon the results of this study. The sample size was smaller than the study was powered for; this related to difficulty with recruitment. Patients were recruited at different durations since diagnosis. MAP could play a role in initiation of IBD, but not be necessary for ongoing perpetuation of the disease process, and as a consequence be no longer detectable years from diagnosis.

The validity of this study could have been further improved by using healthy volunteers as controls instead of patients presenting with diarrhoea for investigation. Many of the former group of patients will eventually be given a diagnosis of irritable bowel syndrome (IBS). The microbiota of patients with IBS may well be different to those of healthy volunteers. Scanu et al (2007) noted that mucosal biopsies from 15 out of 20 patients with IBS were positive for the IS900 sequence.
CHAPTER 2: MICROBIOTA ANALYSIS USING OLIGONUCLEOTIDE MICROARRAY

ABSTRACT

The pathogenesis of inflammatory bowel disease is likely to involve interaction between genetic factors, innate immunity, and the enteric microbiota. Alterations in the composition of the normal commensal microbiota may play a pathogenic role.

A custom 2240 probe oligonucleotide microarray based on 16s rRNA sequences was used to compare the microbiota profiles of patients with inflammatory bowel disease with controls. Twenty mucosal samples obtained from colonoscopic biopsies were analysed – five from Crohn's disease inflamed (CDI) tissue, five from Crohn's disease non-inflamed (CDNI), five from ulcerative colitis (UC), and five healthy control samples. Analysis was performed using principal components analysis and between group analysis.

The microbiota from both Crohn's disease and ulcerative colitis differed significantly from the control group, though not between CDI and CDNI groups. Alterations in the abundance of Faecalibacterium prausnitzii, Shigella flexneri, Dorea longicatena, and Xenorhabdus bovienii were associated with Crohn's disease. Alterations in the abundance of Yersinia pestis and Eubacterium rectale were associated with ulcerative colitis.

The gastrointestinal microbiota differed in mucosal samples from patients with inflammatory bowel disease compared to those taken from controls. The presence of inflammation did not appear to significantly alter the microbiota composition. The abundance of particular organisms including the previously described F. prausnitzii was found to be different in patients with inflammatory bowel disease compared to healthy controls, and new putative aetiological organisms were identified. These findings support the hypothesis that a bacterial 'dysbiosis' may contribute to the pathogenesis of inflammatory bowel disease.
HYPOTHESIS

That the pathogenesis of inflammatory bowel disease relates to a complex interplay between alterations in the gastrointestinal immune system and the bacterial microbiota within the gastrointestinal tract.

Short Rationale:

Linkage studies and association studies have revealed a broad range of single nucleotide polymorphisms associated with both Crohn's disease and ulcerative colitis. The function of the genes affected by these SNPs often relates to the innate immune system, affecting bacterial sensing, autophagy and mucosal barrier function.

The specific role for the microbiota in the pathogenesis of IBD is less well characterised, though it is likely to include either alterations in the abundance of a single pathogenic organism, or alternatively broad alterations in the microbiota termed 'dysbiosis'. The pathogenic effect of dysbiosis may be mediated by changes in the functional and metabolic effects, such as the production of short chain fatty acids such as butyrate. Particular SNPs may potentially alter the susceptibility to particular organisms by affecting a particular immune pathway, and so may correlate with the presence of particular organisms. This would provide evidence to support this model of the pathogenesis of IBD.

AIMS

To assess the microbiota in mucosal biopsies collected during a clinically indicated colonoscopy in a cohort of patients with IBD compared to control patients.

1. To assess the microbiota more broadly using a custom oligonucleotide microarray based on the bacterial 16s ribosomal RNA sequence. This metagenomic type approach aims to broadly describe the organisms in a particular community, and the community of organisms will be compared between the CD, UC and control groups.

2. To compare inflamed samples and non-inflamed samples
METHODS

Patient / sample characteristics

The set of samples available for analysis was the same as that mentioned in the previous section for MAP analysis. However, only a subset of samples could be used as not all samples contained sufficient extracted DNA for the purposes of analysis using the microarray. For simplification, five samples from Crohn’s disease (inflamed), five from Crohn’s disease (non-inflamed), five from ulcerative colitis and five from the control group (‘normal’) were analysed using the oligonucleotide microarray.

Oligonucleotide probe design and microarray preparation

A custom phylogenetic microarray was designed and validated according to the methods described by Kang et al (2010).\textsuperscript{286} Briefly, the probes based on 16s ribosomal RNA sequences from gastrointestinal bacteria identified on the Entrez Nucleotide database situated on the National Center for Biotechnology Information (NCBI) website were included. The particular oligonucleotides selected were derived from a literature search of published papers.\textsuperscript{125,287} Individual probes were then designed using the GoArray oligonucleotide program.\textsuperscript{288} The resultant 40-mer oligonucleotide probes represented diverse taxonomy groups, with differing specificities ranging from species to phylum levels; the majority were targeted at the species level. These were synthesised on to a custom 4X2K probe microarray by Combimatrix (USA). The microarray was synthesized with four replicates of each probe distributed across the array to allow validation of results. Samples were analysed as per the Combimatrix hybridisation and imaging protocol. Resultant microarray images were analysed using GenePix Pro 6.0 software.\textsuperscript{289,290}
Microarray hybridisation

The microarray chip was initially incubated with nuclease free water at 65°C for 10 min, and then incubated in ‘pre-hybridisation’ solution as per the Combimatrix hybridization and imaging protocol. The microarray chip was incubated with this solution at 42°C for 1 hour; this solution was then removed from the hybridization chamber. The chip was filled again with hybridization solution and incubated at 42°C overnight. After hybridization the chip was washed using specific solutions. After removing the cleaning solution, the microarray was loaded into a scanner (see next paragraph) as per the manufacturer’s recommendations. After data acquisition, each microarray chip was able to be used twice again to analyse different samples by using a stripping kit; in total, each chip was able to be used three times.

Signal detection and data analysis

The microarray slides were scanned with an Axon Genepix 4000A microarray scanner (Molecular Devices, Sunnyvale, California). Obtained images were analysed using GenePix Pro 6.0 software (Molecular Devices, FINDREF), and the resultant GenePix Results Format (GPR) files were analysed using GeneSpring 7.3 software (Agilent Technologies, Santa Clara, California) and the ‘R’ statistical package. The data were normalized by using the standard ‘one colour’ option of the GeneSpring program.

Statistical analysis

The microarray data were normalized using the quantile normalization method, then analysed using the R statistical environment. The four different groups were compared using between group analysis (BGA), correspondence analysis (CoA), and Monte–Carlo tests. These were performed using the multivariable analysis, ADE4 and MADE4 software packages. The analysis is classified as a ’supervised’ microarray analysis in that different groups were defined beforehand. The Monte-Carlo permutation test was used to generate P values for differences between samples.
RESULTS

Between class analysis between different groups

20 samples were analysed using the microarray platform; 5 from the CDI group, five from the CDNI group, 5 from the UC group and 5 control samples.

Figure 5 Between group analysis between Crohn's disease inflamed (CDI), Crohn's disease non-inflamed (CDNI), ulcerative colitis (UC), and the control group ('Nor')

Between group analysis (BGA) suggested significant separation between the four sample groups analysed, Crohn's disease inflamed (CDI), Crohn's disease non-inflamed (CDNI), ulcerative colitis (UC) and controls when comparing all bacterial species (Figure 5). The principal components analysis of the four groups was analysed using a Monte Carlo test, giving a $p$-value of 0.005.
The abundance of each of the 2240 organism specific probes present in the microarray within the four different groups was compared using BGA, employing the Monte Carlo test. The organisms showing the largest difference in abundance between the four groups are presented in Table 22 (note there may have been more than one probe detecting a particular organism). Of note, both the *Faecalibacterium* and *Clostridium* species fall within the *Clostridiaceae* family. For some of these organisms, the box chart analysis is presented in graphical form in Figure 6, Figure 7, and Figure 8.

*Faecalibacterium prausnitzii* was analysed specifically and was noted to be decreased in the CDI and CDNI subgroups compared to controls, and was decreased in the UC compared to all three (Figure 6). In the *Clostridum proteolyticum* species, the abundance in ulcerative colitis samples was greatly increased compared to samples from Crohn's disease, CDI and CDNI.
Table 22 Between class comparisons between the four groups for different organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides distasonis</td>
<td>0.0650</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>0.0186*</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>0.3882</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
<td>0.0462*</td>
</tr>
<tr>
<td>Bacteroides distasonis</td>
<td>0.0031*</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>0.1083</td>
</tr>
<tr>
<td>Clostridum proteolyticum</td>
<td>0.0020*</td>
</tr>
<tr>
<td>Clostridium butyricum</td>
<td>0.0008*</td>
</tr>
<tr>
<td>Clostridium limosum</td>
<td>0.0049*</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>0.0522</td>
</tr>
<tr>
<td>Xenorhabdus bovienii</td>
<td>0.0605</td>
</tr>
<tr>
<td>Enterobacter cowanii</td>
<td>0.0272*</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>0.0129*</td>
</tr>
</tbody>
</table>

Species with a p-value of less than 0.05 are marked with an asterix

![Figure 6 Box chart analysis comparing abundance of Faecalibacterium prausnitzii in different groups (P=0.04)](image-url)
Comparison between Crohn’s disease (inflamed) and control group

As opposed to the above comparison between four groups, a more specific comparison was performed for specific organisms between two groups. The Crohn’s disease (inflamed) and the control groups were compared initially using a heat map of the top ten probes showing the strongest association, based on the BGA. The BGA suggested a difference between the
two groups overall ($p = 0.033$), and the abundances of *Faecalibacterium prausnitzii*, *Bacteroides vulgatus*, *Dorea longicatena*, *Bacteroides caecae* and *Allstipes putredines* appeared different in abundance between the two groups on the heat map (Figure 9). However, when the relative abundances of organisms were assessed individually, only the abundances of *Faecalibacterium prausnitzii* and *Dorea longicatena* were noted to be decreased in Crohn’s disease compared to controls, and *Xenorhabdus bovienii* and *Shigella flexneri* were increased in abundance in the Crohn’s group compared to control samples (see Figure 12 and Figure 10).

Figure 9 Heat map analysis comparing Crohn’s disease (inflamed) and control groups
Figure 10 Box chart analysis comparing abundance of *F. prausnitzii* between CDI and control groups ($p$ value = 0.02)

Figure 11 Box chart analysis comparing abundance of *Xenorhabdus bovienii* between Crohn’s disease (inflamed) and normal groups
Figure 12 Box chart analysis comparing abundance of *Shigella flexneri* between CDI and control groups (p value = 0.04)

Figure 13 Box chart analysis comparing abundance of *Dorea longicatena* between Crohn’s disease (inflamed) and normal groups
Comparison between samples from ulcerative colitis and control groups

In the heat map analysis of the top ten probes based on the BGA comparing samples from patients with ulcerative colitis compared to control patients, differences in abundances were seen in a number of organisms including *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, *Bacteroides distasonis*, *Dorea longicatena* and *Bacteroides fragilis* (see Figure 14). BGA suggested that the difference between the groups was statistically significant with a *p*-value of 0.039. When organisms were compared individually, the abundance of only two organisms were noted to be statistically significant, *Yersinia pestis* and *Eubacterium rectale* (see Figure 15 and Figure 16). The difference in the abundance of *F. prausnitzii* between UC and controls on the heat map was *p* = 0.019 using one probe, and *p* = 0.14 using another probe, and did not reach statistical significance when individually compared. This is despite the striking difference in the initial four-group comparison (see Figure 6).
Figure 15 Box chart analysis comparing abundance of *Yersinia pestis* between UC and control groups (*p* value = 0.02)

Figure 16 Box chart analysis comparing abundance of *Eubacterium rectale* between UC and control groups (*p* value = 0.04)

Figure 17 Box chart analysis comparing abundance of *Faecalibacterium prausnitzii* between normal and ulcerative colitis groups
Comparison between Crohn’s disease (inflamed) samples with Crohn’s disease (non-inflamed) samples

The heat map of Crohn’s-disease inflamed (CDI) samples compared to Crohn’s disease non-inflamed (CDNI) samples (see Figure 18) suggested differences in a number of organisms including *Faecalibacterium prausnitzii*, which has previously been discussed. Other organisms include *Lactobacillus vitulinus*, *Oscillospira guillermondii*, *Bifidobacterium bifidum* and *Hydrogenophaga* spp. The between groups analysis did not detect a statistically significant difference between CDI and CDNI when compared as a whole, given a $p$ value of 0.302. As such, no further analysis was performed between these two groups of samples.

Figure 18 Cluster analysis comparing of Crohn’s-disease inflamed vs. non-inflamed
DISCUSSION

This study shows significant differences in the bacterial profiles of the mucosal microbiota between patients with either CD or UC and controls, based on analysis of mucosal samples using a custom 16s rRNA oligonucleotide probe microarray. The use of the oligonucleotide microarray allowed analysis of specimens across a large range of species, and also provided a semi-quantitative analysis of abundance to be performed. Comparing all four groups (CDI, CDNI, UC and controls) using principal components analysis, suggested that a small number of the differences identified between the groups were statistically significant. This analysis showed significant differences between the groups in a number of organisms including members of the Bacteroides, Faecalibacterium, and Clostridium genera.

Subsets of two groups were then compared directly using a heat map based on the BGA. Samples from the CDI group were different to control patients, reaching statistical significance for several organisms. The abundance of F. prausnitzii and Dorea longicatena was decreased in the CDI group compared to controls, and the abundance of Xenorhabdus bovienii and Shigella flexneri was increased in the CDI group compared to controls. Shigella flexneri is a Gram negative organism, of the family Enterobacteriaceae which is known as a gastrointestinal pathogen in humans causing a diarrhoeal illness. Xenorhabdus (also known as Photorhabdus) are Gram-negative gamma proteobacteria that are better known as colonising the guts of soil nematodes.

In the current study, samples from patients with ulcerative colitis were compared to those from control patients, and the BGA suggested statistically significant differences between the two groups. When the organisms were compared specifically, the abundances of Yersinia pestis and Eubacterium rectale were noted to be decreased in the UC group compared to controls. The difference in the abundance of F. prausnitzii was not statistically significant between the two groups overall. When the CDI and CDNI groups were compared using heat map based on the between group analysis, a number of organisms including F. prausnitzii appeared different in abundance, however the BGA did not suggest a statistically significant difference between these two groups.

The major findings of this study is that the microbiota in Crohn’s disease and ulcerative colitis groups appeared to be different from the control group, and that no difference was detected in Crohn’s disease samples between inflamed samples and non-inflamed samples.
This argues against the hypothesis that inflammation is the primary cause of alterations in the microbiota. As discussed in the previous section, the majority of studies suggest that the presence of inflammation does not appear to alter the microbiota significantly. A primary ‘field’ effect in IBD may contribute to pathogenesis, however the possibility that previous inflammation or inflammation in other parts of the bowel may have contributed to the dysbiosis still needs to be considered. Another major finding was that previously unassociated organisms displayed differential abundance between IBD and control patients. \textit{Shigella flexneri}, \textit{Dorea longicatena}, and \textit{Xenorhabdus bovienii} were associated with Crohn’s disease, and \textit{Yersinia pestis} and \textit{Eubacterium rectale} were associated with ulcerative colitis.

This study confirms previously noted findings that \textit{F. prausnitzii} is decreased in abundance in patients with Crohn’s disease compared to controls. This association was not seen in patients with UC compared to controls. Sokol et al (2008) noted that patients with ileal Crohn’s disease who underwent surgical resection were more likely to have post-operative recurrence if the abundance of \textit{F. prausnitzii} was decreased.\textsuperscript{250} Fujimoto et al (2012) found decreased \textit{F. prausnitzii} in the stool samples of patients with Crohn’s disease compared to controls, using terminal restriction fragment length polymorphisms and real time polymerase chain reaction.\textsuperscript{252} \textit{F. prausnitzii} is a known butyrate producer, and butyrate may have ‘anti-inflammatory’ properties, possibly due to decreased pro-inflammatory cytokine expression via inhibition of nuclear factor kappa B activation and degradation of the I kappa B alpha protein.\textsuperscript{159,163}

The findings from this study do support the hypothesis that alterations in the enteric microbiota contribute to the pathogenesis of IBD. The individual organisms identified may form part of this ‘dysbiosis’, or alternatively they may represent individual pathogens. This raises the question of whether a normal commensal or ‘allochthonous’ organism may become ‘pathogenic’ if their abundance is increased or decreased significantly compared to normal variation.\textsuperscript{11} The presence of a dysbiosis in IBD contributing to pathogenesis is entirely compatible with a model of impaired clearance of bacterial pathogens by impaired innate immunity mediated by genetic predisposition. Commensal bacteria may enter into subepithelial tissues instead of being adequately cleared by mucosal defenses. This defective clearance may cause compensatory antibacterial effects from T cells leading to mucosal inflammation. The ongoing inflammation may also be impaired due to genetic
factors, and the inability to properly clear these organisms leads to a cycle of chronic inflammation.\textsuperscript{241,298}

A critical concept is how the host immune system distinguishes between commensal bacteria and pathogens in order to maintain immune ‘tolerance’ to commensals. In some cases pathogens may contain virulence factors that may help the host identify them. Pattern recognition receptors such as toll-like receptors play an important role in regulating this process; TLRs may be involved with cytoprotection, tissue repair and angiogenesis which are important to help protect the gut from injury.\textsuperscript{70} The detection of commensals compared to pathogens is made more difficult as some organisms normally resident in the bowel may become pathogenic under different circumstances, such as antibiotic use in the case of \textit{Clostridium difficile}.\textsuperscript{299} It may be that organisms such as this have an advantage over pathogens not normally resident in the microbiota, as they are more likely to be recognised as commensal bacteria, at least initially.\textsuperscript{150} It is easy to speculate that even small perturbations to this delicate homeostasis may disrupt the symbiosis between the host and the microbiota, with subsequent development of chronic inflammation.

Whether the alterations in the relative abundance of particular organisms or whether a broader dysbiosis is thought to be more important, therapeutic options which directly affect the microbiota include antibiotics, prebiotics, probiotics or even faecal transplantation. ‘Prebiotics’ refer to dietary oligosaccharides that may alter the abundance of particular organisms. Together with probiotics these are termed together as ‘synbiotics’.\textsuperscript{266} Apart from a few specific situations such as probiotics following pouch surgery for colitis or antibiotics for perianal Crohn’s disease, the evidence to support the use of these therapeutic modalities in inflammatory bowel disease is lacking.\textsuperscript{300,301} At this point in time, the majority of treatments used in IBD reduce inflammation by their effect on the immune system. Whether these treatments also affect the bacterial microbiota directly remains an interesting possibility.

**Use of a microarray to assess microbiota**

The use of the novel microarray in this study has enabled a broad range of organisms to be studied in patients with inflammatory bowel disease. The use of a microarray for oligonucleotide hybridisation has advantages over conventional whole genome sequencing techniques such as ‘shotgun’ sequencing or ‘next generation’ techniques which require a
very large amount of DNA to be analysed to attempt to fully describe a community, which are significantly more labour intensive and expensive.\textsuperscript{133} The use of the microarray also has the potential to detect organisms in lower abundance, which may be missed using whole genome sequencing due to getting ‘drowned out’ by the vast volume of other organisms. Some of these uncommon organisms may be of particular importance if they are found to play a pathogenic role despite being low in abundance.

**Limitations of the study**

Although the sample sizes were small in this study, the redundancy in the probes in the microarray and the magnitude of the differences in abundance make the results more robust. Nonetheless, these findings need to be validated in larger sample sets. In terms of anatomical location, samples from the terminal ileum were the focus in order to control for the variable that different organisms may be present in different anatomical areas, however samples were pooled from different areas of the gastrointestinal tract. Patients with Crohn’s disease were at different durations from diagnosis, and had been treated with different medications including immunomodulators, which could have effects on the microbiota. As mentioned in the previous section, sample logistics including refrigeration could have had an effect on results of microbiological analysis.

Another potential limitation is that control patients may have had a diagnosis of irritable bowel syndrome as they had a normal colonoscopy to investigate diarrhoea. As a consequence their microbiota may be different compared to healthy volunteers. Tannock (2010) suggests potential limitations to the use of mucosal biopsies for analysis, as the bowel preparation prior to colonoscopy may have altered the mucosal microbiota. The remaining bowel cleansing solution pools in the bowel prior to the procedure and may bathe the wall of the bowel, as well as the colonoscope and biopsy forceps. The mucosal microbiota may differ from the microbiota within the lumen of the bowel which is not adjacent to the mucosa.\textsuperscript{298}
SECTION TWO:

QUALITY AND SAFETY IN INFLAMMATORY BOWEL DISEASE
HYPOTHESIS

The treatment of inflammatory bowel disease has evolved over time from the use of corticosteroids and 5-aminosalicylate agents to incorporate the use of immunomodulators and monoclonal antibody based therapy. These therapies have dramatically reduced morbidity in many patients with IBD and improved quality of life, however they have also introduced a different range of risks including infection and malignancy. This study examines the hypothesis that

1. That the use of screening for latent infections and vaccination in inflammatory bowel disease is likely to be performed suboptimally by gastroenterologists in Australia.

2. That screening for latent infections and management of infusion reactions to infliximab are likely to be performed suboptimally in tertiary hospital centres.

3. That the use of a clinical decision support system can improve the rate of screening for latent infections prior to the use of anti-TNFα therapy, and as such improve quality and safety for this group of patients.

4. In addition, in patients on a stable dose of the thiopurine medications azathioprine or 6-mercaptopurine that the use of pharmacogenetic testing for the enzyme Thiopurine methyltransferase (TPMT) and the measurement of thiopurine metabolites can improve quality and safety in this group of patients.

Aims

1. To assess the use of vaccination and screening for latent infections in IBD using an electronic survey of gastroenterologists in Australia, distributed through a mailing list for the Gastroenterology Society of Australia.

2. To assess the use of infliximab in a tertiary hospital using a retrospective cohort study, examining use of screening for latent infections, the incidence of infusion reactions, and particular adverse events.

3. To examine the design and implementation of a novel clinical decision support system as a method of implementing guidelines to improve clinical governance in this group of patients.
4. To assess the use of genotyping for TPMT and measurement of thiopurine metabolites in a cohort of patients with IBD on a stable dose of medication.
LITERATURE SEARCH

INTRODUCTION

The mainstay of treatment of inflammatory bowel disease (IBD) has been medications that suppress the immune system. The medications used include corticosteroids, 5-aminosalicylate based agents, immunomodulators such as the thiopurines and methotrexate, and biological agents. The latter term refers to monoclonal antibody based therapies, and in the case of IBD this mainly refers to infliximab and adalimumab, which are antagonists of tumour necrosis factor alpha (TNFα).

Although immunomodulators and biological agents are efficacious in the treatment of IBD, they are not without risks. The risk of serious infections in patients treated with anti-TNFα agents such as infliximab or adalimumab is thought to be increased by a factor of between 1.4 and 2.0. This risk is substantially increased if a combination of two or more of corticosteroids, immunomodulators or anti-TNFα agents are used. Toruner et al (2008) examined 100 consecutive IBD patients with opportunistic infections, and found that the risk of opportunistic infections had an odds ratio (OR) of 2.9 (95% CI, 1.5 – 5.3) using any of these agents, and that using two or three of these drugs the OR increased to 14.5 (95% CI, 4.9 – 43). The risk was greatest in patients over 50 years old. Infections may be conventional bacterial or viral infections, or ‘opportunistic’ infections. The risk of developing tuberculosis, which can be regarded as an opportunistic infection, is five times higher during treatment with TNF inhibitors. Given the increased risk of infection in these patients, strategies to reduce this risk of infection are very important. Two main approaches are screening for ‘latent’ infections, and vaccination. Medications used to treat IBD may also have an increased risk of malignancy, including haematological malignancies such as lymphoma, solid organ malignancies, and skin cancers.

The TREAT study (2006) examined 6290 prospectively recruited patients with IBD to evaluate long-term safety with infliximab. This study initially suggested that the rate of morbidity and mortality in patients treated with infliximab was no higher than those patients treated with conventional immunomodulators over two years. Although the rate of serious infection was increased on univariate analysis, with multivariate analysis there was no significant increase in infections overall. A follow up letter noted the potential bias due to industry funding in the TREAT study, and also presented by
comparison a study performed by Bongartz et al (2006) showing an odds ratio of 2.0 for serious infections in patients with rheumatoid arthritis. A follow up report (2002) of patients in the TREAT registry after a mean of 5.2 years again did not report any overall increase in mortality compared to patients given conventional treatment, though it did confirm an increase in serious infections in patients treated with infliximab (hazard ratio 1.43). An increase in serious infections was particularly noted in patients with moderate to severe disease activity, who were also on narcotic analgesic treatment or prednisolone treatment. Use of narcotic analgesics may have been a surrogate for severe poorly controlled disease. An editorial by Hanauer (2012) regarding the TREAT registry made the point that concurrent use of thiopurines and infliximab appears to not increase the risk of infection if used concurrently without glucocorticoids, and this combination is likely to increase the effectiveness of infliximab use by decreasing immunogenicity, as noted in the SONIC study (2010). The TREAT registry has not shown any increase in malignancy or lymphoma with patients treated with infliximab, as opposed to thiopurines which are associated with an increased risk of lymphoma and non-melanoma skin cancer.

To improve quality and safety relating to the treatment of IBD, a broad range of strategies needs to be considered. ‘Clinical governance’ is a term originating in the National Health Service in the United Kingdom, and can be broadly defined as ‘a system for improving the standard of clinical practice’. It encompasses education, clinical audit, clinical effect, risk management, research and openness (see Figure 19). The Australian Council on Healthcare Standards (ACHS) defines clinical governance as ‘the system by which the governing body, managers and clinicians share responsibility and are held accountable for patient care, minimising risks to consumers, and for continuously monitoring and improving the quality of clinical care’. It is a broad mandate, and relates to both individuals and broader systems.

As part of clinical governance, adverse drug events (ADEs) are in particular focus, as many of these are preventable. In one survey of primary care practices in the United States, 25% of patients had experienced an ADE. 11% of these ADEs were regarded as preventable. Education by way of journal articles, lectures, and textbook chapters form a core part of clinical governance. However at the hospital or clinician level, individualised approaches may be more appropriate to improve quality and safety. It can be quite difficult to change or improve clinicians’ practice in real world situations in terms of improving quality and safety in inflammatory bowel disease. The development of guidelines to change clinical
practice may be effective in this situation. An audit of current clinical practice forms a core part of clinical governance when audit results are compared to ‘best practice guidelines’.

Specific to IBD, strategies to improve quality and safety in the treatment of IBD include vaccination of patients and screening for latent infections. Specific to thiopurines, pharmacogenetic or functional testing of the Thiopurine methyltransferase (TPMT) gene or enzyme, and measurement of thiopurine metabolites may be useful. Specific to the anti-TNFα agent infliximab, measures to reduce the risk of potentially serious infusion reactions can be employed. Furthermore, implementation of quality and safety measures has the potential to be facilitated using a computerised decision support system (CDSS), or through the use of an IBD clinical nurse specialist (CNS).

![Figure 19 The elements of clinical governance (from Starey, 2001)](image)

**TREATMENT OF IBD**

Truelove and Witt initially described the positive results of a controlled trial of cortisone for ulcerative colitis in 1955. The effects of corticosteroids on Crohn's disease was reported much later, in 1979 when the National Cooperative Crohn's Disease Study assessed the effects of azathioprine, salazopyrin and corticosteroids on both active and quiescent Crohn's disease. This study assessed 579 patients and the results suggested that
steroids were particularly effective in ileal Crohn’s disease, sulphasalazine was effective in colonic Crohn’s disease, and response to azathioprine was better than placebo but did not reach statistical significance. These medications did not affect the rate of relapse in patients with quiescent Crohn’s disease in this study.317

The role of azathioprine in Crohn’s disease was initially studied in a small double blind cross over study of 15 patients in 1971, which did not show efficacy.318 A subsequent 24 week double blind controlled trial of 24 patients however did show a positive response to azathioprine, as did a later controlled trial of 51 patients.319,320 In the latter trial one patient died of pancytopaenia presumed to be related to azathioprine. Bouhnik et al (1996) assessed the long term effects of azathioprine in 157 patients with Crohn’s disease who had been taking azathioprine or 6-mercaptopurine for over six months, and who had been in steroid-free clinical remission for over six months. In those who continued the medication the risk of relapse was lower at both one year and five years than those who stopped the medication. If the patient remained in remission for 4 years, the follow up rates of remission were similar whether the drug was continued further or not.321 A Cochrane review suggests efficacy of methotrexate (MTX) for induction of remission in Crohn’s disease, based mainly on a trial using subcutaneous 25mg weekly dosing.322 A study by Feagan et al showed improved maintenance of remission on MTX 15mg subcutaneously weekly, in a group of patients who had achieved induction of remission after a 4 to 6 month period of MTX 25mg subcutaneously weekly.323,324

The successful use of a human/mouse chimeric monoclonal antibody to tumour necrosis factor alpha (‘cA2’) in patients with rheumatoid arthritis stimulated a small study of 10 patients with Crohn’s disease refractory to conventional treatments in 1995. These patients were treated with a single infusion of cA2 in an open-label treatment protocol. The response to the treatment was dramatic; eight out of ten patients had normalisation of their Crohn’s disease activity index (CDAI) and healing of endoscopic ulceration by four weeks post treatment.325 In 1997 a multi-centre randomised controlled trial studied the response to a single infusion of cA2 in 108 patients with moderate to severe Crohn’s disease (defined as a CDAI between 220 and 400). Patients were randomised to placebo, a dose of 5 mg/kg, 10 mg/kg or 20 mg/kg of cA2. This showed significant improvement in both the rates of clinical remission (defined as a decrease in the CDAI by at least 70 points) and clinical remission (defined as a CDAI of under 150).326 cA2 was later termed ‘infliximab’. The effects of infliximab on healing perianal fistulas was later assessed in a
multi-centre randomised controlled trial of infliximab compared to placebo in 95 patients. The primary endpoint (reduction in the number of draining fistulas by at least 50% on two consecutive visits) was reached in 68% of patients receiving 5 mg/kg of infliximab, 56% of patients receiving 10 mg/kg of infliximab, and 26% of patients receiving placebo. Numerous rigorous studies have confirmed the efficacy of anti-TNFα treatment in Crohn’s disease and this is now established therapy.

There is evidence supporting the use of infliximab in acute severe ulcerative colitis (ASUC) refractory to intravenous hydrocortisone as a means of avoiding surgery. The use of infliximab is equivalent to the use of intravenous cyclosporine in this context. Despite these measures the rates of colectomy for ASUC remain high. Meta-analyses of studies assessing induction and maintenance therapy in patients with ulcerative colitis show efficacy overall, particularly in the large Active Colitis Trials (ACT) 1 and 2. Adalimumab, a subcutaneous fully humanised anti-TNFα agent has shown efficacy in Crohn’s disease for induction and maintenance of remission in the CLASSIC-1 and CLASSIC-2 trials. The CHARM study did not show any difference between weekly or fortnightly dosing for adalimumab. In ulcerative colitis, adalimumab did show an effect on clinical remission however the effect was more modest, possibly due to an increased rate of patients previously treated with alternative anti-TNFα agents. A recent study suggests that the 52 week efficacy of adalimumab was similar to infliximab in ulcerative colitis. Certolizumab pegol, a monoclonal antibody (Fab) to TNFα showed significant clinical response though not clinical remission during induction and maintenance treatment in patients with Crohn’s disease in the PRECISE1 study. The PRECISE2 study only randomised patients who had achieved clinical remission after induction treatment with certolizumab pegol; this study did show an improvement in clinical remission with maintenance therapy. The soluble TNF receptor, etanercept has not been shown to be efficacious in inflammatory bowel disease, possibly due to less inhibition of lipopolysaccharide-induced IL-1β production compared with other TNF inhibitors.

TNF-α has been found in the lamina propria of the mucosa in patients with ulcerative colitis and Crohn’s disease in multiple studies. TNF-α induces two major signaling cascades, the apoptotic (cell death) pathway and the inflammatory pathway. TNF-α is thought to play an important role in causing inflammation, which characterises conditions such as Crohn’s disease and rheumatoid arthritis. Infliximab causes apoptosis of T-lymphocytes in the lamina propria of the mucosal wall of the gastrointestinal tract.
Monoclonal antibody therapies targeting integrins (cell surface glycoproteins which mediate the adhesion, migration and activation of immune cells) have also shown efficacy in treatment of inflammatory bowel disease.\textsuperscript{353} The results of the initial induction and maintenance controlled studies of natalizumab, a humanised monoclonal IgG4 antibody against $\alpha$-4 integrin showed similar responses to placebo in the induction study, however higher rates of clinical response and remission in patients receiving active drug in the maintenance phase.\textsuperscript{326} This suggests a slower onset of action than the anti-TNF agents. This study was complicated by the death of a patient due to progressive multifocal leukoencephalopathy (PML), a brain disorder caused by the reactivation of the JC virus. The JC virus is a human polyomavirus often contracted in childhood, which usually remains dormant within the body. Antibodies against the JC virus are detectable in at least 80% of adults. It appears that immunosuppression due to natalizumab reactivates the JC virus contributing to these cases of PML.\textsuperscript{353}

More recently, vedolizumab which binds more specifically to gut specific integrin $\alpha$4-$\beta$7 has shown efficacy for ulcerative colitis (GEMINI 1) and Crohn’s disease (GEMINI 2).\textsuperscript{326,354} Cases of PML have not been reported with vedolizumab. The $\alpha$4-$\beta$7 integrin mediates infiltration of the gastrointestinal tract with memory T cells, binding to mucosal adressin cell adhesion molecule 1 on endothelial cells (MAdCAM-1).\textsuperscript{355} This compares to natalizumab it blocks the $\alpha$4 chain irrespective of the $\beta$ component. This blocks $\alpha$4-$\beta$1 which binds to vascular-cell adhesion molecule 1 (VCAM-1), which may mediate entry of the lymphocytes into the central nervous system.\textsuperscript{353} Ustekinumab is a human monoclonal antibody that binds to the p40 subunit, which is common to the interleukin-12 and 23 cytokines. A phase 2b induction and maintenance randomised controlled trial of ustekinumab suggested increased clinical response though not increased clinical remission in the induction phase, and increased clinical remission in the maintenance phase.\textsuperscript{356}
ADVERSE EVENTS DUE TO MEDICATIONS USED TO TREAT IBD

Development of tuberculosis after anti-TNFα use

Keane et al (2001) reported 70 cases of tuberculosis on a background of approximately 147,000 patients with Crohn’s disease or rheumatoid arthritis treated with infliximab, using data from the United States based Food and Drug Administration Adverse Events Reporting System (FDA AERS). These cases of tuberculosis were often extra-pulmonary or disseminated in nature. This was a voluntary reporting, and there may have been underreporting of cases. The risk of tuberculosis after infliximab was estimated to be four to five times higher than the background rate in patients with rheumatoid arthritis. Studies of patients with RA in the United States suggest a background rate of 6 cases per year per 100,000 patients. The majority of studies assessing the risks associated with the use of biological agents are in patients with inflammatory rheumatic diseases. Wolfe et al (2004) assessed two populations of patients with RA, one who had not been treated with anti-TNFα agents, and one cohort following infliximab treatment. They did not detect an increase in the rate of TB over the background population in the first group (6.2 per 100,000), however the rate was increased to 52.5 per 100,000 patient years of exposure after infliximab.

Tubach et al (2009) found the risk of developing TB was three times higher in a population of French patients with inflammatory arthritis or IBD treated with infliximab or adalimumab, compared to those treated with etanercept. A Canadian cohort study using a database of 112,300 patients with RA found a total of 386 cases of TB. This found the risk of developing TB for patients using biological agents was a relative risk of 1.5 (95% CI, 1.1–1.9), and for disease modifying anti-rheumatic drugs (DMARDS) a relative risk of 1.2 (95% CI, 1.0–1.5). Development of TB in Australia and New Zealand after the use of biological agents is rare, possibly related to low background prevalence of LTBI and uptake of screening for LTBI. The TREAT registry also did not report any cases of TB, though this was a closely monitored population and screening for LTBI was probably carefully performed.

Corticosteroids also increase the risk of TB, as seen in a study of patients treated for systemic lupus erythematosus in Hong Kong, and in a study of patients with rheumatic diseases treated with different doses of steroids. The risk of developing TB after the
use of immunomodulators is more contentious. Aberra et al (2007) examined patients with IBD prior to the biologicals era, and found an increased risk of TB with an odds ratio of 2.36 (95% CI: 1.17–4.74) compared with control subjects even when adjustments for confounders such as corticosteroids and smoking were taken into account. They suggested the use of immunomodulators was the likely cause of the increased risk.³⁶⁵ Hernandez-Cruz et al (1999) did not find an increased risk in RA patients treated with azathioprine or methotrexate.³⁶⁶ Westhovens et al (2006) examined patients with RA treated with methotrexate (MTX) alone, or MTX in combination with infliximab. They found that the rate of serious infections did not differ between the two groups, however there was an increased risk of infections in patients receiving a higher dose of infliximab (10 mg/kg) compared to 3 mg/kg dosing.³⁶⁷

Wallis et al (2004) report an increased risk of granulomatous infections in patients treated with infliximab or etanercept using the FDA AERS system. Granulomatous infections were reported in 239 per 100 000 patients treated with infliximab, and 74 per 100 000 patients treated with etanercept. These infections were mainly cases of tuberculosis, however cases of candidiasis, coccidioidomycosis, nocardiosis, histoplasmosis, and listeriosis were also reported.³⁶⁸ Winthrop and Siegel (2004) raised doubts about the accuracy of this study as the numerator included patients from all around the world, and the denominator included cases only from the USA.³⁶⁹ Winthrop et al (2008) surveyed members of the Infectious Diseases Society of America Emerging Infections Network (EIN). 426 responding members reported 1876 mycobacterial infections, including not only M. tuberculosis, but a number of other non-tuberculosis mycobacterial (NTM) infections in patients treated with biological agents, including M. avium complex, M. chelonae, M. marinum and M. abscessus.³⁷⁰

**Testing methods for detecting LTBI**

Screening for latent tuberculosis infection (LTBI) prior to the use of anti-TNFα therapy reduces the risk of developing TB by 80%; this appeared to be independent of the screening method.³⁷¹,³⁷² LTBI is usually acquired by inhaling respiratory droplets from an active case of TB. These droplets are inhaled into the alveolar spaces in the lungs where they are absorbed by the alveolar macrophages in particular.³⁷³ The mycobacteria are then encapsulated within granulomas in the body, and may dormant for many years or decades, and only reactivate with advanced age or immunosuppression. TNFα facilitates the formation of granulomas.³⁷⁴ One third of the world’s population is thought to be affected
The definition of latent tuberculosis is unfortunately inexact; it may include individuals who have been exposed to TB and have completely cleared the organism, and also include those who are incubating actively replicating mycobacteria, though are clinically asymptomatic. Imaging results from CT or FDG-PET of patients with LTBI may correspond to those found in patients with active TB. In 90% of patients the disease remains latent, however in others active disease develops, often within two years of acquiring the infection.

The development of TB after the use of anti-TNFα therapy in the majority of cases is due to reactivation of latent tuberculosis infection (LTBI) rather than primary infection. 72% of cases occurred within 90 days of treatment, consistent with this mode of reactivation. Screening for LTBI prior to the use of anti-TNFα therapy reduces the risk of developing TB by 80%; this appeared to be independent of the screening method. LTBI is usually acquired by inhaling respiratory droplets from an active case of TB. These droplets are inhaled into the alveolar spaces in the lungs where they are absorbed by the alveolar macrophages in particular. The mycobacteria are then encapsulated within granulomas in the body, and may be dormant for many years or decades, and only reactivate with advanced age or immunosuppression. TNFα facilitates the formation of granulomas.

One third of the world’s population is thought to be affected by latent tuberculosis infection (LTBI). The definition of latent tuberculosis is unfortunately inexact; it may include individuals who have been exposed to TB and have completely cleared the organism, and also include those who are incubating actively replicating mycobacteria, though are clinically asymptomatic. Imaging results from CT or FDG-PET of patients with LTBI may correspond to those found in patients with active TB. In 90% of patients the disease remains latent, however in others active disease develops, often within two years of acquiring the infection.

Testing for LTBI has conventionally been through the use of the tuberculin skin test (TST), also called the ‘Mantoux’ test. This involves intradermal injection of ‘purified protein derivative’ (PPD), containing over 200 antigens derived from heat-killed M. tuberculosis. A reading is then made 48 – 72 hours later, and a positive result depends upon the patient’s background. For example, the cut-off may be 5mm for immunosuppressed or HIV infected patients, or close contacts of smear positive TB, or 10mm for healthcare workers, individuals from endemic regions, or intravenous drug users. In low risk individuals or those with previous BCG vaccination, a cut-off of 15mm may be used. The positive result
is thought to represent immunological memory to *M. tuberculosis*, regarded as evidence of representing LTBI.\(^2\) This test has been used for over 100 years – and numerous studies demonstrate that a positive result correlates with the risk of developing active TB in the future, and that people with a negative result have a low risk. In addition, there is evidence that treatment of LTBI in patients with a positive TST results in a reduced risk of developing active TB in the future.\(^3\) The TST however may result in a false positive result due to environmental mycobacteria of past bacille Calmette-Guérin (BCG) immunisation, or false negative results due to immunosuppression or anergy.\(^4\) Repeated testing can lead to a larger result termed ‘boosting’. The presence of surrounding erythema may confound the interpretation of the degree of induration by less experienced operators.\(^5\) Mow et al (2004) suggest the presence of anergy in patients with Crohn’s disease, however this has been disputed as being no higher than the control population.\(^6\) There is also a subjective element to the reading of the TST, and the two test method leads to reduced patient adherence.

Interferon gamma release assays (IGRA) are blood tests based on the *in vitro* release of interferon-gamma (IFN-\(\gamma\)) specific to *M. tuberculosis* from T cells. Whole blood is collected in three tubes; one for testing for the patient, a positive mitogen control, and a negative control. The main tube is subjected to antigens specific to *M. tuberculosis*, including early-secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). The QFT-G also includes a third antigen, TB7.7.\(^7\) These antigens were identified using genomic sequencing of *M. tuberculosis*, and are more specific than the PPD; they are not found in BCG vaccination or environmental mycobacteria. Antigen presenting cells (APC) process the antigen and present them to T cells specific to these antigens. If they are present, they secrete IFN-\(\gamma\), which can be measured. There are two commercial assays available, the QuantiFERON-TB Gold In-Tube (QFT-GIT, Qiagen, Hilden, Germany) which uses a whole blood ELISA, and the T-SPOT.TB (Oxford Immunotec, Abingdon, UK) which uses an enzyme linked immunospot assay (ELISPOT).\(^8\) The QFT-GIT replaces a slightly older assay called the QuantiFERON-TB Gold (QFT-G). These tests have the advantage that only one visit is necessary, and there are no operator-dependent factors in interpretation of the results.\(^9\)

Dasgupta and Menzies (2005) note that the chest radiograph (CXR) is not a particularly sensitive test for detecting LTBI, only showing findings in 10-20% of patients with LTBI. Those patients with findings on CXR have a higher risk of reactivation however.\(^10\) The
clinical history also forms an important part of the assessment of the risk of LTBI. High risk subjects may be considered for treatment of LTBI, even if their screening tests are negative.\textsuperscript{358}

\textit{Studies comparing testing for LTBI with IGRA and TST}

Although evidence to support the use of interferon gamma release assay based testing instead of conventional guidelines based on Mantoux testing is not available specifically in populations of patients with inflammatory bowel disease, there is evidence in surrogate populations. The lack of a gold standard for LTBI makes validation of results more difficult.\textsuperscript{389} The only gold standard is the outcome of developing active TB in the future, which requires longitudinal cohort studies to study. In addition, treatment of LTBI would need to be withheld in these studies, which may have reached a point of ethical preclusion.\textsuperscript{390} Patients with culture proven active TB cannot be used as a gold standard, as many cases of active TB have negative testing on IGRA or TST, thus diminishing the sensitivity of the test for detection of LTBI.\textsuperscript{391}

There are a large number of studies comparing the performance of IGRA and the TST, however these studies suffer from significant heterogeneity in underlying BCG prevalence, background rate of LTBI in the community, disease state (e.g. IBD vs. rheumatic diseases), and degree of immunosuppression. In addition, the majority of studies comparing these two modalities have been retrospective rather than prospective.\textsuperscript{392} The background rate of LTBI in a particular population and the rate of acquiring LTBI \textit{de novo} play a pivotal role in determining pre-test probability. There is evidence to support the notion that IGRA have better specificity than the TST, as expected given the specificity of the antigens tested in IGRA. The TST may be affected by a broader range of antigens including environmental mycobacteria, and the BCG in particular. Given all this, in populations with a high background rate of BCG vaccination, the IGRA are likely to hold a significant advantage in terms of specificity.\textsuperscript{393}

Determination of which test holds better sensitivity is more difficult, given the lack of a gold standard for LTBI. Some studies with a low background rate of BCG suggest a higher rate of positive results with IGRA compared to the TST, however it is difficult to ascertain whether this is due to lower sensitivity of the TST or decreased specificity of IGRA (the latter seems less likely for reasons outlined above). The current treatment status of the patient is important as immunosuppressants and biological agents may reduce the rate of
positive results from both the TST and IGRAs, perhaps affecting the TST more than the IGRA.

Pai et al (2008) performed a meta-analysis of 38 articles to determine the sensitivity and specificity of IGRAs compared to the TST in immunocompetent individuals. The pooled sensitivity was 76% for all QuantiFERON studies, and 90% for the T-SPOT.TB. The pooled specificity of the QuantiFERON tests was 99% amongst participants who were not BCG vaccinated, and 96% amongst those who had undergone BCG vaccination. The pooled specificity of the T-SPOT.TB was 93%. Studies assessing the sensitivity and specificity of the TST were very variable, however it was found to have a pooled sensitivity of 77%. Specificity was 97% amongst non-BCG vaccinated individuals, and low and highly variable amongst BCG vaccinated individuals.394

An alternative approach is to test patients with both the TST and an IGRA. Surprisingly, in studies in which both are performed, there is little cross-over in positive results, even if the background population prevalence of BCG vaccination is low.395,396 Furthermore, in studies comparing two different IGRAs, positive results between the QFT-G and the T-SPOT.TB are also discordant.397,398 Winthrop et al (2012) suggest an approach where both the TST and an IGRA are performed in populations with high background risk, and use of an IGRA alone in immunocompetent patients in low risk populations.392 Ferrara et al (2006) studied 393 consecutively enrolled subjects in Italy who had suspected activation of latent TB using the ELISPOT, the QFT-G and the TST. They found the IGRA tests had a higher sensitivity than the TST, however there was discordance between the ELISPOT and the QFT-G results in some patients.399

Matulis et al (2008) studied 142 patients in Switzerland with inflammatory rheumatic conditions, 89% of whom were treated with immunosuppressive DMARDs. Risk factors for LTBI were used as the comparator. In the multivariate analysis, the IGRAs correlated much more strongly with the risk factors than the Mantoux test did (odds ratio of 29 vs. 4.8). The Mantoux test on the other hand correlated much better with BCG vaccination (odds ratio of 5.8 vs. 1.8). In the Swiss population, 83% of people have undergone BCG vaccination.400 Piana et al (2006) examined 138 immunosuppressed patients with haematological disorders who were exposed to a case of smear positive TB. Of those who returned with a positive Mantoux test, 21/24 patients had a positive T-SPOT.TB test; of the three negative tests, two had previous vaccination with the bacille Calmette-Guerin (BCG) that can cause a false positive Mantoux. Conversely, of those with a negative Mantoux test, only 57/91
patients had a negative T-SPOT.TB.\textsuperscript{401} Thus the IGRA picked up almost all positive Mantoux tests, and was positive in over one third of the negative Mantoux tests. The latter could have been false negative Mantoux tests in the context of immunosuppression. The alternative of false positive T-SPOT.TB results seems less likely as there were less positive T-SPOT.TB results than positive Mantoux results.

Kik et al (2010) compared the positive predictive value of the QFT-G, T-SPOT.TB, and the TST in 339 healthy individuals in the Netherlands exposed to cases of smear positive TB who subsequently had a TST of 5mm or over and were not treated for LTBI. Nine cases developed active TB. The positive predictive value of the QFT-G, T-SPOT.TB, and for a TST result either ≥ 10mm or 15mm were found to be similar (approximately 3 to 4% for each). In this study, the sensitivity of the TST using either a 10mm or 15mm cutoff was higher than the use of an IGRA, for predicting development of TB. These results may have been biased towards the TST by the fact that all patients included had to have a TST of 5mm or over.\textsuperscript{402} Diel et al (2008) studied 601 contacts of cases of smear positive TB, and found that the IGRA performs better than the TST for predicting the risk of developing active TB.\textsuperscript{403} Raval et al (2007) studied 130 patients developing TB after administration of infliximab from the FDA AERS between 2001 and 2006. Of these patients, 89 had concurrent immunosuppression, 33 had a history of latent or active TB, and 25 were born or had spent an extended time in an endemic area. In a subset of 67 patients who underwent a TST prior to treatment, 34 had a negative TST.\textsuperscript{404}

Patients with HIV can form a useful surrogate group to study the performance of these tests in an immunosuppressed population. Cattamanchi et al (2011) performed a meta-analysis and did not find an increase in sensitivity using IGRAs over the TST in patients with HIV.\textsuperscript{405} The ELISPOT test was found to have a higher sensitivity than the TST in a cohort of South African children, and higher specificity than the TST in a cohort of Zambian patients with HIV.\textsuperscript{406,407} Rangaka et al (2007) studied HIV infected patients in Africa known to have pulmonary TB, and a separate group with HIV without known TB. In this population, the IGRA appeared to have better sensitivity than the TST.

**Existing guidelines for screening for LTBI**

Existing guidelines for screening for LTBI are heterogeneous in their recommendations. The majority of patients with IBD planned for treatment with anti-TNFα agents are already on an immunomodulator, and often corticosteroids as well. Existing guidelines for
screening for latent tuberculosis infection in patients on immunosuppressive therapy are most relevant to this group of patients. The British Thoracic Society (BTS) guidelines suggest asking the patient about risk factors for LTBI and performing a chest radiograph initially (see Figure 20). A Mantoux test does not form part of the initial screening in these guidelines reflecting the view that “the high incidence of anergy in patients with Crohn’s disease who take immunosuppressants makes tuberculin skin testing unreliable and unnecessary”.\textsuperscript{408} The recommendations in the BTS guidelines are based on epidemiological data as to baseline risk of tuberculosis, and recommendations are based on risk in different ethnic groups.

A different approach is recommended by the Centers for Disease Control (CDC) based in the United States. They suggest screening initially with a Mantoux only, and performing a CXR only if the Mantoux is positive and treatment for LTBI is planned, to exclude active tuberculosis (updated in 2010).\textsuperscript{393} The CDC guidelines do contain the proviso that treatment of LTBI should be considered regardless of the Mantoux if sufficient risk factors for LTBI are present. More recently, both the BTS and the CDC have suggested an IGRA can be used in place of the Mantoux test in this context.

The American College of Rheumatology (2012) recommends screening all patients initially with either a TST or an IGRA. The latter is preferred if the patient has a history of BCG vaccination. A history for risk factors for LTBI is taken, and those with significant risk factors may have testing repeated if initial tests are negative. If the TST or IGRA is negative and there are no significant risk factors, then the patient can proceed with therapy. If testing is positive then a chest radiograph (CXR) is performed and if normal then the patient is treated for LTBI for at least one month prior to the commencement of biological therapy.\textsuperscript{409}
The use of anti-TNF therapy and the risk of drug induced hepatitis during chemoprophylaxis (see text and British Thoracic Society guidelines described in full by the British Thoracic Society algorithm). (For recommendations about the prevention of TB in the small minority of these, currently exceptional patients, other risk factors are identified for the small minority of patients with Crohn’s disease not taking concomitant immunosuppressive therapy, and in those with ulcerative colitis in whom infliximab is being considered, see British Thoracic Society (2005)).

Figure 20 British Thoracic Society algorithm for screening for LTBI for patients on immunomodulators prior to the use of anti-TNFα therapy (Rampton et al, 2005)

Figure 21 Guidelines from the Centers for Disease Control for screening for latent tuberculosis infection prior to the use of anti-TNFα agents
Treatment of LTBI

If a positive result is found on screening tests, treatment of LTBI is generally recommended. There are different regimens to treat LTBI, the most common being nine months of isoniazid (INH) 300mg monotherapy. Other regimens include rifampin monotherapy or rifampin and pyrazinamide; these regimens may have a shorter duration of therapy. **379** Large-scale studies from the United States Public Health Service suggest that the efficacy of INH treatment varies between 25 to 92%, with an average of 60%. The variation was put down to patient adherence. **379** Theis and Rhodes (2008) suggest that patients with LTBI treated with INH and who subsequently receive anti-TNFα therapy still have a 19% chance of developing active TB. **358**

Initial studies reporting the risk of hepatotoxicity due to INH were based on a heterogeneous population of patients (possibly including a proportion of alcoholics), in which minimal information about subjects were collected. These studies suggested a rate of hepatotoxicity between 5 and 20 per 1000 patients, though this may have included patients with only modest elevations of liver transaminases. **410** Previous guidelines suggested avoiding INH treatment in patients over 35 years of age due to a significantly increased risk of hepatotoxicity. A study of 11 141 patients in Seattle, Washington treated with INH monotherapy defined hepatotoxicity as symptoms of hepatitis, elevation in liver transaminase enzymes to over five times the upper limit of normal, and resolution upon cessation of the medication. Patients were monitored clinically, and routine liver function tests were not measured. They were tested only when patients became symptomatic. The study showed an incidence of 0.10% of hepatotoxicity in patients starting INH, and 0.15% in patients who completed the course of therapy. The rate of hepatotoxicity did increase with age, though overall these were significantly lower rates than previously reported. **411-413**

Screening for latent viral hepatitis

The European Crohn’s and Colitis Organisation (ECCO) guidelines recommend screening for hepatitis B (HBV) infection. This should include testing for not only hepatitis B surface antigen (HBsAg) but also core antibody (HBcAb) and surface antibody (HBsAb). Those patients negative for HBsAg though positive for HBcAb are termed ‘seronegative’ patients. Although they do not exhibit active viraemia or surface antigen positivity, these patients are thought to harbor the HBV virus within the liver. Reactivation of HBV in these
'seronegative' patients has been documented in patients treated with the anti-CD20 antibody rituximab as well as infliximab.\textsuperscript{414,415} As such, screening for HBV infection prior to the use of biological therapies is critically important to prevent reactivation.\textsuperscript{416-426} Testing for HBsAb is also useful in order to assess whether seroimmunity (for example due to vaccination or past infection) is present. Those who are seronegative to HBV should be considered for vaccination, particularly if they are increased risk for acquisition due to lifestyle or occupational factors.

**Other infections**

The ECCO guidelines (2008) also recommend ensuring that the primary care physician performs routine screening for cervical cancer. The human papilloma virus vaccination should be offered to young females in accordance to community recommendations. There are insufficient data to recommend an increased frequency of screening, which has been discussed in other guidelines. Other routine screening such as mammogram screening for breast cancer should be ensured in accordance with community recommendations. General preventative measures such as skin cancer prevention should also be adhered to, especially in those taking azathioprine which may increase the risk of skin cancers.\textsuperscript{311}

**Malignancy**

As mentioned previously, the TREAT registry suggests that the risk of solid malignancies and lymphoma is not increased with the use of anti-TNF\(\alpha\) agents. Haynes et al (2013) noted the risk of ten common solid malignancies was not increased using anti-TNF\(\alpha\) agents, as part of the SABER collaboration. This collaboration included patients with IBD, RA, and seronegative spondyloarthopathies such as psoriatic arthritis and ankylosing spondylitis.\textsuperscript{427} The thiopurine agents have been associated with an increased risk of lymphoma, and non-melanoma skin cancers (NMSC). The risk of lymphoma appears to relate to the duration of thiopurine use, and the risk increases with the age of the patient (though a subset of young male patients appear to at risk of an uncommon hepatosplenic T-cell lymphoma).\textsuperscript{311,312}
VACCINATION IN IBD

Specific vaccines used in IBD

Pneumococcal and influenza vaccination

Recommendations for influenza vaccination in the US are longstanding, and date back to 1960 when recommendations were made for vaccination of people with chronic diseases, the elderly, and pregnant women, however these recommendations were made without any substantial evidence of vaccine efficacy or effectiveness in these groups. The Advisory Committee on Immunization Practices (ACIP) has maintained that placebo controlled trials on these groups of patients thought to be high risk of influenza would not be ethical, and so there has not been trial based evidence to study efficacy in these groups.\textsuperscript{428} Vaccinations for influenza include trivalent inactivated vaccine (TIV) and a live attenuated influenza vaccine (LAIV). In 2010 the ACIP made formal recommendations for yearly TIV vaccination in all people aged over six months old, or LAIV for healthy non-pregnant people aged between 2 and 49 years old.\textsuperscript{428} Due to different influenza antigens in different seasons, vaccination on a yearly basis is important. A meta-analysis of 31 studies assessing the efficacy of influenza vaccination proven on RT-PCR or viral culture showed a moderate protective effect in some seasons, however evidence for protection in adults aged 65 or over or those with chronic illness was lacking.\textsuperscript{429}

The United States Centers for Disease Control (CDC) suggest that patients with chronic diseases such as rheumatoid arthritis who are thought to be at an increased risk of infection should undergo influenza vaccination on a yearly basis, and the 23-valent polysaccharide pneumococcal vaccine (‘Pneumovax’) as a single dose.\textsuperscript{430} These guidelines do not recommend a booster dose of the Pneumovax. Similar recommendations for influenza and pneumococcal vaccine are recommended for all people aged over 65 years old in the community in Australia.\textsuperscript{431}

The 23-valent pneumococcal vaccine (also known as ‘23vPPV’, or ‘Pneumovax23’) protects against 23 of the 91 known pneumococcal serotypes, primarily via a B cell dependent immune response via release of IgM.\textsuperscript{432} There are two major clinical sequelae of infection with \textit{Pneumococcus}, pneumococcal pneumonia and less commonly pneumococcal bacteraemia, also known as ‘invasive pneumococcal disease’ (‘IPD’). The existing understanding that Pneumococcal vaccination decreases the rate of both bacterial
pneumonia and IPD has been questioned by a meta-analysis in 2004, and a Cochrane meta-analysis in 2008,\textsuperscript{433,434} The Cochrane meta-analysis suggested that 23vPPV is useful to prevent IPD in adults, though not all-cause mortality or all cause-pneumonia. These findings and recommendations were less convincing for patients with chronic diseases, however this may be related to smaller numbers of patients studied in this group.

An alternative vaccine against pneumococcus is the conjugate 7-valent pneumococcal vaccine (‘PCV7’ which is a conjugate of polysaccharide and adjuvant proteins, which induces a more vigorous B and T cell response, though protects against fewer serotypes than the 23-valent vaccine.\textsuperscript{432,435} Both the PCV7 and the 23vPPV are on the recommended schedule of vaccination for children in Australia (see Table 23).

\textit{Varicella vaccination}

Primary varicella zoster virus (VZV) infection occurs primarily in childhood, though it can also occur in older patients who are serologically naïve to the infection. VZV may remain dormant in the nerve root ganglia in the spinal cord and brain after primary infection, and be reactivated later in life to cause herpes zoster (HZ) infection or ‘shingles’. This is characterised by often painful vesicles in a dermatomal distribution. HZ infection may lead to the syndrome of post-herpetic neuralgia (PHN), a painful condition characterised by lancinating pain. McDonald et al (2009) examined risk factors for varicella zoster infection amongst veterans with rheumatoid arthritis (RA). They found the risk factors to be severe comorbid medical conditions, older age, prednisolone use, and medications to treat RA. Amongst the patients treated with anti-TNFα agents, the risk of VZV was lower amongst those treated with etanercept and adalimumab. The incidence of HZ was 9.96 per 1000 patient years.\textsuperscript{436}

Winthrop (2010) reviewed previous studies related to the rate of HZ in patients treated with biological agents, most of which dealt with patients with inflammatory rheumatic conditions. They found that patients with RA appeared to have an increased risk of HZ over the background population, and those treated with steroids had a higher risk again. For patients treated with biological agents, some studies showed an increase in risk and some showed no increase in risk.\textsuperscript{380,437,438} Garcia-Doval et al (2010) reviewed the incidence of HZ amongst Spanish patients treated using anti-TNF therapy as part of the BIOBADASER database, and found an incidence of 6.5 per 1000 patient-years. The rate of hospitalisation was very infrequent, with a total incidence of only 32 per 100 000 patient-years. In
addition, the rate of disseminated HZ was low. The authors did not recommend vaccination for this group of patients.\textsuperscript{339}

There are two different live attenuated vaccines for varicella infection. ‘Varivax’ is designed for use in children who have not previously had chicken pox to avoid primary infection, whereas ‘Zostavax’ contains the same live attenuated virus at a much higher dose, which has shown efficacy in reducing shingles in older patients.\textsuperscript{440} There are no prospective studies to assess whether the Zostavax is effective in reducing HZ amongst patients treated with anti-TNF\textsubscript{α} agents. As it is a live vaccine, it should not be administered in patients with significant immunosuppression. Winthrop et al (2010) suggest vaccination should be deferred for at least a month in patients treated with at least 20mg of prednisolone for 2 weeks or more, or treated with biological agents. They suggest that the use of immunomodulators such as the thiopurines or methotrexate do not form a contraindication to varicella vaccination.\textsuperscript{380} Zhang et al (2012) questioned the increased risks with biologicals in a retrospective cohort study of 463 541 patients in Alabama, USA and found 633 patients given HZ vaccination and subsequently given a biological agent within the following 42 days. There were no cases of HZ within these patients, and after 42 days there was a lower risk of HZ overall.\textsuperscript{441} Zhang et al (2011) found similar findings in a cohort study of patients from the US based Aetna health insurance database.\textsuperscript{442}

**Efficacy of Vaccination during Immunomodulator or TNF Inhibitor Therapy**

Existing studies have suggested that titres post-vaccinations are reduced in this group of patients, however the clinical implications of these reduced titres are uncertain. It is possible that despite low titres or even absent titres there will be an adequate humoral response when exposed to the specific antigen at a later date, due to adequate T cell memory.\textsuperscript{289} This same concept also applies to the question of whether post vaccination titres should be measured, for example after vaccination for hepatitis B.\textsuperscript{443,444} Conversely, post-vaccination titres are often lower in immunosuppressed patients and post-pneumococcal vaccination titres are used by clinical immunologists to discriminate between normal and abnormal humoral immunity.\textsuperscript{435} For routine vaccination, there is limited data to support the measurement of post-vaccination titres routinely, though in some high-risk situations such as for health care workers who perform venipuncture or surgeons, ensuring adequate hepatitis B vaccination may be more important.\textsuperscript{435}
Existing guidelines for vaccination in IBD

Sands et al (2004) put forward recommendations regarding vaccination in patients with IBD, in part based on recommendations for other patients with chronic diseases such as rheumatoid arthritis. These recommendations include that all patients should have their routine vaccination history reviewed at diagnosis, and undergo ‘catch-up’ vaccination if appropriate. The possibility of reduced efficacy of vaccination in patients on immunomodulators or TNF inhibitor therapy was noted, and it was suggested that ‘adequate immune response should be ascertained’ by way of post vaccination titres. Specific vaccinations were not put forward in this article, rather that vaccination ‘should be in accordance’ with standard recommendations (see Figure 23).445

1. Standard recommended immunisation schedules for children and adults should be generally adhered to.

2. At diagnosis, children and adults should have complete review of immunisation history for completeness. All patients with incomplete series should commence catch-up vaccination.

3. Adults who cannot provide a clear history of chickenpox should have serological testing for varicella. Nonimmune individuals should receive varicella vaccine. Children who are not immune by vaccination or acquired immunity through infection should receive varicella vaccine.

4. Live bacterial or viral vaccines should be avoided in immune compromised children and adults with IBD. This includes:
   i. Treatment with glucocorticoids (≥ prednisolone 20mg/day equivalent, or 2mg/kg/day if less than 10kg, for 2 weeks or more, and within 3 months of stopping
   ii. Treatment with effective doses of 6-mercaptopurine or azathioprine (effect on safety not established) and within 3 months of stopping
   iii. Treatment with methotrexate (effect on safety not established) and within 3 months of stopping
   iv. Significant protein-calorie malnutrition.

5. Whenever possible, adequate immune response (as reflected by serological response) should be ascertained for individuals who have required immunisation while immunosuppressed. Repeat dosing may be considered when immune response to immunisation is insufficient.

Figure 22 Recommendations regarding Immunisation in Patients with Inflammatory Bowel Disease (Sands et al, 2004)

Melmed et al (2006) compared vaccination practice in IBD compared to existing guidelines using a self-administered questionnaire regarding vaccination in a group of patients with
This was based on existing recommendations for vaccination for influenza, pneumococcus and varicella from the Centers for Disease Control Morbidity and Mortality Weekly Report 'Prevention and Control of Influenza' (2004). This study showed that uptake of vaccination was very low, despite the presence of risk factors and indications for vaccination. The most commonly reported reasons were lack of awareness and concern regarding side effects of vaccination. This study had the limitation that questionnaire answers were self-reported and there was no verification of responses by the medical staff.

The European Crohn's and Colitis Association (ECCO) published guidelines for the prevention and diagnosis of opportunistic infections in patients with inflammatory bowel disease in 2008. A summary of the extensive recommendations is in Figure 23. These were consensus guidelines with involvement of representatives of different countries within the European Union. These guidelines suggest an initial detailed interview to assess for risk factors for different infections, clinical examination then a detailed laboratory panel for screening for infection. This broad initial screen included screening for HCV, HBV, HIV and CMV serology. More specifically, they recommend screening for *Strongyloides* in relevant patients and screening for latent tuberculosis with the tuberculin skin test and chest radiograph 'in accordance with each country’s specific guidelines’. These guidelines were significantly broader than those previously published, and in many cases represent expert opinion rather than a meta-analysis of the evidence.
Detailed interview
- History of travel and/or living in tropical areas or countries with endemic infections
- History of bacterial infections,
- History of fungal infections: oral and vaginal candidosis, intertrigo
- Risk of latent or active tuberculosis:
  - date of the last BCG vaccination
  - history of contact with tuberculosis patients
  - country of origin, prolonged stay in countries where tuberculosis is endemic
  - history of latent or active tuberculosis and treatment given
- History of VZV infection
- History of herpes simplex virus infection: frequency and severity of recurrences
- Immunisation status for tetanus, diphtheria and poliomyelitis, and date of vaccination (was the patient vaccinated against these diseases within the past 10 years?)
- Immunisation status for rubella, measles and mumps, and date of vaccination
- Immunisation status for hepatitis B and, in vaccinated patients, testing for the presence of hepatitis B antibodies
- Future plans to travel abroad to endemic areas (to consider whether the patient may need live virus vaccines such as the one for yellow fever)

Clinical examination
- Identification of systemic and/or local possibly active infections
- Evaluation of dental status
- Gynaecological visit and pap smear

Laboratory tests
- Neutrophil count
- Lymphocyte count and, in the case of lymphopenia, CD4 lymphocyte count
- C-reactive protein
- Urine analysis in patients with a history of urinary tract infection, and urinary symptoms
- VZV serology in patients without a clear history of varicella immunisation
- CMV serology
- HCV, HBV and HIV serology*
  - In patients with HCV or HBV chronic viral infection, alanine aminotransferase (ALT) assay, and determination of the stage of liver fibrosis and of necroinflammatory activity
  - In patients with HIV, CD4 cell count and viral load
- Eosinophil count, stool examination and strongyloides serology for patients having lived in a tropical area

Other procedures
- Tuberculin skin test (according to each country’s specific guidelines)
- Pulmonary chest x ray
*Patients with a history of hepatitis B vaccination should be tested for the presence of hepatitis B antibodies.
CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; VZV varicella zoster virus.

Figure 23 European Crohn’s and Colitis Organisation recommendations regarding screening and vaccination (Viget et al, 2008)

Existing guidelines for vaccination in the general population in Australia

There are no published guidelines for screening and vaccination for patients with inflammatory bowel disease specific to Australia. For the general population, the Department of Health and Aging have a vaccination schedule, reproduced in Table 23. Vaccination for hepatitis B has been added to the schedule for all children in Australia in recent years. In addition, the use of a vaccine against human papillomavirus has been shown to reduce the risk of cervical cancer and now forms part of the schedule.\footnote{449}
<table>
<thead>
<tr>
<th>Age</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>Hepatitis B (hep B)</td>
</tr>
</tbody>
</table>
| 2 months  | Hepatitis B (hep B)  
Diphtheria, tetanus and acellular pertussis (DTPa)  
*Haemophilus influenzae* type b (Hib)  
Inactivated poliomyelitis (IPV)  
Pneumococcal conjugate (7vPCV)  
Rotavirus |
| 4 months  | Hep B, DTPa, Hib, IPV, 7vPCV  
Rotavirus |
| 6 months  | Hep B, DTPa, Hib, IPV, 7vPCV  
Rotavirus |
| 12 months | Hep B, Hib, MMR  
Meningococcal C (MenCCV) |
| 12-24 months | Hepatitis A* |
| 18 months | Varicella (VZV) |
| 18-24 months | Pneumococcal polysaccharide (23vPPV)*  
Hepatitis A* |
| 4 years   | DTPa, IPV, MMR  
Inactivated poliomyelitis |
| 10-13 years | Hepatitis B (hep B)  
Varicella (VZV) |
| 12-13 years | Human Papilloma Virus (HPV) |
| 15-17 years | DTPa |
| 15-49 years | Influenza*, 23vPPV* |
| 50 yrs and over | Influenza*, 23vPPV* |
| 65 yrs and over | Influenza, 23vPPV |

* Aboriginal and Torres Strait Islander children in high risk areas only

Table 23 Immunisation Program Schedule for Australia (c/o Australian Government Dept. of Health & Aging)
INFLIXIMAB INFUSION REACTIONS

Infusion reactions are thought to occur in approximately 5-15% of patients treated with infliximab (closer to 6% in most studies), and severe reactions occur in approximately 1%.\textsuperscript{450-452} In the ACCENT-1 trial, infusion reactions occurred in 6% of patients on 5 mg/kg dosing, 4% of patients on 10 mg/kg dosing, and 3% of patients given a single 5 mg/kg dose then randomised to placebo.\textsuperscript{453} Hamzaoglu et al (2010) found infusion reactions in 6% of 1794 infusions assessed.\textsuperscript{454} Lees et al (2009) found a higher rate of infusion reactions at 13.4% amongst 620 patient-years of follow up on infliximab.\textsuperscript{455} The severity of infusion reactions can be characterised as mild, moderate or severe (see Table 24).\textsuperscript{456} The definition of a severe reaction has been variably reported in the literature, including those reactions requiring admission to hospital, requiring discontinuation of infliximab, and reactions judged life threatening.\textsuperscript{457,458} Conventionally, infusion reactions to infliximab are classified as acute or delayed, where reactions within 24 hours are classified as ‘acute’. Delayed reactions are thought to be mediated by a type III hypersensitivity reaction, similar to a serum sickness type reaction, though without the end organ damage sometimes associated with this syndrome. These delayed reactions may have characteristic features such as myalgias, rash, arthralgia and rash.\textsuperscript{456} Kugathasan et al (2002) note the development of delayed reactions to infliximab is associated with the second infusion being administered more than 20 weeks after the first infusion.\textsuperscript{459} The use of this episodic type approach is less common than a three dose induction dosing regimen followed by regular 8 weekly maintenance infusions.

A number of mechanisms for infusion reactions to infliximab have been postulated. One putative mechanism is the development of antibodies to the infliximab molecule. Originally called human antichimeric antibodies, these are more recently referred to as ‘antibodies to infliximab’ (ATIs). The development of ATIs have been correlated with the development of infusion reactions to infliximab, however there is not consistent data to support this.\textsuperscript{457,460} Potential strategies to reduce the rate of development of ATIs include scheduled maintenance therapy instead of episodic therapy, the use of concomitant immunomodulator therapy, and possibly the use of premedication with corticosteroids and antihistamines.\textsuperscript{456,461} A study of patients with inflammatory rheumatic conditions showed higher rates of infusion reactions in those patients treated with infliximab monotherapy without methotrexate coadministration.\textsuperscript{462}
Steenholdt et al (2011) examined patients after a severe infusion reaction and found that patients had increased circulating levels of IgG ATIs, however IgE antibodies were not detected. This suggests that the mechanism of severe infusion reactions may not be a true classical IgE mediated anaphylaxis. In that particular study, severe infusion reactions were noted in 8% of patients, however this was substantially higher in patients on episodic treatment compared to those on scheduled maintenance therapy (17% vs. 3%). The rate of infusion reactions is higher with the second infusion than with the first with episodic therapy.

The majority of infusion reactions to infliximab are thought to be related to activation of cells by Fc-IgG receptors, or activation of the complement system via immune complexes. Despite this non-IgE mediated mechanism, a reaction consistent with anaphylaxis can occur secondary to infliximab administration. The World Allergy Organisation defines anaphylaxis as 'a severe, life-threatening generalized or systemic hypersensitivity reaction. This might be sub-classified as allergic (e.g. mediated by IgE, IgG or immune complexes) or non-allergic. This definition is notable in that it clarifies that an IgE based mechanism is not necessary for the diagnosis of anaphylaxis. A symposium of the National Institute of Allergy and Infectious Disease and the Food Allergy and Anaphylaxis Network in 2006 attempted to create a consensus definition as to the clinical criteria for diagnosing anaphylaxis; their findings are summarised in Table 25. Note that only one of the three major criteria needs to be fulfilled for the diagnosis of anaphylaxis. They suggest that the term 'anaphylactoid' is outdated, and discouraged its use.

There is no direct evidence to support the use of premedication with hydrocortisone to reduce the risk of infusion reactions to infliximab. Farrell et al (2003) noted an association between patients given hydrocortisone premedication and reduced ATIs, and patients with lower levels of ATIs were more likely to be in clinical remission. A direct association between hydrocortisone and clinical remission was not seen. This study also noted lower immunogenicity in patients treated with a three-dose induction regimen instead of single-dose induction, and in those patients concurrently being treated with immunomodulators. Lee et al (2010) studied 2165 consecutive infusions and noted no difference in the rate of infusion reactions whether corticosteroids were given prior to the infusion or not, though did note a lower rate of reactions with concomitant immunomodulator use. This study also showed that one hour infusion regimens for
infliximab could be performed safely.\textsuperscript{467} A study of patients with rheumatoid arthritis showed no decrease in infusion reactions with betamethasone premedication.\textsuperscript{468}

**Table 24 Classification of infusion reactions (courtesy of Mayer et al)**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Flushing, dizziness, headache, diaphoresis, nausea or palpitations</td>
</tr>
<tr>
<td>Moderate</td>
<td>Chest discomfort, dyspnoea, hypotension or hypertension (&gt;20mmHg), increased temperature, palpitations or urticaria</td>
</tr>
<tr>
<td>Severe</td>
<td>Hypotension or hypertension, increased temperature with rigors, dyspnoea with wheezing, stridor, flushing</td>
</tr>
<tr>
<td>Delayed</td>
<td>Rash or urticaria, myalgias, flu-like symptoms, joint stiffness and pain, headache. Can occur between 1 and 14 days post infusion, but usually occurs after 5-7 days</td>
</tr>
</tbody>
</table>

**Existing guidelines for management of infusion reactions**

The approach to managing infusion reactions depends initially upon the severity of the reaction. Cheifetz et al (2003) suggested guidelines for management of infusion reactions (see Figure 24). Many of the reactions were thought to be rate related, and that these reactions could be alleviated by reducing the rate of subsequent infusion. Mild reactions are treated by slowing the rate initially, and moderate reactions are treated by either stopping the infusion or reducing the rate and giving a first generation antihistamine as well as paracetamol. In the case of severe reactions, the infusion reaction is stopped and supportive measures are given. In some cases, the infusion can be restarted depending upon the severity of the reaction.\textsuperscript{451} Han and Cohen (2004) describe a fairly similar approach.\textsuperscript{18}
Guidelines for managing infusion reactions at the infusion (Cheifetz et al, 2003)

Management of Anaphylaxis

The diagnosis of anaphylaxis is critically important as fatalities can occur. Anaphylaxis to infliximab is not ruled out by a negative skin patch testing, or by a negative serum tryptase level, as the sensitivity of these tests for anaphylaxis is low. Serum IgE levels to infliximab is experimental and not validated. It can be difficult to differentiate severe infusion reactions with anaphylaxis in some cases. Adrenaline should be administered via the intramuscular route if anaphylaxis is suspected as this has been shown to reduce mortality due to anaphylaxis. Anaphylaxis should be considered in all severe infusion reactions, and involvement of an immunologist or clinical allergy specialist is prudent in some cases. If desensitisation is to be attempted, it should be in a centre with appropriate expertise and supervision.
Table 25 Clinical criteria for diagnosing anaphylaxis (Sampson et al, 2006)

<table>
<thead>
<tr>
<th>Anaphylaxis is highly likely when any one of the following 3 criteria is fulfilled:</th>
</tr>
</thead>
</table>
| 1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula)  
   and at least one of the following:  
   a. Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)  
   b. Reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence) |
| 2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):  
   a. Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)  
   b. Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)  
   c. Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)  
   d. Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting) |
| 3. Reduced BP after exposure to known allergen for that patient (minutes to several hours):  
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person’s baseline PEF, Peak expiratory flow; BP, blood pressure. |
Adverse events due to thiopurine medications

The thiopurine medications azathioprine (AZA) and 6-mercaptopurine (6-MP) are widely used for the treatment of inflammatory bowel disease (IBD). Randomised controlled trial evidence supports the use of these thiopurine medications for maintenance of remission in Crohn's disease and ulcerative colitis.⁴⁷⁰,⁴⁷¹ Although effective medications, approximately 15 - 28% of patients will develop adverse events after thiopurine use, though this rate seems higher than that experienced in clinical practice and is likely to include minor symptoms.⁴⁷² Serious adverse events include myelosuppression, pancreatitis, and hepatotoxicity. Thiopurines are also associated with an increased risk of lymphoma and skin cancer.³¹²,⁴⁷³ Improving the quality and safety relating to thiopurine use involves optimising the efficacy of these drugs, whilst also minimising the risk of developing adverse events.

The metabolism of the thiopurine medications involves a complex pathway with multiple enzymes involved (see Figure 25).⁴⁷⁴,⁴⁷⁵ The pharmacologically active end metabolites are the 6-thioguanines (6-TGN) which are incorporated into DNA or RNA of lymphocytes as fraudulent bases, inhibiting their activity.⁴⁷⁶ In addition, 6-TGNs can inhibit Rac1, which can lead to apoptosis of T-cells.⁴⁷⁷ This inhibition of lymphocyte activity leads to decreased inflammation.⁴⁷⁸ The prodrug AZA is initially broken down into 6-MP, possibly via glutathione S-transferase. A conversion factor of 2.08 should be taken into account when considering equivalent doses (with AZA having the higher dose). 6-MP may subsequently be further metabolized by three competing enzymes, thiopurine S-methyl transferase (TPMT), xanthine oxidase (XO), or hypoxanthine phosphoribosyl transferase (HPRT).⁴⁷⁶

XO catabolises 6-MP into 6-thiouric acid (6-TU), a biologically inactive metabolite. The activity of XO can be inhibited by the use of allopurinol, an interaction that can be utilised for selected patients. HPRT converts 6-MP into 6-thioinosine-monophosphate (6-TIMP). 6-TIMP is phosphorylated into the metabolites 6-thioguanine monophosphate (6-TGMP), 6-thioguanine diphosphate (6-TGDP) and 6-thioguanine triphosphate (6-TGTP). These two metabolites are termed the ‘6-thioguanines’ (6-TGN), and are the pharmacologically active metabolites.
TPMT affects the pathway at multiple points including the metabolism of 6-mercaptopyrimidine into the by-product 6-methylmercaptopyrimidine (6MMP) as seen in Figure 25.\textsuperscript{479} 6-MMP is an inactive metabolite, however high levels have been associated with hepatotoxicity due to thiopurines.\textsuperscript{480} The gene encoding the TPMT enzyme is found on chromosome six.\textsuperscript{481} Patients with heterozygous mutations often require lower doses, as they are more prone to myelosuppression on standard doses. Patients with homozygous mutations almost universally develop myelosuppression, which may be severe enough to cause fatality. Testing of TPMT may help to prevent these potentially serious side effects. Testing of the TPMT gene can be performed using either pharmacogenetic testing for single nucleotide polymorphisms of the gene which identifies the majority of patients who have the “slow metabolizer” phenotype, or using a functional assessment of the \textit{TPMT} enzyme activity (also sometimes called ‘phenotyping’). Genotype is of course invariable over time within an individual, and only needs testing once.
Genotyping of the TPMT gene

At least 28 mutations of the TPMT gene have been identified. The most common mutations are the TPMT *2, *3A, *3B, and *3C polymorphisms. These mutations are found in 80 – 95% of patients with decreased TPMT activity. In Caucasians, the *1/*1 wild type genotype occurs in approximately 86 – 97% of the population. 3-14% of patients have a heterozygous mutation of TPMT, and the risk of a homozygous mutation is between 1 in 178 to 1 in 3736. It is commonly quoted that approximately 10% of the Caucasian population harbours a heterozygous mutation, and 1 in 300 patients have a homozygous
mutation of TPMT. There are significant differences in the abundance of genotypes between different races, for example in a study in 1999, variants of TPMT were found in 10.1% of Caucasians, 2.0% of South-west Asians, and 4.7% of Chinese patients. All mutants found in the Chinese patients were TPMT*3C. TPMT*3A was found more often in the Caucasian population. A study of 192 Japanese patients showed the TPMT*3C allele in 0.8% of patients, and TPMT*2, TPMT*3A and TPMT*3B were not found. Patients from Ghana by comparison had a 14.8% allele frequency for TPMT*3C, and TPMT*2, TPMT*3A and TPMT*3B were not detected.

Evidence for the most effective dose includes 2.0 – 2.5 mg/kg for AZA, and 1.0 – 1.5 mg/kg for 6-MP. An American Gastroenterological Association Institute Technical Review suggested 2.0 – 3.0 mg/kg dosing for AZA. Gardiner et al (2008) suggested 3.0 mg/kg AZA dosing in TPMT wild type homozygotes and 1.0 mg/kg dosing in heterozygotes, though starting at 2.0 and 0.5 mg/kg dosing respectively to reduce the risk of intolerance. Patients with a heterozygous mutation are more likely to have myelosuppression on standard doses of a thiopurine due to reduced TPMT activity causing increased levels of 6-thioguanine. These patients often require a lower dose of thiopurine to maintain remission, compared to the conventional 2.0-2.5 mg/kg dosing. Patients with a homozygous mutation are at very high risk of myelosuppression due to absent TPMT enzyme activity, and should generally not be treated with thiopurine medications, though there are case reports of very low dose use in these patients. Whilst the use of TPMT genotype testing for patients on thiopurine drugs is well established in some locations, it is still not widely used in Australia. TPMT testing is used variably in the United Kingdom. A survey carried out in a Melbourne teaching hospital showed that TMPT testing was only carried out in 6% of the cases prescribed thiopurine drugs across all departments in the hospital over a two month period.

**Measurement of TPMT enzyme activity (phenotyping)**

An alternative approach to TPMT genotyping is the measurement of TPMT enzyme activity, also called 'phenotyping'. The results of phenotyping are often categorised as high, medium or low metabolisers. Enzyme activity is most commonly measured using high-performance liquid chromatography (HPLC) followed by an enzymatic assay. Alternatively a radiochemical assay or tandem mass spectrometry can be used. These assays have the potential to be affected by recent blood transfusions, and possibly due to the presence of the thiopurine drug itself. TPMT activity is affected by the age of the red blood cell in a
study of children with acute lymphoblastic leukaemia.\textsuperscript{502} There may be significant variation in test results between different laboratories. Results may be recorded as per milliliter of packed red blood cells or per gram of haemoglobin. Loit et al (2011) note that the results between the two indices are not comparable, and that consistent cutoff values for low, intermediate or high metabolisers have not been established.\textsuperscript{503}

Variability of TPMT enzyme activity over time within the one individual was found to be low.\textsuperscript{504} Winter et al (2007) suggest that concordance between phenotype and genotype amongst intermediate metabolisers was poor in this study at only 65%. One patient in this study developed severe leukopaenia and was found to have a heterozygous genotype only. Measurement of TPMT enzyme activity may be preferable to genotyping, as the latter may miss some cases with low enzyme activity; genotyping for common mutations may only pick up 85% of common mutations as previously mentioned.\textsuperscript{505} However, the variability of activity measurements over time and subject to extraneous influences makes genotype the preferred measure for many clinicians.

\textit{Measurement of thiopurine metabolites}

A complementary approach to improving quality and safety is the measurement of blood levels of the thiopurine metabolites 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP) in red blood cells. Previous studies have suggested that patients with 6-TGN levels above 450 pmol/\(8 \times 10^8\) erythrocytes are more likely to develop myelosuppression, and that those with levels less than 235 pmol/\(8 \times 10^8\) erythrocytes are less likely to be in remission. This has resulted in a suggested ‘therapeutic range’ for 6-TGN of 235-450 pmol/\(8 \times 10^8\) erythrocytes; however, it should be noted that there is only a rough correlation between these parameters and toxicity, and they should be used as a guide rather than a strict therapeutic range.\textsuperscript{506} 6-methylmercaptopurine (6-MMP) levels above 5700 pmol/\(8 \times 10^8\) erythrocytes have been associated with abnormal liver function tests.\textsuperscript{480,507} Again, there is only a rough correlation with toxicity however it has resulted in a provisional toxicity threshold of \(> 5700 \text{ pmol/}\(8 \times 10^8\) erythrocytes\) for 6MMP. This may be particularly useful in patients who develop abnormal liver function tests on a thiopurine medication to confirm whether it is medication related; a reduction in dosage may lead to an improvement in liver function tests but may also reduce the therapeutic 6TGN level. Measurement of thiopurine metabolites may also identify thiopurine ‘shunters’ who benefit from co-administration of allopurinol (see below).\textsuperscript{508}
Haines et al (2011) measured thiopurine metabolites in 63 IBD patients taking thiopurines with ongoing symptoms, and found that 29% were underdosed, 11% were noncompliant, 52% were refractory to treatment (having therapeutic or elevated metabolite levels), and 10% were 6MMP ‘shunters’. When the clinical course was changed according to an algorithm guided by the metabolites, 87% had an improvement in clinical outcome. This study provides good evidence to support the use of thiopurine metabolites in patients with ongoing disease activity. An example of such an algorithm is provided in Table 26.

<table>
<thead>
<tr>
<th>6TGN</th>
<th>6MMP</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>Underdosing or non-adherence</td>
<td>Check adherence, increase dose</td>
</tr>
<tr>
<td>Low</td>
<td>Normal or high</td>
<td>Underdosed due to preferential 6MMP production</td>
<td>Consider adding allopurinol or switch therapy</td>
</tr>
<tr>
<td>Normal</td>
<td>Low, normal or high</td>
<td>Appropriate dose</td>
<td>Switch therapy</td>
</tr>
<tr>
<td>High</td>
<td>Low, normal or high</td>
<td>Dose too high</td>
<td>Switch therapy</td>
</tr>
</tbody>
</table>

**Thiopurine shunters**

Approximately 10% of patients are not able to reach therapeutic levels of 6-TGN despite dose escalation, due to development of elevated 6-MMP levels. These patients are termed preferential 6-MMP metabolisers or ‘shunters’ and are more likely to develop hepatotoxicity due to thiopurines. Sparrow et al (2007) used 100mg of allopurinol, combined with 25 – 50% of the previous dose of thiopurine in these patients. By inhibition of XO, allopurinol reversed this ‘shunting’ and reduced 6-MMP levels, and led to a lower disease activity overall. The mechanism of why the use of a XO inhibitor decreased the levels of 6-MMP is not entirely clear, and it does not appear that allopurinol achieves this by inhibiting TPMT. Fazal et al (2013) report on three patients with IBD who successfully underwent pregnancy whilst taking allopurinol. Due to the potential for adverse events from this combination, close attention with weekly blood tests initially has been advocated.
Other thiopurine relating tests

Polymorphisms of an alternative enzyme involved with thiopurine metabolism, *inosine triphosphate pyrophosphate (ITPA)* have been associated with increased risk of severe febrile neutropaenia in paediatric patients treated with 6-mercaptopurine for acute lymphoblastic leukaemia (though *TPMT* was noted to be a stronger predictor). However in a study in 2004 as well as a meta-analysis in 2007, polymorphisms of *IPTA* were not correlated with thiopurine toxicity in patients with inflammatory bowel disease.\(^{515-517}\)

**CLINICAL DECISION SUPPORT SYSTEMS (CDSS)**

**Introduction**

Bates et al (2003) noted a large gap between evidence based medicine and implementation of guidelines into clinical practice; adherence with published guidelines is often poor. Clinical decision support systems (CDSS) have been defined as ‘any electronic system designed to aid directly in clinical decision making, in which characteristics of individual patients are used to generate patient-specific assessments or recommendations that are then presented to clinicians for consideration’.\(^{518}\) As Kawamoto et al (2005) describe, these systems have been shown to ‘improve prescribing practices, reduce serious medication errors, enhance the delivery of preventative care services and improve adherence to recommended care standards’. They have also been shown to ‘be more effective and more likely to result in lasting improvements in clinical practice’ compared to other approaches'. Sims et al (2001) note that the use of CDSSs in clinical practice has strong potential to improve the uptake of evidence-based medicine.\(^{519}\)

Clinicians’ prescribing behaviour can be altered beneficially via the use of a CDSS.\(^{520}\) These systems can vary in complexity; for example, a simple example of a CDSS that can be effective is a computerised reminder to physicians based on a patient’s medication list.\(^{521}\) A more complex example of a CDSS that has gained popularity is the computerised physician order entry (CPOE) system, in which clinicians can prescribe medications electronically. CPOE includes electronic ordering of diagnostic tests including blood tests and radiology tests in addition to prescription of medications.\(^{522}\) Despite these advances, uptake of CDSS in clinical environments is low.\(^{523}\) In the United States, uptake of a CPOE
system is between 4.3% to 15% of hospitals. Barriers to implementation of a CPOE are significant, including a large up-front capital investment, support of key stakeholders including clinicians and pharmacists, and support from hospital administration.524

Studies assessing the use of a CDSS to improve quality and safety

There are few reports of the use of a CDSS tailored specifically for IBD. CDSS have been used successfully in other disciplines with varying levels of success, including the intensive care unit, diabetes management, and to manage cardiovascular risk factors.525-527 The use of CDSSs for antibiotic stewardship has been particularly productive.528-530 A common use of a CDSS is to improve the quality of medication prescribing. This includes drug interactions, and also can incorporate patient factors such as renal or hepatic function.531-534 CDSSs are often integrated with CPOE, allowing drug interactions between prescribed drugs to be detected. Several studies failed to show an improvement in outcomes when assessing the use of a CDSS in managing patients in primary care with chronic diseases such as hypertension, asthma, and angina.535-537 Cleveringa et al (2013) describe that the management of type 2 diabetes can be improved in primary care with the use of a CDSS if combined with performance feedback and case management.538

Several systematic reviews of the use of a CDSS have attempted to assess whether they improve clinical outcomes and patient outcomes. Roshanov (2011) conducted a systematic review of the use of CDSS on ordering of diagnostic tests and found that some interventions did modify the behaviour of clinicians, however it was difficult to predict which interventions were going to be effective and which were not.539 Hunt (1998) performed a review of 68 controlled trials regarding CDSSs and found that they can enhance clinical performance, particularly in the case of medication dosing and preventative care, however the use for diagnosis was lacking.540

Garg et al (2005) performed a systematic review of one hundred randomised and non-randomised studies assessing the use of a CDSS on practitioner performance and patient outcomes, in a large range of clinical situations. In 64% of these studies there were improvements in preventative care, improved diagnosis, drug dosing, drug prescribing, or disease management. 52 of these studies assessed clinical outcomes to a limited extent, and only 7 showed improved health outcomes. Many studies may have been underpowered to show this effect. The authors discuss the argument that the gold
standard for a CDSS should be multicentre randomised trials demonstrating improved outcomes with use of a particular CDSS, however this approach would be very difficult in terms of logistics and resources for the majority of CDSS projects and thus not feasible.\textsuperscript{541}
CHAPTER 3: VACCINATION AND SCREENING FOR INFECTIONS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: A SURVEY OF AUSTRALIAN GASTROENTEROLOGISTS

Publications arising from this section:


ABSTRACT

Patients with inflammatory bowel disease treated with corticosteroids, immunomodulators and tumour necrosis factor alpha (TNF-α) inhibitor monoclonal antibody therapy have an increased risk of infections, which may be conventional or opportunistic in nature. There is the potential to reduce the risk of conventional infections such as community-acquired pneumonia or influenza by means of vaccination. In addition, screening for latent tuberculosis infection or hepatitis B prior to the use of TNF-α inhibitor therapy has the potential to reduce potentially devastating flares of these infections. Guidelines have been produced to address vaccination and screening, however they are not presently consistent in their recommendations, and the evidence base to support these recommendations is variable.

This study examined the practice of gastroenterologists in Australia by means of a 13 question structured electronic questionnaire. This showed that the majority of clinicians screened patients for latent tuberculosis prior to the use of TNF-α inhibitors, though the methodology was heterogeneous. Hepatitis B was tested by more than half of respondents prior to TNF-α inhibitor use, and some respondents screened patients prior to corticosteroid or immunomodulator use. The majority screened with hepatitis B surface antigen, and many correctly screened with the core antibody as well to assess for ‘seronegative’ disease. Vaccination for hepatitis B, influenza and pneumococcus was performed infrequently and the timing of vaccination with relation to treatment was heterogeneous. Provisional recommendations are made regarding vaccination and screening for infections in patients with inflammatory bowel disease.
HYPOTHESIS

The treatment of inflammatory bowel disease has evolved over time from the use of corticosteroids and 5-aminosalicylate agents to incorporate the use of immunomodulators and monoclonal antibody based therapy. These therapies have dramatically reduced morbidity in many patients with IBD and improved their quality of life, however they have also introduced a different range of risks including infection and malignancy. This study aims to examine the hypothesis:

1. That the use of screening for latent infections and vaccination in inflammatory bowel disease is likely to be performed suboptimally by gastroenterologists in Australia.

Aims

To assess the use of vaccination and screening for latent infections in IBD using an electronic survey of gastroenterologists in Australia, distributed through a mailing list for the Gastroenterology Society of Australia.
METHODS

A structured questionnaire was designed using an electronic questionnaire website (http://www.esurveypro.com/). Respondents fill in the questionnaire online and the data is collected electronically for later assessment. There were fourteen questions relating to vaccination and screening for latent infection in IBD. There was a mix of different question types including simple ‘yes’ / ‘no’ answers, questions with multiple options, and some questions with a ‘matrix’ of answers relating to different subsections. The questionnaire was designed to be a descriptive analysis of this area and no sample size or power calculation was performed.

The survey was initially advertised via the hardcopy quarterly newsletter for the Gastroenterology Society of Australia (GESA). In addition, the survey was distributed to members of the email newsgroup Inflammatory Bowel Disease Australia (IBD-A), a special interest group of GESA. The IBD-A newsgroup is by its nature comprised of gastroenterologists with a particular interest in the management of inflammatory bowel disease. A total of 44 people replied to the survey.

RESULTS

The results for each question are presented below. For each question the number of respondents and the percentage answering each question were noted. Note that not all respondents filled in each subsection and the totals in the final column may be less than the total number of respondents. For some sections, respondents may be able to answer more than one section.
1. Have you managed patients with inflammatory bowel disease who are being treated with tumour necrosis factor alpha (TNF) inhibitors such as infliximab or adalimumab?

Almost universally, respondents had used TNF inhibitors, as expected.

2. Have you commenced patients with inflammatory bowel disease on TNF inhibitor treatment yourself?

The majority of clinicians had commenced TNF inhibitors themselves.

3. Do you routinely screen patients with inflammatory bowel disease for latent tuberculosis infection, including asking about risk factors? (you can select more than one answer)

Almost two thirds of respondents screened patients for latent TB specifically prior to use of TNF inhibitors. As respondents may have answered yes to more than one of the options, the total number of respondents who performed screening cumulatively cannot be accurately estimated.
4. Referring ONLY to the patients you screen for LTBI, what screening methodology do you use?

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Patients with risk factors for LTBI only</th>
<th>Never</th>
<th>Number of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>History for risk factors</td>
<td>0% (45)</td>
<td>4% (2)</td>
<td>0% (0)</td>
<td>41</td>
</tr>
<tr>
<td>Chest x-ray</td>
<td>80% (36)</td>
<td>14% (6)</td>
<td>4% (2)</td>
<td>41</td>
</tr>
<tr>
<td>Tuberculin Skin Test (Mantoux)</td>
<td>40% (11)</td>
<td>18% (5)</td>
<td>40% (11)</td>
<td>27</td>
</tr>
<tr>
<td>QuantIFERON-TB Gold blood test</td>
<td>60% (27)</td>
<td>17% (7)</td>
<td>12% (5)</td>
<td>30</td>
</tr>
<tr>
<td>Tspot.TB blood test</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>103% (23)</td>
<td>23</td>
</tr>
</tbody>
</table>

Number of Respondents: 44

Respondents who skipped this question: 0

This is a complex question as it stratified into ‘all patients’ and ‘patients with risk factors for latent tuberculosis infection only’. The vast majority asked all patients being screened about risk factors. The majority of clinicians performed a chest x-ray in all patients being screened, and a smaller number performed a chest x-ray only if risk factors were present. Less than half performed a Tuberculin skin test and 69% performed a QuantIFERON-TB Gold, suggesting that some clinicians may have been using both methods.

5. Do you routinely screen any patients with inflammatory bowel disease for hepatitis B, including asking about risk factors? (you can select more than one answer)

- Never: 17.19% (11)
- Yes, prior to corticosteroid use: 12.50% (8)
- Yes, prior to immunomodulator use: 18.76% (12)
- Yes, prior to TNF inhibitor use: 42.19% (27)
- Yes, all patients with inflammatory bowel disease: 9.38% (6)

In this question it should be noted that the screening methodology is not specified, and the screening may have consisted of asking about risk factors only. In general, there was a low rate of screening amongst patients, with the exception of approximately 40% of patients planned for TNF inhibitor therapy being screened.

6. Referring only to the patients for whom you do screen for hepatitis B (as mentioned in the previous question), what screening methodology do you use? Note – if you never screen for hepatitis B, please enter ‘never’ for each category.
For those patients being screened, 69% screened with hepatitis B surface antigen, 58% with surface antibody, and over half tested for hepatitis B core antibody. It should be noted that hepatitis B core antigen cannot be measured in the blood and was included to see if any respondents answered yes to this regardless! The majority of clinicians who screened for hepatitis B screened all patients rather than targeting patients with risk factors only.

7. Do you recommend any of the following vaccinations routinely for patients with IBD, and if so for which subgroups of patients? (you can select more than one answer)

About one third never recommended hepatitis B or influenza vaccination for any patient. Less than 20% recommended vaccination to patients on immunomodulators or TNF inhibitor therapy. Few recommended vaccination on corticosteroids. Some clinicians recommended vaccination for all patients with IBD regardless of therapy. About a quarter of clinicians recommended hepatitis B vaccination only if there were risk factors present.

8. If you offer a patient vaccination for hepatitis B, influenza or pneumococcus, WHEN would you be happy to vaccinate them? (You can select more than one answer)
This question dealt with the timing of vaccination. This was very heterogeneous, with the most common (30%) being prior to commencing an immunomodulator, steroids, or a TNF inhibitor.

9. Do you ask patients with inflammatory bowel disease about routine childhood vaccinations? (can choose more than one answer):

Only 18% of clinicians asked all patients about childhood vaccinations being up to date. A few clinicians asked patients prior to immunomodulator or TNF inhibitor therapy specifically.

10. Do you think vaccinations for patients with IBD are the responsibility of:

The majority felt vaccination was the responsibility of the general practitioner and gastroenterologist together. About 27% of respondents had not thought about whose responsibility vaccination is.

11. What proportion of your practice relates to treating patients with inflammatory bowel disease?

Clinicians had a variable amount of IBD work.
12. At what level of seniority in Gastroenterology are you practising?

Mainly consultant gastroenterologists, in particular over half had more than ten years’ experience.

13. Where does the majority of your work take place?

14. Thank you for participating in this survey. Please leave any comments or clarifications below.

(Qualitative answers)

Some sample answers are as follows: -

“These answers reflect my practice until now, and before the ECCO guidelines were released. From now on I will be adopting a practical approach to immunisation and intend to ask/vaccinate about “The Big 5” - HBV, HPV, VZV, Strep. Pneumoniae, Influenza- prior to commencement of immunomodulators/ biologicals. [...].”

“I would be interested to hear an evidence base for vaccination or not.”

“I am very concerned about HPV infection in women (and men I guess as well!) on biological therapy as I have had patients with very aggressive intraepithelial neoplasia in cervix and vaginal vault after treatment, who are in their twenties. Therefore I now ask about this vaccine!”

“In all patients I screen for the herpes viruses so that I can immunise for varicella prior to commencement of steroids/immunomodulators. I also like to know EBV status so that if negative they can be warned. Same for CMV and pregnancy. All my patients so far have been hep B immunised within 10 years so I hadn’t thought of screening in a low risk Australian borne pop”
DISCUSSION

These results are the first published description of the use of screening for latent infections and vaccination in patients with by gastroenterologists within Australia. The results suggest that clinicians who treat patients with TNF inhibitors are often performing screening for infections; however there is significant heterogeneity in the methodology used. Vaccination is performed less frequently, possibly reflecting the lack of clear guidelines in this area. It should be noted that this is a biased population with the majority of respondents having an interest in inflammatory bowel disease, with many of the respondents having over ten years’ experience as a gastroenterologist. The rates of screening and vaccination in the general gastroenterology community could conceivably be lower.

Screening for Latent Tuberculosis Infection (LTBI)

Approximately two thirds of respondents screened for latent tuberculosis infection just prior to the use of TNF inhibitors. If those respondents screening prior to the use of corticosteroids, immunomodulators and those who screened all patients were included, a total of 98.28% screened for latent tuberculosis infection. This is an excellent result as best practice recommendations suggest that all patients being commenced on TNF inhibitors should have screening for LTBI.\textsuperscript{371,543} Specifically, screening for LTBI prior to immunomodulator and corticosteroid use was less common at 13.79% and 6.90% respectively, reflecting the lack of evidence to support this practice at present. A small number of clinicians (12%) reported screening all patients for LTBI regardless of treatment. This approach is difficult to advocate, as it may create the situation of positive test results in patients who are not necessarily planned for TNF inhibitor treatment. There are few data to suggest an increased risk of reactivation of LTBI with immunomodulators such as azathioprine and methotrexate; however it may be that this area has not been adequately studied. Corticosteroids on the other hand are well known to have the ability to reactivate LTBI. Chan and Yosipovitch (2003) suggest that screening for LTBI should be performed prior to prolonged corticosteroid use, however the risk of LTBI is likely to be significantly higher in Singapore where the study was performed, than in Australia.\textsuperscript{544}

The particular methodology used to screen for LTBI was heterogeneous. Nearly all clinicians surveyed asked all these patients about risk factors for LTBI. The majority of
respondents (80%) screened all identified patients with a chest x-ray (CXR), and 15% performed a CXR only if risk factors were present. Use of the QuantiFERON-TB Gold (an interferon gamma release assay) was performed in 27/44 (69%) of patients, and the Tuberculin Skin Test was used in 11/44 patients (40%). Some patients may have been tested with both. Smaller percentages of respondents screened only patients with risk factors using these techniques. The T.Spot.TB was not used by anyone reflecting the lack of availability of this assay in Australia. It should be noted that the QuantiFERON-TB Gold is not available at all institutions in Australia, which may have limited use of this test.

Existing guidelines for screening for latent tuberculosis infection in patients on immunosuppressive therapy (the majority of patients prior to TNF inhibitor therapy) are heterogeneous in nature (see literature search). The author proposes provisional guidelines for screening for LTBI in an Australian population based on an IGRA (see Figure 26). These guidelines suggest asking all patients about risk factors for latent tuberculosis. An IGRA is performed in all patients, as well as a chest x-ray (unless in a low risk group, defined as Australian born and under 50 years old without risk factors for LTBI).545

**Summary of our recommended screening protocol**

1. History for tuberculosis risk factors* from all patients
2. Interferon-γ release assay: all patients
3. Chest x-ray: all patients, unless in low-risk group†

Any of

1. Chest x-ray changes
2. Prior personal history of TB (inadequately treated or incompletely documented)
3. Positive Interferon-γ release assay‡

YES

NO

Refer for treatment of LTBI +/- further investigation
No further screening§

LTBI = latent tuberculosis infection. * Tuberculosis (TB) risk factors are history of birth or residence in a TB-endemic area, history of contact with active tuberculosis, or prior personal history of tuberculosis. † Low-risk group = Australian-born, younger than 50 years, with no TB risk factors.
‡ Indeterminate results: consider repeat test in 2 weeks if not immunosuppressed, or discuss with infectious diseases specialist.
§ If TB risk factors, maintain heightened vigilance for signs and symptoms of TB infection during tumour necrosis factor inhibitor therapy. Consider performing a Mantoux test as well to increase sensitivity.

---

**Figure 26 Provisional guidelines for screening for latent tuberculosis infection in an Australian population based on Interferon Gamma Release Assay testing**
Screening for Hepatitis B

Respondents performed screening for hepatitis B less frequently. Approximately 40% of respondents screened for hepatitis B prior to TNF inhibitor use, and less than 20% screened prior to corticosteroid and immunomodulator use. In terms of screening methods, two thirds of respondents tested for hepatitis B surface antigen (HbsAg), and slightly less tested for surface antibody (HbsAb). Hepatitis B core antibody (HbcAb) was tested by over half the respondents, correctly reflecting the concerning ability of TNF inhibitors to reactivate hepatitis B surface antigen negative disease. This reactivation may similarly occur after the use of rituximab, a monoclonal antibody used for treatment of lymphoma and other conditions. This is thought to reflect an intrahepatic reservoir of hepadnavirus in patients who do not have circulating antigenaemia. Consensus recommendations suggest screening with HbsAg and HbcAb to identify those with past or previous infection, and HbsAb to identify those who may be serologically naïve and candidates for hepatitis B vaccination.

Although screening for hepatitis B prior to immunomodulator or corticosteroid use is not commonly performed, corticosteroids are well known to be potent agents to reactivate hepatitis B; a receptor on the hepatitis B virus is in fact activated by corticosteroids. Given the significant risks of inducing a flare of viral hepatitis, a reasonable approach is to screen for hepatitis B in all patients prior to commencement of corticosteroids and immunomodulators. If hepatitis B infection is present, this warrants a full assessment on its own merits, which may involve a hepatologist. This should include a quantitative hepatitis B viral load, and may include assessment of the likelihood of advanced fibrosis, which may consist of non-invasive imaging such as the Fibroscan, or even a liver biopsy in selected cases. Depending up the results, it may be appropriate to commence antiviral treatment for hepatitis B prior to the use of immunomodulators or TNF inhibitors; involvement of a hepatologist with an interest in viral hepatitis would be appropriate.

Vaccination in IBD

Vaccination practice amongst Gastroenterologists was quite variable (questions 7 to 10). Regarding hepatitis B vaccination, 18% recommended this prior to the use of TNF inhibitors, 10% prior to immunomodulators, 2% prior to steroids, and 14% to all patients with inflammatory bowel disease. 30% never recommended hepatitis B vaccination. This is likely to reflect the lack of consensus guidelines for hepatitis B vaccination in patients
with IBD. The percentages of physicians recommending influenza and pneumococcal vaccination were similar to that recommending hepatitis B vaccination. This is lower than the recommendations of the ECCO guidelines; however the evidence base to support these recommendations will be discussed further.

Question 8 asked about the timing of when clinicians would be happy to vaccinate patients if vaccination is recommended. 20% of respondents did not routinely vaccinate any patients at all. 12% offered vaccination whilst the patient is on corticosteroid therapy, 15% whilst on an immunomodulator, and 10% whilst on TNF inhibitor therapy. 30% were happy to vaccinate prior to commencing any of these treatments. These categories were not mutually exclusive, so some clinicians would have answered to more than one of these categories. This question largely relates to the safety and efficacy of vaccination during particular treatments. As previously mentioned, the efficacy of vaccination during immunomodulator or TNF inhibitor therapy may be reduced, however guidelines recommend vaccination regardless as they may offer at least partial efficacy. There is a large body of literature to support the safety of vaccination (apart from live attenuated vaccines which are contraindicated) during immune suppressive therapy. If possible, vaccination should be performed prior to immunosuppressive therapies, as it may be more efficacious. Nonetheless, some guidelines suggest that vaccination should be performed in this group of patients regardless of current therapy, as it may confer at least partial protection despite potentially reduced efficacy.443,444,547

The majority of clinicians surveyed did not ask patients about routine childhood vaccinations. Over half of respondents felt that the responsibility for vaccination was to be shared between the Gastroenterologist and the patient’s General Practitioner (GP), and only 6% each felt that it was either the Gastroenterologist’s or GP’s responsibility alone. Given that GPs may be hesitant to organise vaccinations for patients with IBD on immunosuppressive medications, a reasonable approach is for the gastroenterologist to give specific recommendations about vaccination for these patients, and to ask the GP to check whether their baseline vaccination status is up to date. Given that GPs are often better set up to administer and record vaccination, this also offers a good opportunity to participate in shared care of these sometimes complex patients.
Future directions

Given the low prevalence of LTBI in Australia, one approach to patients planned for an immunomodulator or corticosteroid use only is to risk stratify using epidemiological risk factors and then screen only those patients with risk factors. This targeted strategy may avoid unnecessary investigation in patients with a low pre-test probability for LTBI prior to immunomodulator use. However, this approach is not validated. In a different population with a higher incidence of LTBI such as in some countries in South East Asia, universal screening for LTBI prior to corticosteroid or immunosuppression use may be more appropriate.

This raises the important question of whether the background prevalence of LTBI in a population should alter the screening methodology. Australia has a low prevalence of LTBI overall (the exact prevalence has not been formally assessed); however in subgroups such as migrants from endemic areas, the prevalence of LTBI may be significantly higher. In addition, older patients born in Australia prior to World War II may be at increased risk given the increased prevalence of tuberculosis in Australia during this period. The changing demographics in Australia and the increasing rate of travel to endemic areas such as Asia and Africa means that the place of birth and travel history should be carefully scrutinised.

Recommendations

Existing guidelines provide recommendations regarding screening and vaccination, however there are different levels of evidence behind these recommendations. This is particularly evident in screening for LTBI, where there is significant heterogeneity behind the methodologies. Nonetheless, the statement that assessment of risk of LTBI should be performed for all patients prior to treatment with TNF inhibitors can be backed up with good evidence, though it does not specify the methodology. Guidelines based on the newer interferon gamma release assays may offer better sensitivity and specificity than existing Mantoux based testing, and are easier to perform and are more convenient for the patient. The author offers new provisional guidelines based on the IGRA; however, at this point, evidence to support their superiority over existing guidelines is lacking. Performing screening in general may be more important than the particular methodology. Whether to screen patients treated prior to prescription of corticosteroids or immunomodulators for LTBI is more contentious. The approach of screening patients with risk factors is
reasonable, though even this approach has not been validated. With respect to hepatitis B, a reasonable approach is to perform screening on all patients with inflammatory bowel disease using HBsAg and HBcAb. HBsAb can be also tested to identify a group with immunity, as previously mentioned. Given the ease of performing these tests, they can be performed soon after diagnosis.

In the Australian population of patients with inflammatory bowel disease on either immunosuppressive medications or TNF inhibitor therapy, a reasonable approach is to recommend influenza vaccine on a yearly basis, as well as a single 23-valent pneumococcal vaccination. Patients who are seronegative for hepatitis B should be offered a three dose course of hepatitis B vaccination. General practitioners should be asked to ensure that patients’ other routine vaccinations are up to date, with the proviso that live attenuated vaccinations (such as the measles / mumps / rubella vaccination) are contraindicated if immunosuppressed.

<table>
<thead>
<tr>
<th>Table 27 Summary of Screening Recommendations for Patients with IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatitis B</strong></td>
</tr>
<tr>
<td><strong>Latent Tuberculosis Infection</strong></td>
</tr>
<tr>
<td><strong>Strongyloides, Hepatitis C, HIV</strong></td>
</tr>
<tr>
<td><strong>Cervical Cancer</strong></td>
</tr>
<tr>
<td><strong>Other cancers</strong></td>
</tr>
</tbody>
</table>
Although not discussed in the questionnaire, other ‘latent’ infections should be considered depending upon risk factors. The ECCO guidelines (2008) suggest that all patients should be screened for hepatitis C and HIV. Given the relatively low incidence in Australia, an alternative approach would be to only test those with risk factors. Testing for *Strongyloides* in patients from endemic areas or frequent travelers to these areas (tropical and subtropical areas, West Africa, the Caribbean and South East Asia) is important as steroids and immunomodulators can reactivate this latent parasite infection, causing significant morbidity.

The ECCO guidelines (2008) also recommend ensuring that the primary care physician performs routine screening for cervical cancer. The human papilloma virus vaccination should be offered to young females in accordance to community recommendations (see Table 28). There are insufficient data to recommend an increased frequency of screening, which has been discussed in other guidelines. Other routine screening such as mammogram screening for breast cancer should be ensured in accordance with community recommendations. General preventative measures such as skin cancer prevention should also be adhered to, especially in those taking azathioprine which may increase the risk of skin cancers.311

Varicella zoster vaccination was not discussed in this survey; however, case reports of either primary varicella infection (‘chicken pox’) or herpes zoster (‘shingles’) have been recorded in patients treated with TNF inhibitor therapy. The mechanism of the two are different; primary infection is often acquired in childhood, whereas shingles represents reactivation of the latent virus which remains dormant in the nerve root ganglia in the spinal cord and brain after primary infection. This most commonly occurs in older patients. There are two different vaccines, both of which are live attenuated. ‘Varivax’ is designed for use in children who have not previously had chicken pox to avoid primary infection, whereas ‘Zostavax’ contains the same live attenuated virus at a much higher dose, which has shown efficacy in reducing shingles in older patients. Depending upon the age of the patient, administration of Varivax or Zostavax can be considered, however given that these infections are uncommon and the potential risk of a live attenuated virus if steroids are given soon after vaccination, it is difficult to justify offering this vaccination to all patients. The decision should be individualised.
<table>
<thead>
<tr>
<th>Vaccination Type</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>Three dose vaccination for all seronegative patients at diagnosis or before steroid or immunomodulator use</td>
</tr>
<tr>
<td>Influenza</td>
<td>Yearly vaccination for patients on corticosteroids, immunomodulators or biological agents (or preferably prior to treatment with these agents).</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>Single 23-valent pneumococcal vaccination for patients on corticosteroids, immunomodulators or biological agents (or preferably prior to treatment with these agents).</td>
</tr>
<tr>
<td>Varicella Zoster</td>
<td>Avoid vaccination in the immunosuppressed, as this is a live vaccine. Consider with caution only in patients who are not taking corticosteroids, immunomodulators or biological agents. Patients on these drugs must not be treated with these agents for two months. Patients considered for vaccination must be seronegative, without a history of chickenpox, and not have been previously vaccinated.</td>
</tr>
</tbody>
</table>
CHAPTER 4: AN AUDIT OF TUMOUR NECROSIS FACTOR ALPHA INHIBITORS USED FOR INFLAMMATORY BOWEL DISEASE IN A TERTIARY HOSPITAL

ABSTRACT

An audit was performed of all patients with inflammatory bowel disease being treated with infliximab between 2002 and 2010 at a tertiary metropolitan hospital was performed. 219 infusions were administered in 53 patients. About one third of patients had episodic treatment, and the rest had maintenance therapy. Screening for latent tuberculosis infection was performed in 75.4% of patients, though one significant adverse event of pulmonary tuberculosis was recorded in a patient who did not undergo screening. Screening for hepatitis B and hepatitis C was performed infrequently. Infusion reactions were common in this group, with an overall rate of 15.1%, higher than reported in the literature. Of the severe reactions, there was one case of laryngeal oedema, and one case of Stevens-Johnson syndrome. One unusual case of anaphylaxis complicated by later development of bilateral parotidomegaly was recorded. On the basis of these findings, new guidelines for screening for latent infection and management of infusion reactions were developed with the view to improving clinical governance relating to infliximab use.

Publications arising from this section:


HYPOTHESIS

The treatment of inflammatory bowel disease has evolved over time from the use of corticosteroids and 5-aminosalicylate agents to incorporate the use of immunomodulators and monoclonal antibody based therapy. These therapies have dramatically reduced morbidity in many patients with IBD and improved their quality of life, however they have also introduced a different range of risks including infection and malignancy. This study examines the hypothesis:

1. That screening for latent infections and management of infusion reactions to infliximab are likely to be performed suboptimally in tertiary hospital centres.

Aims

To assess the use of infliximab in a tertiary hospital will be assessed using a retrospective cohort study, examining use of screening for latent infections, the risk of infusion reactions, and particular adverse events.
METHODS

A comprehensive retrospective audit was performed of all patients being treated with infliximab from 2002 (when the first patient was treated) to 2008 by way of a chart review for each patient. Approval was obtained from the human research ethics committee (HREC) at Melbourne Health in Victoria, Australia. Patients included in the study were treated at The Royal Melbourne Hospital, a tertiary referral hospital in a metropolitan setting. A list of patients treated with infliximab was obtained from the pharmacy at The Royal Melbourne Hospital (courtesy of Mr. James Dwyer, Deputy Director of Pharmacy). Files were retrieved from medical records at The Royal Melbourne Hospital and data were entered into a custom Microsoft Access database.

Data were collected regarding the number of infusions, infusion reactions, screening for latent infections, and whether episodic or maintenance infliximab was given. In addition, data were recorded concerning the phenotype of patients, their concurrent medications, and other adverse events if available.

Data from the Microsoft Access database were exported into Microsoft Excel format for analysis. For descriptive statistics, histograms were created using the Microsoft Excel ‘data analysis’ package. Chi square analysis was performed using the Microsoft Excel CHITEST() function.

RESULTS

Patient details

A total of 219 infliximab infusions were recorded from 53 patients. The distribution of the ages of the patients is shown in Figure 27. Although the most common age groups were 21-30 and 31-40, there was a significant number of patients were aged between 41 and 70, reflecting the bimodal incidence of Crohn’s disease.
Disease characteristics for each patient were recorded using the Montreal Classification (see Figure 28). Disease location was evenly spread between ileal, ileocolonic and colonic disease (see Figure 28).
<table>
<thead>
<tr>
<th>Age Modifier</th>
<th>Disease Location</th>
<th>Disease Behaviour</th>
<th>Perianal Disease Modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;16 years old (A1)</td>
<td>Ileal (L1)</td>
<td>Inflammatory (non-stricturing and non-penetrating) (B1)</td>
<td>Presence of perianal disease (P)</td>
</tr>
<tr>
<td>17-40 years (A2)</td>
<td>Colonic (L2)</td>
<td>Stricturing (B2)</td>
<td></td>
</tr>
<tr>
<td>&gt; 40 years old (A3)</td>
<td>Ileocolonic (L3)</td>
<td>Penetrating (excluding perianal fistulae) (B3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isolated upper (L4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In terms of disease phenotype (see Figure 29), the majority of patients exhibited inflammatory phenotype (B1) or stricturing phenotype (B2). Only two patients were documented as having a penetrating phenotype (B2). This includes entero-enteric or entero-cutaneous fistulae, though excludes perianal fistulae. Upper GI phenotype was not documented in any patient. Note that phenotype information was not available for every patient. Six patients had perianal disease (p).

![Figure 29 Disease Behaviour (Montreal Classification)](image)
**Characteristics of Infusions**

A total of 231 infusions were given to patients. The mean number of infusions given was 5.133, with a standard deviation of 3.841 (see Figure 30). There were outliers, with two patients given nine infusions, one patient was given 10 infusions, and six patients were given over ten infusions in total. These infusions had funding from three main sources – the Drug and Therapeutics Committee (DTC) at The Royal Melbourne Hospital, via the Medicare funded Pharmaceuticals Benefit Scheme (PBS) after its approval for Crohn’s disease in 2008, and as part of an in-house study assessing MRI imaging and response to infliximab (‘MRI / infliximab’). Of the 219 total infusions, 108 were obtained via the DTC, 59 via the PBS, and 49 via the MRI / infliximab study.

![Figure 30 Total Number of Infliximab Infusions Per Patient](image)

Whether treatment was given as episodic or maintenance treatment was recorded in 26 out of 47 patients. Of these 26, maintenance therapy was given in 19 and episodic therapy was given in 7. Data regarding infliximab doses is seen in Figure 31. The mean dose was 333.8mg, with a standard deviation of 76.47mg. The most common dose was 300mg. The recommended dose for Crohn’s disease is 5 mg/kg per infusion. Unfortunately weight information was not recorded consistently, and as a consequence weight based dosing information is not available.
Figure 31 Infliximab Doses (in milligrams)

**Screening for Latent Tuberculosis Infection**

Amongst the total of 53 patients, screening for latent tuberculosis infection (LTBI) was documented in 40 patients (75.5%). The most common modalities of screening for LTBI were the QuantiFERON-TB Gold (QFT-G), an interferon gamma release assay and a chest radiograph. 29 patients had a QFT-G performed (see Figure 32). Only two patients out of 29 (6.9%) had a positive QFT-G result. 20 out of 29 were negative (69.0%), and 7 out of 29 were indeterminate (24.1%). Of the two patients with positive QFT-G results, both were treated for latent tuberculosis infection with isoniazid for nine months, in consultation with the infectious diseases service.
A chest radiograph was documented in 22 of the 53 patients. Out of these, 20 were reported as normal, and two patients had minor upper lobe scarring. These radiographs were reviewed with a radiologist, and advice from the Infectious Diseases department was sought. These patients both had a negative QFT-G and minimal risk factors for latent tuberculosis infection. Neither patient was recommended for treatment with Isoniazid.

There was one case of pulmonary tuberculosis infection after treatment with infliximab. This patient had a background of being born in Chile, an endemic area for tuberculosis. This patient was offered screening for tuberculosis as part of her workup for infliximab, which she unfortunately did not have performed. The patient was later commenced on infliximab as an inpatient where screening tests were not performed. After the third dose of infliximab, she developed fevers and a cough and a chest radiograph showed pulmonary infiltrates. She underwent a bronchoscopy, which was positive for acid-fast bacilli, consistent with the diagnosis of pulmonary tuberculosis. She was treated for tuberculosis (rifampicin, isoniazid, pyrazinamide and ethambutol), and the infliximab and methotrexate were stopped during treatment. She subsequently made a good recovery and was later commenced back on the methotrexate.
Screening for Latent Viral Hepatitis

Testing for Hepatitis B was documented in only six patients. In all six, the hepatitis B surface antigen (HBsAg) was negative. One patient was core antibody (HBcAb) positive. This patient did not have a reactivation of hepatitis B after infliximab therapy, but surprisingly had a reactivation of hepatitis B at a later date after treatment with azathioprine, requiring treatment with entecavir. Two patients were documented as serologically immune for hepatitis B. One patient was documented as serologically non-immune, and subsequently vaccinated for hepatitis B. Three patients were tested for hepatitis C. No patients had positive serology. Testing for the human immunodeficiency virus (HIV) was performed in only 1 of 47 patients; the result was negative.

Infusion reactions

Of the total of 53 patients, the presence or absence of infusion reactions was documented in 29 patients. Of these 29 patients, 4 experienced a moderate reaction and 3 experienced a severe reaction. This is an overall rate for infusion reactions of 27.6% amongst patients with documentation regarding the presence or absence of infusion reactions, or 15.1% of the total number of patients. This contrasts with previous suggestions of lower rates of infusion reactions (an infusion reaction rate of 5% has been previously reported). Mild infusion reactions were not documented, possibly consistent with milder reactions not generally being documented in the nursing notes during an infusion. Infusion reactions are classified according to Table 30. A table listing these reactions in detail is shown in Table 32.

Table 31 categorises these infusion reactions in those patients in whom whether infliximab infusions were given in an episodic or maintenance regimen was documented. A 3x2 Chi square analysis was performed (the column for ‘mild’ reactions was not included given both numbers were zero), giving a Chi square result of 1.555 with 2 degrees of freedom. The P-value for this analysis was 0.4596; this was not statistically significant given an alpha of 0.05. Thus no association between whether episodic or maintenance therapy was evident and the incidence of infusion reactions, though overall numbers were small.
Table 30 Classification of infusion reactions (courtesy of Mayer et al)\textsuperscript{456}

<table>
<thead>
<tr>
<th>Mild</th>
<th>Flushing, dizziness, headache, diaphoresis, nausea or palpitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>Chest discomfort, dyspnoea, hypotension or hypertension (&gt;20mmHg), increased temperature, palpitations or urticaria</td>
</tr>
<tr>
<td>Severe</td>
<td>Hypotension or hypertension, increased temperature with rigors, dyspnoea with wheezing, stridor, flushing</td>
</tr>
<tr>
<td>Delayed</td>
<td>Rash or urticaria, myalgias, flu-like symptoms, joint stiffness and pain, headache. Can occur between 1 and 14 days post infusion, but usually occurs after 5-7 days</td>
</tr>
</tbody>
</table>

Table 31 Infusion reactions in episodic vs. maintenance therapy

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Mild</th>
<th>Mod</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Episodic</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Maintenance</td>
<td>16</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Reaction Severity</td>
<td>Pre-medication</td>
<td>Episodic or Maintenance Therapy</td>
<td>Nature of Reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>-------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Hydrocortisone 100mg IV, Phenergan 12.5mg IV.</td>
<td>Maintenance</td>
<td>Development of urticaria 24-48 hours post each dose. Also developed allergy to mice manifested as urticaria when in proximity to mice in the workplace. Hives reduced with premedication with phenergan.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Hydrocortisone 100mg IV</td>
<td>Maintenance</td>
<td>Chest discomfort, dyspnoea each infusion which resolves with stopping infusion. Loss of efficacy, successfully changed to adalimumab.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Hydrocortisone 100mg IV, Phenergan 50mg IV</td>
<td>Episodic</td>
<td>Chest discomfort, rash, mild dyspnoea. Retrialed slower with premedication successfully. Later bridged to maintenance infliximab on PBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Hydrocortisone 100mg IV, Phenergan 50mg IV</td>
<td>Episodic</td>
<td>Dyspnoea, flushing, pallor. Infliximab ceased. Surgery then successfully changed to adalimumab.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>Patient refused premedication</td>
<td>Episodic</td>
<td>Developed laryngeal oedema at second infusion. Infliximab ceased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>Hydrocortisone 100mg IV, Phenergan 12.5mg IV</td>
<td>Induction regimen (previous 3 doses 5 years earlier)</td>
<td>Back pain, dyspnoea, pruritus, flushing. No haemodynamic instability. Given paracetamol. Symptoms worsened. Treated with hydrocortisone and phenergan; not given adrenaline. Subsequently developed bilateral parotidomegaly (see further details)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 32 Infusion Reactions to Infliximab**

The use of premedication prior to administration of infliximab was variable. The most common regimen used was a combination of intravenous hydrocortisone (most commonly 100mg), and oral cetirizine, a second-generation non-sedating antihistamine. Promethazine, a first generation antihistamine with sedative properties was used less commonly (see Figure 33). The occurrence of infusion reactions depending upon which premedication was given is presented in Table 33. This suggests the most infusion reactions in patients treated with promethazine, less in those treated with hydrocortisone, and none in patients treated with cetirizine. Chi square analysis was performed on this 3x3 contingency table, giving a Chi-square of 13.34 with four degrees of freedom, and a p-value of 0.0097. This was statistically significant given an alpha of 0.05.
The last case was unusual in that the patient developed signs and symptoms consistent with anaphylaxis after an infliximab infusion, and later developed bilateral parotidomegaly. This had not previously been documented in the literature, and was subsequently published as a case report in *Inflammatory Bowel Diseases* journal.548 This case is presented in more detail as follows:

A 56 year old man had a background of longstanding ileocolonic Crohn’s disease, with an ileostomy due to past colectomy. He presented with a flare of his ileal disease, proven on imaging. Three doses of infliximab had previously been administered five years prior. He was treated with 5mg/kg infliximab with good result; intravenous hydrocortisone 100mg and promethazine 12.5mg were given as premedication. During the second dose of infliximab given two weeks later he developed acute lower back pain, and was given oral paracetamol. Within minutes he developed a sensation of breathlessness, throat tightness and flushing with pruritus. Wheeze was noted on auscultation. Blood pressure, heart rate, respiratory rate and oxygen saturations were normal.
There was no stridor, urticaria, or tongue or facial angioedema. The infusion was stopped, and he was treated with further intravenous hydrocortisone and promethazine. He was not given adrenaline, and a serum tryptase was not requested. The patient’s symptoms improved and he was discharged home several hours later.

Later that day the patient noticed bilateral swelling in the parotid areas. There were no associated sicca symptoms, fever or pain. A computed tomography scan of the neck and thorax confirmed parotid swelling and ruled out sialectasis or lymphadenopathy. Mumps serology was consistent with past infection. Anti nuclear antibodies and antibodies to extractable nuclear antigens were negative. An immunologist reviewed the patient who considered the initial infusion reaction to be consistent with anaphylaxis. Paracetamol allergy was ruled out by a graded oral challenge. Skin prick testing to neat infliximab was negative. The patient was changed to subcutaneous adalimumab, which was well tolerated and resulted in clinical remission. The parotid swellings resolved spontaneously over a period of six weeks.
DISCUSSION / ANALYSIS

Patient characteristics

219 infusions given to 53 patients were documented. The ages of patients were fairly evenly distributed. The majority of patients had either inflammatory or stricturing phenotype; only two patients had a penetrating disease phenotype. The large number of patients with stricturing disease treated was surprising, given initial concerns that stricturing disease was a contraindication to infliximab. This related to concerns that infliximab may cause bowel obstruction in these patients. These concerns appear to have not borne out over time, and many patients with stricturing disease can be treated safely with infliximab, though a small risk of bowel obstruction remains. This may be more common in patients with fibrotic rather than inflammatory strictures. Given that the alternative is usually surgery in cases such as this, the use of TNF inhibitors in this situation is not unreasonable. There were no cases of obstruction noted in this audit.

Five patients with perianal disease were treated in this cohort. Since this audit, perianal Crohn’s disease has been added as a separate indication for infliximab under section 100 of the Pharmaceutical Benefits Scheme in Australia. Infliximab has been shown to be efficacious for perianal Crohn’s disease in the ACCENT II study. A local review suggested significant unmet demand for TNF inhibitor treatment for perianal Crohn’s disease in Australia prior to the PBS listing for this indication.

Screening for Latent Tuberculosis Infection

Screening for latent tuberculosis infection was performed moderately well in this cohort of patients; however one serious adverse event with a case of pulmonary tuberculosis did occur. Existing guidelines suggest that all patients should be screened for latent tuberculosis infection prior to infliximab therapy. Infliximab is estimated to increase the risk of developing tuberculosis by a factor of five. In this study, most patients who underwent screening were tested with a QuantiFERON-TB Gold and a chest radiograph. The QuantiFERON-TB gold result was performed in 29 out of 53 patients. Of these patients, the QFT-G was positive in two patients, who were subsequently treated with nine months of isoniazid in parallel to treatment with infliximab. There were a large number of indeterminate results (7/29 or 24%) which is substantially higher than previously
reported in the literature, which varies between 2 and 15%.\textsuperscript{553,554} This may have related to the large number of patients being treated with immunomodulators in this study.

The case of the patient developing pulmonary tuberculosis illustrates the significant risks of not performing screening for latent tuberculosis infection prior to treatment. The recognition of an increased rate of tuberculosis with infliximab therapy was reported in 2001 when the United States based Food and Drug Administration reported 70 cases of tuberculosis on a background of approximately 147 000 patients with Crohn’s disease or rheumatoid arthritis treated with infliximab.\textsuperscript{357} This was a voluntary reporting program, and there may have been underreporting of cases. The risk of tuberculosis after infliximab was estimated to be four to five times higher than the background rate in patients with rheumatoid arthritis. Comparable incidence data was not available for patients with inflammatory bowel disease though were assumed to be similar. Tuberculosis after infliximab is often extra-pulmonary or disseminated in nature and can certainly be fatal in some cases.

Screening for latent tuberculosis infection prior to the use of TNF inhibitor therapy reduces the risk by 80%; this appeared to be independent of the screening method.\textsuperscript{371} As discussed in section three, existing guidelines for screening for latent tuberculosis infection are heterogeneous in their recommendations. The British Thoracic Society guidelines are based on epidemiological data as to baseline risk of tuberculosis, and recommendations are based on risk in different ethnic groups. The Mantoux does not play a significant role in screening these patients. Guidelines from the United States based Centers for Disease Control (CDC) on the other hand are primarily based on the Mantoux test. More recently, both the BTS and the CDC have suggested an IGRA can be used in place of the Mantoux test in this context. Provisional guidelines for screening for latent tuberculosis infection in Australia based on an the QuantiFERON-TB Gold IGRA were written by the author as discussed in section three of this thesis.\textsuperscript{545}

**Screening for latent viral hepatitis**

Screening for hepatitis B and hepatitis C was performed infrequently in this group of patients. Of the six patients who were screened for hepatitis B, two were found to be serologically immune, and one was found to be serologically naive and vaccinated. Notably, one patient was found to be hepatitis B core antibody positive, though surface antigen negative. Reactivation of hepatitis B in these ‘seronegative’ patients has been
documented in patients treated with the anti-CD20 antibody rituximab as well as due to infliximab.\textsuperscript{414,415} In this case, it was unusual in that the reactivation did not occur after infliximab, it occurred later after treatment with azathioprine, which has not been associated with reactivating seronegative hepatitis B.

**Infusion Reactions**

In this series, seven patients out of fifty-three patients in total had documented infusion reactions to infliximab (13.2%). Four patients developed moderate infusion reactions (7.5%), most commonly characterised by chest discomfort and dyspnoea in this study. In one case of a moderate infusion reaction, the patient developed urticaria after administration with infliximab. This was notable as it was accompanied by the development of urticaria when mice were nearby. This may well have represented cross-reactivity with the murine component of infliximab, a chimeric mouse-human antibody.

There were three cases of severe infusion reaction to infliximab (5.6%). One case developed a rash which was biopsy proven to be consistent with Stevens-Johnson syndrome, a condition characterised by fever, sore throat and fatigue, which can progress to severe ulceration of the mucous membranes. In one case (described earlier in more detail), the reaction was a severe reaction thought to be consistent with anaphylaxis, and the patient later developed the unusual complication of bilateral parotidomegaly. In this case, the bilateral parotidomegaly may have represented an unusual reaction to infliximab, or alternatively may have represented an intercurrent viral illness that lowered the patient’s threshold to developing anaphylaxis to infliximab, a phenomenon known as ‘summation anaphylaxis’. A third case developed laryngeal oedema at the second infusion, and infliximab was subsequently ceased. This is consistent with studies suggesting reactions are more common at the second infusion than the first, presumably due to sensitisation to the antigen.\textsuperscript{386}

In this audit the incidence of infusion reactions (13.2%) appeared higher most studies, which report an incidence of closer to 6%. The high prevalence of patients treated with infliximab in an episodic manner rather than as maintenance therapy may have been the reason for the high prevalence of infusion reactions, despite the lack of a statistically significant association. The high use of episodic treatment predated the listing of infliximab on the pharmaceutical benefits scheme for Crohn’s disease. At the time this audit was performed, the majority of infusions were given prior to approval by the
Pharmaceutical Benefits Scheme in Australia, and as a consequence were given as episodic rather than maintenance therapy. This is likely to relate to financial reasons, and in addition the increased immunogenicity with episodic treatment was not recognised at the time as being clinically significant.\textsuperscript{457}

**Premedication use with infliximab**

The rate of infusion reactions with the use of different premedication agents with was statistically significant ($p$-value of 0.01) when the 3x3 contingency table in Table 33 underwent Chi square analysis. For cetirizine, there were no infusion reactions in the 18 patients given this premedication. For hydrocortisone, 5 out of 24 (20.8\%) had a moderate or severe reaction, and for patients given promethazine, 4 out of 6 (66.7\%) had a moderate or severe reaction. For the use of promethazine the very high rates of infusion reactions may have been confounded if promethazine was given more frequently to patients with previous mild or moderate infusion reactions. Alternatively, the possibility remains that the use of this first generation antihistamine could be the cause of symptoms or signs which could be interpreted as infusion reactions.

Baert et al (2003) noted that fewer infusion reactions in patients treated with a three-dose induction regimen instead of single dose induction, and in those patients concurrently being treated with immunomodulators. This study also found that those patients with an ATI level of 8 μg per millilitre or greater before an infusion were more likely to have a shorter duration of response.\textsuperscript{555}

**Development of new Guidelines for the Management of Infusion Reactions**

A new protocol for managing infusion reactions to infliximab at The Royal Melbourne Hospital was developed as an outcome of this audit (see Table 34). Prior to this audit being performed, all infusion reactions led to the infusion being initially stopped. However a review of the literature suggested that for minor reactions it might be more appropriate to slow down the infusion rather than ceasing it altogether. Symptoms can be treated as required with paracetamol for example. This approach to slowing the infusion rather than stopping it may have the benefit that antibodies to the infliximab are less likely to be produced. Infusions reactions that are assessed as moderate in severity are stopped for 20 minutes and the patient is reviewed by medical staff. It is reasonable to treat with a single dose each of cetirizine and intravenous hydrocortisone. The infusion is then restarted as a ‘slow protocol’. For reactions assessed as severe, treatment is analogous to that of acute
anaphylaxis. Although this approach may involve patients being over treated, it has the advantage that true cases of anaphylaxis are treated adequately with intramuscular adrenaline which has been shown to improve the outcomes of anaphylaxis.\textsuperscript{469}

Cheifetz et al (2003) suggest that patients with mild or moderate reactions should be able to undergo re-treatment with infliximab with appropriate prophylaxis.\textsuperscript{451} Although the evidence to support the use of premedication with corticosteroids and antihistamines for all patients treated with infliximab seems minimal; this practice seems reasonable in those patients with previous infusion reactions to reduce immunogenicity. Having said that, for patients at increased risk of reactions such as those on episodic therapy or who have stopped therapy for a long period of time, the use of premedication is also not unreasonable to reduce immunogenicity.\textsuperscript{556} The higher rate of infusion reactions with promethazine, suggests that monotherapy with intravenous hydrocortisone or dual therapy in combination with cetirizine is a reasonable approach. Patients with severe reactions may also be potential candidates for an ‘induction of tolerance protocol’ for subsequent infusions, with slowly escalating rate of infusion each time it is given.\textsuperscript{557} Clearly such an approach would not be appropriate in patients with high-risk features such as laryngeal oedema, severe bronchospasm or Stevens-Johnson syndrome in whom the drug is then contraindicated.
Table 34 Proposed new guidelines for management of infusion reactions

<table>
<thead>
<tr>
<th>Mild:</th>
<th>Slow infusion rate to 10mL/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Fever</td>
<td>Treat symptoms (e.g. Cetirizine 10mg for pruritus if not given as premedication, paracetamol, IV fluids)</td>
</tr>
<tr>
<td>Chills</td>
<td>After 20 minutes increase rate to 20mL/hr., then follow ‘slow infusion protocol’ as for first infusions.</td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Observations every 10min until WNL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moderate:</th>
<th>Stop infusion for 20 min. Resident or registrar to review patient. Administer cetirizine 10mg PO and hydrocortisone 100mg IV.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest pain</td>
<td></td>
</tr>
<tr>
<td>Shortness of breath (mild)</td>
<td>If symptoms resolve, restart infusion at rate of 10mL/hr.</td>
</tr>
<tr>
<td>Hypertension</td>
<td>After 15 min, increase to 20mL/hr. and continue as per ‘slow infusion protocol’.</td>
</tr>
<tr>
<td>Hypotension (drop of &lt; 20mmHg) in isolation</td>
<td></td>
</tr>
<tr>
<td>Urticaria</td>
<td>Observations every 5 min until WNL</td>
</tr>
<tr>
<td>Elevated temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> if multiple of these symptoms or signs occur together, consider a severe reaction</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severe:</th>
<th>Stop infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial / tongue / laryngeal oedema</td>
<td>Give adrenaline 0.5mg (1:1000) intramuscularly (not SC or IV). May need repeat doses</td>
</tr>
<tr>
<td>Throat tightness / hoarse voice +/- cough (may represent laryngeal oedema)</td>
<td>Call Code Blue if haemodynamically or respiratory instability, or concerned. Registrar or consultant to urgently review patient.</td>
</tr>
<tr>
<td>Acute bronchospasm or wheeze, stridor</td>
<td><strong>Administer:</strong> Hydrocortisone 100mg IV. Start IV normal saline 250mL/hr. Administer Zyrtec (cetirizine) 10mg oral if not given as premedication</td>
</tr>
<tr>
<td>Low SaO₂, or respiratory distress</td>
<td><strong>Consider depending upon status:</strong></td>
</tr>
<tr>
<td>Severe chest discomfort</td>
<td>- Nebulised salbutamol (if bronchospasm; auscultate chest)</td>
</tr>
<tr>
<td>Hypotension ( &lt; 90 mmHg, or ≥ 30% drop from baseline blood pressure. Alternatively, a drop of 20mmHg if baseline BP &lt; 100mHg)</td>
<td>- Bolus IV fluids +/- supine position if haemodynamically unstable</td>
</tr>
<tr>
<td></td>
<td>- Oxygen via Hudson mask</td>
</tr>
<tr>
<td></td>
<td>- Intubation / airway management may be necessary if severe laryngeal oedema or respiratory compromise</td>
</tr>
<tr>
<td></td>
<td><strong>Further management:</strong></td>
</tr>
<tr>
<td></td>
<td>Send serum tryptase at 1hr post reaction and 3 hrs. post reaction. Duration of monitoring will depend upon clinical outcome. Patient may need admission and monitoring overnight.</td>
</tr>
</tbody>
</table>

WNL = within normal limits
CHAPTER 5: A QUALITATIVE STUDY OF THE USE OF A CLINICAL DECISION SUPPORT SYSTEM TO IMPROVE QUALITY AND SAFETY IN INFLAMMATORY BOWEL DISEASE

ABSTRACT

Quality and safety in inflammatory bowel disease often lags behind best practice guidelines. Clinical governance aims to improve on these measures using a number of different measures including clinical audit, research and development, openness, risk management, clinical effectiveness and education and training. As this thesis has put forward so far, the clinical governance related to the use of anti-TNFα agents in inflammatory bowel disease in many situations falls short of best practice guidelines. Previous chapters show that screening for latent tuberculosis infection and hepatitis B prior to the use of anti-TNFα agents is performed suboptimally amongst gastroenterologists in Australia, and there is significant heterogeneity in screening methodology. In addition, the approach towards vaccination of this group of patients was also short of best practice guidelines.

This study aims to assess the implementation of a clinical decision support system (CDSS) relating to screening for latent infections prior to the use of anti-TNFα agents in IBD. It examines the development of such a system, and the barriers to implementation. Alternative approaches to development of a CDSS including the principles of ‘change management’ are discussed. An indirect outcome of the study was the appointment of an IBD clinical nurse specialist (CNS), which has improved quality and safety within the department.
HYPOTHESIS

The treatment of inflammatory bowel disease has evolved over time from the use of corticosteroids and 5-aminosalicylate agents to incorporate the use of immunomodulators and monoclonal antibody based therapy. These therapies have dramatically reduced morbidity in many patients with IBD and improved their quality of life, however they have also introduced a different range of risks including infection and malignancy. This study aims to examine the hypothesis:

1. That the use of a clinical decision support system can improve the rate of screening for latent infections prior to the use of anti-TNFα therapy, and as such improve quality and safety for this group of patients. In addition, in patients on a stable dose of the thiopurine medications azathioprine or 6-mercaptopurine, and

Aims

To examine the design and implementation of a novel clinical decision support system will be examined as a method of implementing guidelines to improve clinical governance in this group of patients.
METHODS

This work was collaboration with Dr. Kirsty Buising of the Victorian Infectious Diseases Service (VIDS) at The Royal Melbourne Hospital. A clinical decision support system (CDSS) for inflammatory bowel disease patients was planned using the existing ‘Guidance’ system based at The Royal Melbourne Hospital (RMH). The Guidance system was initially designed by the Victorian Infectious Diseases Service (VIDS) to improve the quality and safety related antimicrobial prescribing at RMH. Published data supports the use of the Guidance system to reduce inappropriate antibiotic prescribing at the hospital, with consequent cost savings to the hospital.\textsuperscript{529,530} The existing Guidance system comprises of a number of ‘modules’ related to approval of different medications, for example one particular module may relate to approval for the fluoroquinolone ciprofloxacin.\textsuperscript{529} As an example, a resident medical officer can log in to the Guidance system, select this module and enter the UR number of the particular patient (see Figure 34). The module would then ask the clinician a number of questions regarding the appropriate use of this antibiotic. If the results fulfill the criteria for approval, an authorisation code was subsequently provided.

In this study, a module was designed relating to screening for latent tuberculosis infection (LTBI) prior to the use of anti-TNF\(\alpha\) agents in patients with CD. As the project progressed the scope of the project broadened to increasing screening for latent hepatitis B infection (HBV), and also to include a separate module to facilitate filling in the regulatory government requirements from Medicare to apply for infliximab or adalimumab under the section 100 scheme.\textsuperscript{558} In addition, patient phenotype and medical data were collected for each patient. The author designed the module with the lead supervisor, and Dr. Buising. The use of the module was presented to a departmental meeting, and clinicians were invited to use the module. Data regarding the use of the module was subsequently collected, and clinicians were qualitatively interviewed regarding their use of the Guidance module.
Figure 34 Guidance DS Main Screen
RESULTS

The development of this module involved the creation of separate modules for screening for LTBI, as well for completion of section 100 regulatory requirements. The content of the screening module was based on existing guidelines based on the use of an IGRA and chest radiograph previously described by the author.\textsuperscript{545} These screening guidelines formed an algorithm, and as such were able to be converted into an interactive module within the Guidance environment. The use of a CDSS for IBD had not been tried before at RMH, and in fact there are minimal reports of this approach in IBD published in the literature. Screening for HBV as well as collection of phenotype and medication data was included at a later date.

Initial development and testing of the module was performed using the Guidance ‘test’ website. The module was revised after testing to either improve the system in terms of usability, explanatory content or add functionality. In addition, errors were also identified and rectified. The module went through a number of cycles of revision. As the module matured, it was presented to a combined meeting of VIDS and the department of gastroenterology at RMH for discussion and feedback. In addition, it was presented at the unit meeting of the department of gastroenterology at St Vincent’s Hospital, Melbourne where the Guidance system is also in use.

**Module for screening for latent infections**

Development of the screening module resulted in a module with seven separate ‘pages’. The introductory screen explains the need to screen for these infections, and what the module involves (see Figure 35). The next screen relates to screening for LTBI specifically. A simplified flowchart of the screening algorithm is shown on the right side of the screen (see Figure 36). Depending upon the clinical details, particular investigations may be suggested (usually a QuantiFERON-TB Gold blood test and chest radiograph). When the relevant investigations are performed, the clinician can return to this page at a later date with the view to entering the results into the module.

Based on the results of these questions, a recommendation is made by the system regarding the likelihood of LTBI, and whether treatment of LTBI with isoniazid should be considered prior to the use of an anti-TNF\(\alpha\) agent. In general, the module either suggests
that LTBI is unlikely and that the clinician can continue to prescribe the anti-TNFα agent, or that testing is suggestive of LTBI and further advice needs to be obtained (see Figure 37). In the latter case, the clinician is prompted to discuss the case with an infectious diseases physician, and the outcome of the discussion could then be entered into the system. As such, this module provides an opportunity not just to act as a CDSS but also as a mechanism to prospectively collect data that can later be used potentially for quality assurance or research purposes.

![Figure 35 Screening Module - Introduction Screen](image-url)
Figure 36 Screening Module - Tuberculosis Screen

Figure 37 Screening Module - Tuberculosis Outcome Screen
Screening for hepatitis B (HBV)

After the initial presentation at RMH, feedback from clinicians suggested that screening for HBV should be included in the module (Figure 38). This included testing for hepatitis B surface antigen, surface antibody and core antibody (given that anti-TNFα agents can potentially reactivate surface antigen negative disease to cause an acute flare of HBV, with potentially serious morbidity as a result).420

In addition, due to the feedback from the combined unit meeting at RMH, questions relating to disease phenotype and medications were included in the screening module. The rationale for the inclusion of these questions was to enable the prospective collection of accurate data regarding these patients, as previously mentioned. The particular questions asked related to disease location, extraintestinal manifestations, and the presence of complications such as stricturing disease and fistulising disease (Figure 39). Adequate information to classify patients using the Montreal classification for Crohn’s disease was also collected. On a separate screen, systematic questions about the patient’s medication history were present, including whether each medication was currently being used, never used, ceased because of inefficacy or ceased because of intolerance (Figure 40).

Figure 38 Screening Module - Hepatitis B
Figure 39 Screening Module - Phenotype information

Figure 40 Past Medication History
**Medicare (section 100) application module**

Anti-TNFα agents such as infliximab and adalimumab fall under the umbrella of the 'Highly Specialised Drugs Program' of the Australian Commonwealth Government. These are defined as ’medicines for the treatment of chronic conditions which, because of their clinical use or other special features, are restricted to supply through public and private hospitals having access to appropriate specialist facilities’. This program was established in 1991 through an initiative of the Australian Health Ministers’ Advisory Council, as a result of ‘concerns raised by the States and Territories about the rapid growth in the use of high cost drugs provided through the public hospital system’. The program was established on the basis of Section 100 of the National Health Act (1953).

In addition to the module for screening for infections, a module was created to enable the application form for infliximab under the section 100 scheme to be filled in electronically. The aims for the development of such a module included the development of a database of these patients, which could later be analysed for both quality and safety and research purposes (the data within the Guidance system could be queried for such a purpose). As mentioned previously, this five page form is normally required to be filled in by hand. This module was designed to enable the information be entered through the Guidance system, where the information would be stored in the Guidance database (see Figure 41). The author and supervisor corresponded with administrative officers at Medicare who gave approval to submit the results of such a module in printed form, as opposed to manual completion of the paperwork. Several applications were sent using this method (the printed page with the relevant names and signatures was still required to be sent). An additional module to enable the Crohn’s disease activity index (CDAI) to be calculated electronically was also designed. The CDAI is a critical component of application forms to Medicare to apply for infliximab (see Figure 42).
Figure 41 Section 100 module for infliximab

Figure 42 Crohn's Disease Activity Index Calculator
Implementation of the CDSS

After approximately three months of testing and revision, these modules were transferred to the 'live Guidance system where they were accessible to end-users. Clinicians who were interested in using the program were educated in person. During the development of the system, the use of this module was discussed at with Melbourne Health Drug and Therapeutics Committee, which advised that all IBD patients prescribed with anti-TNFα agents should preferably managed in a dedicated IBD specialty outpatient clinic in order to improve the quality and safety related to these agents.

The possibility of making screening and entering of data using these modules compulsory to authorise infliximab or adalimumab was discussed with the pharmacy department at RMH. A provisional plan for the relevant pharmacist to check for ‘approval’ on the Guidance system, in a similar manner to checking for approval for particular antibiotics was made. Although this idea was received positively, it became evident that pharmacists were not actually checking the results of this module. Possible factors contributing to this may have included the pharmacist’s perception that checking for screening for LTBI and HBV was the responsibility of the clinician, not the pharmacist. In addition, checking these results involved additional workload. Another important factor to consider was the release of adalimumab, a subcutaneous anti-TNFα agent onto the Pharmaceutical Benefits Scheme for CD in 2008. The use of adalimumab meant that patients could fill their scripts in pharmacies outside the hospital. As such, the pharmacists would not be checking the screening results for these patients anyway, which may have been regarded as inconsistent.

Figure 43 An example of results from the screening for LTBI module
Outcomes and barriers to implementation of modules

Subsequent to the development of these modules, the percentage of patients screened for LTBI and HBV went from a background rate of less than 50% to 100% of patients being screened. It should be noted that not all patients with IBD treated with anti-TNFα agents were entered into the CDSS by clinicians. Informal qualitative interviewing of gastroenterologists within the department was performed to assess barriers to implementation and whether these modules were felt to be useful.

Some clinicians felt that the exact role of the system was not made clear. This may have partially related to the different separate modules forming part of the system, namely the screening module, the section 100 module and the CDAI module. There was also some confusion as to whether use of the module was to become compulsory in order to use biological agents, a concept that met with some understandable resistance. Some clinicians felt that the use of the section 100 module was slower than filling in the paperwork by hand, and that the use of the CDSS represented additional workload rather than decreasing it. In addition, the pharmacists did not embrace this approach as discussed above. Lastly, some clinicians felt that they were adequately educated regarding screening protocols and did not need to use this system any further.

An indirect outcome of the use of the CDSS was discussions with members of the rheumatology and dermatology departments at RMH, given that both departments treated patients with anti-TNFα agents (for inflammatory rheumatic conditions and psoriasis). This resulted in the recognition that both these other departments had a clinical nurse specialist (CNS) involved closely with the treatment of these patients. These nurses facilitated the screening for infections prior to the use of anti-TNFα agents, and also followed up the results. The approach of these two nurses differed considerably; the dermatology CNS saw the patients directly, ordered screening testing and discussed the issues with the patient, and a consultant reviewed the decision-making at the end of the process and attended to other medical issues. In the rheumatology unit the clinicians performed much of the education and screening of patients, and the clinical nurse specialist played more of an administrative role in following up results and completing regulatory paperwork.
A number of factors led to the decision to subsequently submit a business case for the creation of a position of IBD CNS in the department of gastroenterology at RMH. This included the recognition that quality in safety related to multiple aspects of treatment of patients treated with anti-TNFα agents was being suboptimally performed, the recognition of a successful model of a CNS in the dermatology and rheumatology departments, and in part due to the failure of implementation of the IBD CDSS. The creation of this position led to a significant improvement in quality and safety related to this group of patients.

**Qualitative interview of IBD CNS**

An interview of the IBD CNS at RMH was performed in January 2014 via telephone. The CNS noted significant changes in practice at RMH since the implementation of this position four years ago. It was clarified that since the position had been created, the number of IBD patients treated with biological agents being seen at the IBD clinic at RMH increased from approximately twenty to over one hundred. The CNS was responsible for conducting screening for latent infections in these patients and following up the relevant results, and also arranging vaccination of these patients if appropriate. She described an important role in education of these patients as well. In the IBD clinic the CNS often saw these patients either before or after the clinician saw the patient. In some instances assistance with filling in the regulatory paperwork for the PBS (such as completing the CDAI) was performed with the approval of the relevant clinician. A substantial workload involved the logistics relating to ensuring infliximab infusions were booked at appropriate dates, and also ensuring follow up in clinic in order to complete the PBS regulatory requirements in a timely manner.

An additional outcome was the improved communication with patients as part of a telephone advice service. On many days over twenty telephone calls were received, and the CNS was required to troubleshoot their problems. In some situations if a patient was particularly unwell, liaison with clinicians to potentially organise treatment (such as corticosteroids, or changing the timing or dosing of anti-TNFα agents) in a timely manner may have helped to avoid hospital admission. In other cases, arranging hospital admission directly to the ward avoided the need for the patient to attend the emergency department, improving the efficiency of the use of hospital resources.
The changing role of the IBD CNS involved this nurse completing a Master of Advanced Nursing Practice degree. Amongst its aims, this degree aimed to accredit for the role of ‘nurse practitioner’ (NP). This role involved leadership in the management of patients with chronic diseases to an advanced nursing level, with the ability to perform more complex tasks including management of patients in a more independent manner than usual. The NP role includes the ability to prescribe a limited number of medications independently. Perceived barriers to implementation of this position were thought to include not being taken seriously within the department, people’s perceptions of the role of nurses and the relatively new role of the NP in particular, difficulties implementing cultural change and not being involved with decision making, both within the department and more broadly.
DISCUSSION

‘Healthcare systems are best described as complex adaptive systems. As such, they are a collection of individuals who are free to act in ways that are not totally predictable.’

Barach P, Johnson JK. Understanding the complexity of redesigning care around the clinical microsystem (2006)

This study aimed to improve the clinical governance related to the use of anti-TNFα agents in patients with IBD. Guidelines were developed to manage aspects of quality and safety such as screening for LTBI and HBV prior to the use of anti-TNFα agents. In terms of implementing these guidelines into clinical use, studies suggest that conventional approaches to educate clinicians through the use of guidelines are often ineffective. As a consequence, the use of a clinical decision support system (CDSS) to help implement these guidelines was examined. Although an unconventional approach, the use of a CDSS has been shown to have efficacy in areas such as antibiotic stewardship and reducing drug interactions. One aim of this project was to examine the feasibility of development of such a CDSS initially. The use of a CDSS relating to screening for infections prior to the use of anti-TNFα agents in IBD has not been previously described in the literature.

From the technical point of view the development of the module was completed successfully, though it was a complex process involving multiple cycles of development, revision and updates. The aims of involving clinicians in the use of the CDSS and subsequent improvement in quality and safety were unfortunately suboptimally realised. A number of factors contributed to this including the lack of involvement of stakeholders from the beginning or on an ongoing basis. On reflection, in order to successfully implement a project such as this, the principles of 'change management' must be employed.

Indirectly related to the outcomes of this study was the implementation of an IBD clinical nurse specialist (CNS) position, which has led to improvements in quality and safety in this group of patients. This involved development of a business case to outline a role for the CNS. Previous studies have shown that implementation of an IBD CNS can lead to improved outcomes. The CNS was able to take on the role of organising screening for
LTBI and HBV, vaccination of patients and organising the section 100 paperwork to Medicare. In addition to improving workflow through the outpatient clinics, this enabled significant improvements to quality and safety. Although the implementation of an IBD CNS ‘superseded’ the CDSS in some respects, the use of a CDSS still holds significant promise for playing a role in the management of IBD in the future. The potential barriers to implementation of the CDSS and alternative approaches to implementation are discussed.

Clinical outcomes relating to screening for latent infections prior to the use of anti-TNFα agents improved, regardless of the fact that the uptake of use of the CDSS was limited. The key outcome measures of screening for LTBI and HBV were achieved in this study, though possibly indirectly through education of clinicians rather than through direct engagement with the CDSS. An attempt to better manage infliximab infusion reactions was facilitated by the development of guidelines for management of infusion reactions. A number of factors may have led to these improved outcomes, including the role that it played in education of clinicians by involving some clinicians in the use of the CDSS, as well as discussion of these issues at departmental meetings and discussions with individual clinicians.

Barriers to implementation of the CDSS

A number of barriers to implementation of these modules existed. The reasons for this were discussed with clinicians through informal qualitative interviewing. Complexity of modules and the time to fill in the modules were key issues. Expansion of the screening module to include supplementary screens querying the clinician about patient phenotype data increased the workload for clinicians even further. On reflection, a simple module without the phenotype data may have afforded better adherence. The interface was thought to be not straightforward for some users, particularly in those who perceived their computer related skills to be suboptimal.

Attempts to make the use of this module mandatory were unsuccessful. There was a lack of buy-in from both clinicians and pharmacists with respect to this. Partially this related to the confusion about the role of the CDSS. Although the concept of gaining ‘permission’ by the Guidance module to proceed with prescribing of biological agents was potentially laudable in terms of creating a mechanism to ensure screening had been adequately performed, this concept met with some resistance. An alternative approach to the
development of a CDSS would be the creation of a specific IBD specialty clinic, which could be attended by multiple clinicians. This may have the effect of improving quality and safety by creating consensus on how to perform screening. The IBD CNS plays an important role in the specialty IBD clinic in arranging and enforcing these guidelines and protocols. Although the uptake of the CDSS was suboptimal, the creation of guidelines and the education of clinicians through meetings related the CDSS might have had an effect on quality and safety in the long term, independent of the use of the CDSS.

The barriers for implementation of clinical research into practice are complex and multifactorial. Haines and Donald (1998) describe a large gap between research findings related to clinical care, and implementation into clinical practice. Continuing medical education (CME) activities may have little effect on changing the practice of physicians. Grol (2000) suggests that ‘Implementation of guidelines in daily care is so complex that this field demands specific scientific research...’ on the methods of best implementing them into clinical practice. Cabana et al (1999) suggest that the barriers to behaviour change can be divided into a number of areas including lack of familiarity with guidelines and lack of awareness of the presence of guidelines.

The attitudes of clinicians plays an important role, and may include lack of agreement with specific guidelines (or with guidelines in general), not believing guideline recommendations will improve outcomes, believing they cannot perform the guideline recommendations themselves, and lack of motivation. External factors such as patient preferences, characteristics of guidelines (including the presence of contradictory guidelines in some situations), and environmental factors (including lack of time or resources) play an important role in barriers to uptake as well (Figure 44). Involvement of stakeholders is of critical importance. ‘Safety and quality requires collective action from a range of stakeholders working towards common, clearly understood goals. This is achieved by robust planning, underpinned by consultation and communication’.
Figure 44 Barriers to physician adherence to practice guidelines in relation to behaviour change (Cabana et al, 1999)

Role of change management

Implementing change in a clinical context is a complex process, and ‘change management’ aims to handle this complexity by evaluation, planning and implementation of tactics, strategies and operations in order to best facilitate this. Change affects all organisations whether public or private, and may be due to external reasons such as changing technology or for internal reasons such as in response to costs or performance issues. Many projects trying to effect change fail because they neglect to take into account the human dimensions of change. This includes why people are unhappy with the change process, a lack of understanding of the process of change and lack of access to tools to help effect these changes. Although some may regard change as an exciting opportunity, others may regard it as a disruption, loss or threat. Organisational change can potentially lead to confusion, anger, paranoia and insecurity. It is important to recognise how people will respond to implementation of change. Stakeholders may not be aware of why changes are necessary, feel there are other more important issues to deal with, not agree with the change, disagree with the process of implementation, feel implied criticism as implementation of changes, and feel there is an increased workload involved.

Strategies to improve quality and safety are not straightforward. Clinical units need to review the evidence from randomised clinical trials periodically, and perform audits to assess whether a gap exists between high quality evidence and clinical practice. Henderson-Smart et al (2003) report on the successful implementation of clinical guidelines into a neonatal intensive care unit in Sydney. They suggest that a key factor is agreement amongst consultant physicians that consistency in care is important, and that all
staff are involved and buy-in to this process. ‘Replacing competition with collaboration and consensus’ amongst staff, and agreement on a process for systematic evaluation, implementation and evaluation of outcomes are key factors to improving the quality of clinical care.\textsuperscript{574} Although a single intervention to effect change may be effective, multiple approaches may be more effective.\textsuperscript{575} Guidelines are more likely to be implemented if they are not controversial, if they are clear and not confusing, if they do not demand a change in existing routines, and if they are evidence-based.\textsuperscript{576} Outcomes of changes must be measured in order to assess whether an intervention has been successful or not; key performance indicators should be decided upon and measured periodically.\textsuperscript{568} Barriers to implementation of a CDSS include the fact that clinicians may regard a CDSS as a loss of physician autonomy.\textsuperscript{577,578}

\textbf{Potential improvements to the CDSS}

To improve acceptance of the CDSS in IBD, a number of approaches could be pursued. Kawamoto et al (2005) identified factors that were correlated with the ability of decision support systems to significantly improve patient care. This included automated provision of decision support as part of the clinical workflow, provision of recommendations rather than just assessments, provision of decision support at the time and location of decision making, and computer-based decision support.\textsuperscript{518} It should be noted that this CDSS did fulfill some of these criteria, however not all. The integration of such a system into clinical workflow is a barrier that could be potentially difficult to overcome. One approach would be to integrate these modules into an electronic medical record (EMR) system, if and when one was implemented in the future. Some hospitals in Australia are beginning to implement EMR systems in order to improve quality and safety, communication between doctors, and reduce reliance on paper records with attendant savings on storage and consumables.

A number of factors related to the design of a CDSS have an important bearing on its acceptance in a clinical context. Kawamoto et al (2005) conducted a meta-analysis of 88 randomised controlled studies assessing the effectiveness of CDSSs. The following four features were found to be important in an effective CDSS: decision support at the time and place of decision making, integrating CDSS into the physician’s workflow, the CDSS offering recommendations rather than just assessments, and computer assessment of eligibility for services. Bates et al (2003) describe ‘ten commandments’ for implementation of a CDSS
based on their previous successes and failures. This is described in Table 35.\textsuperscript{577} Horsky et al (2013) also emphasised the importance of the 'human-computer interface' (HCI) when designing a CDSS. As an example, they comment on the danger of excessive alerts which can lead to clinicians automatically dismissing alerts without reading them, even if safety critical.\textsuperscript{531} Herasevich et al (2013) note that over-use of alarm based decision support in the intensive care unit lead to 'alarm fatigue' and subsequent ignoring of alarms. Drawing an analogy with the use of CDSS with electronic medical records, they suggest using a rules-based approach where 'high priority information' is delivered in a timely manner, and the background 'noise' of insignificant alerts is reduced.\textsuperscript{579} Scheepers-Hoeks et al (2013) found that when implementing a CDSS in an intensive care unit, active alerts such as ‘pop-ups’ and pharmacy interventions were more effective than passive alerts. Clinicians in this study also preferred these alerts.\textsuperscript{525}

Characteristics of the interface including visual and perceptual characteristics can have a significant effect on usability, including content, language, colour scheme and typography.\textsuperscript{580} Phansalkar et al (2010) also emphasise the importance of tailoring the interface for the users of the CDSS in order to improve usability.\textsuperscript{581} Although CDSSs have historically used disparate platforms with different interfaces, Sen et al (2012) describe a movement towards an integrated architecture for CDSSs which may reduce some of this heterogeneity, and improve the user-friendliness of the interface.\textsuperscript{582} Systems where users were prompted to use the CDSS were found to be more effective than systems where the user had to initiate contact with the CDSS by themselves.\textsuperscript{525} A longer lead time may have also improved uptake of this CDSS, as over time clinicians may have developed more familiarity with the product.

In summary, the approach to implementation of a CDSS as part of this process was suboptimal and did not sufficiently involve clinicians during the inception and design phase. Closer interaction with these clinicians would be paramount if a further study was considered. A number of other barriers to implementation were discussed, including the potential complexity of the software and the possible increase in workload associated with the use of the CDSS. Alternative approaches including the fundamentals of ‘change management’ can inform an alternative approach to development of a CDSS, and careful attention must be paid to understanding the ‘human’ element of such a project. This includes a broad range of reservations and fears clinicians may have about such an
implementation, including concerns about loss of clinical autonomy. The aspects of design of a CDSS are discussed, and also the evidence base to support the use of a CDSS in general.

Table 35 ‘Ten commandments’ for effective CDSS (Bates et al 2003)

<table>
<thead>
<tr>
<th>‘Commandment’</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Speed is everything’</td>
<td>The speed taken to click between screens is important to users</td>
</tr>
<tr>
<td>‘Anticipate needs and deliver in real time’</td>
<td>This includes ‘latent needs’ such as identifying decreased renal function that requires a reduction in dose of a medication</td>
</tr>
<tr>
<td>‘Fit into the user’s workflow’</td>
<td>Standalone guidelines are rarely used, even if high quality. When prescribing vancomycin electronically, the presentation of a guideline did affect usage by clinicians</td>
</tr>
<tr>
<td>‘Little things can make a big difference’</td>
<td>Usability makes a large difference on clinician acceptance</td>
</tr>
<tr>
<td>‘Recognize that Physicians Will Strongly Resist Stopping’</td>
<td>Clinicians will resist suggestions to stop ordering a particular test or medication, if no alternative is suggested</td>
</tr>
<tr>
<td>‘Changing direction is easier than stopping’</td>
<td>If given an alternative as the default option (e.g. a less expensive antibiotic) then the clinician will often follow this suggestion</td>
</tr>
<tr>
<td>‘Simple interventions work best’</td>
<td>Guidelines that are a single screen only are most effective</td>
</tr>
<tr>
<td>‘Ask for additional information only when you really need it’</td>
<td>Asking the clinician to enter the patient weight, for example reduces clinician adherence</td>
</tr>
<tr>
<td>‘Monitor impact, get feedback, and respond’</td>
<td>If the uptake of a particular intervention is low then feedback from clinicians should be obtained with the view to modifying the system if appropriate</td>
</tr>
<tr>
<td>‘Manage and maintain your knowledge-based systems’</td>
<td>Updated information from new guidelines need to be updated within the system</td>
</tr>
</tbody>
</table>

Implementation of an inflammatory bowel disease clinical nurse specialist

The IBD CNS plays an important role in this ‘clinical microsystem’ that relates to treatment of patients with IBD.\textsuperscript{561,562} Indirectly related to this study, an IBD CNS was appointed at The Royal Melbourne Hospital. A qualitative interview with this CNS helped to define this role, its effect on quality and safety, and barriers to acceptance. This role involved
managing aspects relating to biological agents, and also relating to other immunomodulators. The CNS provides education and reassurance, and acting as a first ‘point of call’ for these potentially complex patients. This helps to improve communication with patients with IBD, who sometimes have difficulty making contact with clinical staff. By making contact early, patients may receive earlier investigation and treatment if necessary and potentially reduce morbidity.

The IBD CNS is engaged directly in outpatients and offers support to the house team attending inpatients. She also maintains the IBD database, conducts a cigarette smoking cessation program, runs a point of care faecal calprotectin service, facilitates transition of children from paediatric IBD care, and monitors results such as TPMT genotyping and thiopurine metabolites. The role as nurse practitioner (NP) has further broadened the role of the IBD CNS into playing a role in inpatient management of patients, and prescribing of medications in some circumstances. The NP role has the potential to change our approach towards treating complex patients with chronic diseases.

There has been a paucity of well design studies to support outcome measures related to the implementation of an IBD nurse to date, however a recent well designed study by Leach et al (2013) recently examined the effect of implementation of an IBD in a tertiary hospital with a busy IBD service. They noted that over a 12 month period the interventions of the nurse led to a decrease in emergency department admissions, hospital admissions and outpatient attendances in clinic. The net savings to the hospital were calculated as $136 535 over this period after salaries and associated expenses were taken into account.

Nightingale et al (2010) also confirmed the cost-effectiveness of an IBD nurse on service delivery.

In terms of an examination of the literature concerning the IBD nurse, Hernández-Sampelayo et al (2010) reviewed 284 articles and documents (including those produced by professional societies) related to this topic. Although the results were heterogeneous and the design of many studies was often poor, they noted some broad patterns including the availability of a telephone service for patients with IBD as being associated with improved health outcomes, and emphasised the multidisciplinary nature of an IBD service. Smith et al (2002) suggest that quality of life for IBD patients can be improved by the use of an IBD nurse, however this study did not show that this was sustained after six months. A Cochrane review (2009) primarily assessing this single study by Smith et al noted methodological limitations in this study.
The IBD clinical microsystem

As part of this study, the Drug and Therapeutics Committee at RMH recommended that patients with inflammatory bowel disease treated with anti-TNFα therapy were treated in a specialist IBD outpatient clinic. Barach et al (2006) describe the concept of the ‘clinical microsystem’ in order to improve the safety of the treatment of a group of complex patients, such as patients with IBD. These Microsystems are a ‘group of clinicians and staff working together with a shared clinical purpose to provide care for a population of patients’. This can be achieved by the development of an IBD specialist clinic within hospitals.

Involvement of a clinical nurse specialist, as well as other allied health staff such as dietitians may help to treat these patients in a more integrated manner. Barach et al describe a ‘...design construct in which social systems can cut across traditional discipline boundaries’. This emphasises the potential rigidity of existing segregation into disciplines and clinical roles. By working together in a holistic manner, patients’ outcomes can potentially be improved. As an example, by liaising with representatives from the dermatology and rheumatology departments who were involved with biological agents, our body of knowledge with how to treat these patients was improved. Treatment with biological agents meant that there was significant overlap with different departments, which have traditionally been quite disparate.

Design of a clinical microsystem may be aided by ‘process mapping’, whereby the processes undertaken within the microsystem are mapped out in flowchart form. An example of process mapping as applied to the section 100 applications for infliximab for Crohn’s disease is shown below in Figure 45. This algorithmic expression of a complex process enables a clearer outline of how to apply for these medications. By putting this in graphical format, complexity is reduced.
Figure 45 Process Mapping for Application for infliximab under Medicare 100 scheme (simplification)
CHAPTER 6: THIOPURINE METHYLTRANSFERASE AND THIOPURINE METABOLITE TESTING IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE ON A STABLE DOSE OF THIOPURINE MEDICATIONS

Publication arising from this section:


**ABSTRACT**

Quality and safety relating to the use the thiopurine medications azathioprine and 6-mercaptopurine has potential to be improved by the use of pharmacogenetic tests relating to the *Thiopurine methyltransferase* gene (*TPMT*), as well as due to testing for thiopurine metabolites. The enzyme encoded by the *TPMT* gene forms part of a complex metabolic pathway with the product of the 6-thioguanines (6-TGNs), metabolites which affect the function of lymphocytes to reduce inflammation. Measurement of 6-TGN and the byproduct 6-methylmercaptopurine (6-MMP) in red blood cells can help guide clinical treatment. Despite their contemporary availability, uptake of these tests has been low in hospitals in Australia. This study examined 42 patients with inflammatory bowel disease from a metropolitan teaching hospital in Melbourne who were on a stable dose of thiopurine medication. Patients underwent *TPMT* genotyping as well as measurement of 6-TGN and 6-MMP metabolites.

9.52% of patients had a heterozygous mutation of *TPMT*, consistent with the published 10% rate in the literature. No association was found between 6-TGN levels and disease activity contrary to previous studies. There was also no association between 6-TGN levels and mean cell volume or lymphocyte count, which are sometimes used as surrogate markers for therapeutic thiopurine levels. No difference was noted in 6-TGN levels or 6-MMP levels in patients with heterozygous mutations compared to controls. In addition, no difference in thiopurine drug doses (in azathioprine equivalents) was detected between heterozygotes and control patients. The study makes recommendations as to the role that *TPMT* genotyping and thiopurine metabolite testing may play in optimising the
management of patients with inflammatory bowel disease treated with thiopurine medications.

**HYPOTHESIS**

The treatment of inflammatory bowel disease has evolved over time from the use of corticosteroids and 5-aminosalicylate agents to incorporate the use of immunomodulators and monoclonal antibody based therapy. These therapies have dramatically reduced morbidity in many patients with IBD and improved their quality of life, however they have also introduced a different range of risks including infection and malignancy. This study aims to examine the hypothesis:

1. That the use of pharmacogenetic testing for the enzyme TPMT and the measurement of thiopurine metabolites can improve quality and safety in this group of patients.

**Aims**

To examine the use of genotyping for TPMT and measurement of thiopurine metabolites in a cohort of patients with IBD on a stable dose of medication. To compare these results with assessment of clinical disease activity, drug dosage, and biochemical and haematological parameters.
METHODS

Patient recruitment

Ethics approval was obtained from The Royal Melbourne Hospital (Melbourne Health) human research ethics committee (HREC). Patients were recruited from inflammatory bowel disease specific or general gastroenterology clinics of three metropolitan teaching hospitals. The Ethics Committee at each participating hospital approved the research protocol. Patients aged between 18-80 years were eligible for inclusion if they were currently taking a thiopurine medication, and had been on this therapy for at least three months. The dose was required to be stable for at least four weeks.

Data collection

Details of clinical variables relating to inflammatory bowel disease were collected including phenotype, disease activity, extraintestinal manifestations and use of ‘rescue’ medications such as corticosteroids. Medication information included which drug was taken, dosage, and weeks on the current dose. The azathioprine dose was converted to mg/kg based on the patients’ body weight, and for patients on 6-mercaptopurine the dose was converted to azathioprine equivalents by multiplying by 2.08. Recent blood test results recorded included haemoglobin, total white cell count, lymphocyte count, platelet count, alanine transaminase (ALT), serum albumin level and C-reactive protein (CRP). The Harvey Bradshaw Index (HBI) was computed from clinical symptoms for patients with Crohn’s disease and the Simple Clinical Colitis Activity Index, (SCCAI) was computed for patients with ulcerative colitis. Because of the increase frequency of TPMT*3C in South East Asians, the ethnic group of each patient was recorded as Caucasian, South East Asian or other.

Genetic testing

Analysis of TPMT genotype was performed using a single base extension multiplex reaction using a DNA sequencer. This identified TPMT *2, *3A and *3C alleles. This is expected to detect about 80-95% of known deficient alleles. On the basis of these results, patients were categorized into no mutations i.e. ‘wild type’, one mutation i.e. heterozygous, or two mutations i.e. homozygous mutations. Those with one deficient allele or heterozygous
mutations were classified as intermediate metabolisers and those with two deficient alleles or homozygous mutations were classified as poor metabolisers.\textsuperscript{494}

Table 36 Example of genotyping result and analysis for a single patient

<table>
<thead>
<tr>
<th>Tests:</th>
<th>Results:</th>
<th>Genotype:</th>
<th>Summary:</th>
</tr>
</thead>
<tbody>
<tr>
<td>238G&gt;C (Ala80Pro)</td>
<td>G/G</td>
<td>TPMT*1/*1</td>
<td>NORMAL</td>
</tr>
<tr>
<td>460G&gt;A (Ala154Thr)</td>
<td>G/G</td>
<td></td>
<td>(EXTENSIVE METABOLISER)</td>
</tr>
<tr>
<td>719A&gt;G (Tyr240Cys)</td>
<td>A/A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Metabolite testing**

6-TGN levels and 6-MMP levels were assayed as previously described by high performance liquid chromatography (HPLC) methods.\textsuperscript{493} Each patient was tested for both of these metabolites. The red blood cells were washed in saline and counted to normalise to $8 \times 10^8$ red blood cells. The reference target range used for 6-TGN was 235-450 pmol / $8 \times 10^8$ red blood cells.

**Statistical analysis**

Data collected regarding each patient was written into a custom data collection sheet for this study for each patient. The cumulative data was entered into a Microsoft Excel spreadsheet. The Microsoft Excel data analysis ‘toolpack’ was used for data analysis. In addition, the publically available ‘SSC-stat v2.18’ plug in pack for Microsoft Excel from the Statistical Services Centre; University of Reading, United Kingdom (2007) was also used for data analysis.

**RESULTS**

42 patients were recruited from The Royal Melbourne Hospital. 3 patients had to be excluded as insufficient clinical or biochemical data could be obtained for these patients.

The mean age of patients recruited into the study was 42.23, with a standard deviation of 17.64. The ages varied from a minimum of 17 to a maximum of 80 years old. A histogram of the age distribution is noted in Figure 46.
Figure 46 Histogram of age distribution of patients (total number 39)

The dosage of thiopurine (adjusted to azathioprine equivalents in milligrams per kilogram of body weight) is noted in Figure 47. This shows significant heterogeneity in dosing when expressed in terms of mg/kg; the weight based dosing varied greatly, between 0.5mg / kg to 2.8 mg/kg. This may reflect the variability in approaches to dosing patients with thiopurines. Common approaches include non-weight based dosing, e.g. 100mg for all patients, or weight based dosing, e.g. 2.0-2.5mg/kg.
Thiopurine metabolite results

Figure 48 shows the distribution of 6-thioguanine levels in all patients. The mean 6-TGN level was 265.6 pmol / $8 \times 10^8$ erythrocytes with a standard deviation of 156.1. The nominal ‘reference range’ in previous studies is listed as 235-450 pmol / $8 \times 10^8$ erythrocytes. In this graph we can see that the majority of patients fall within this range, though several are within the 400-600 pmol / $8 \times 10^8$ erythrocytes range. In terms of outliers, one patient had a 6-TGN level of 715 pmol / $8 \times 10^8$ erythrocytes.
In Figure 49 we see a histogram of the frequency of 6-methylmercaptopurine values. The ‘reference range’ has been quoted as less than 7500 pmol / \(8 \times 10^8\) erythrocytes. Levels above this range have previously been correlated with abnormal liver function tests. In this sample of patients, the vast majority have 6-MMP levels below 3000 pmol / \(8 \times 10^8\) erythrocytes, though a few patients do have values above this level.
Figure 50 Scatter plot comparing paired 6-TGN and 6-MMP values

A scatterplot of matched 6-TGN and 6-MMP levels is shown in Figure 50. There is minimal correlation between the two parameters, with a correlation coefficient of 0.074. This is biologically plausible, as 6-mercaptopurine is metabolised into 6-MMP based on TMPT activity, and the rest is available to be metabolised into 6-TGN (unless metabolised by other pathways).
Genotyping results

Out of the 39 patients remaining after 3 were excluded for insufficient clinical information, there were three patients with *1/*3A heterozygous mutations of TPMT. If the three patients who were excluded due to insufficient clinical and laboratory information were included, there were four patients with *1/*3A heterozygous mutations. The rest of the patients were homozygous ‘wild type’ with a *1/*1 genotype. This is a rate of 7.69% heterozygous mutations (or 9.52% if the additional three patients are included), consistent with the published 10% incidence rate for heterozygous mutations. Figure 51 shows a comparison of the distribution of 6-TGN levels in patients with normal (wild type) TPMT genotype, and those with heterozygous TPMT mutations (the outer lines of the box chart show the range, the boxed area has the 25th and 75th percentile and the middle line is the mean). There are only three patients in the latter group; however the 6TGN levels appear significantly higher in the latter group. However, comparing the two groups using a two-sample t-test (assuming equal variances), the mean 6TGN level in the wild type group was 259.8 pmol / 8 × 10^8 erythrocytes and the mean 6-TGN level in the heterozygote group was 328.3 pmol / 8 × 10^8 erythrocytes. This gave a two-sided P-value of 0.475; a statistically significant difference between the two groups was not detected in this particular sample.

Similarly, in Figure 52, 6-methylmercaptopurine levels are compared in patients with ‘wild type’ homozygote TPMT genotypes, with those patients with heterozygous genotypes. Again, only three patients are in the latter group. Comparing the two groups using a two-sample t-test (assuming equal variances), the mean 6MMP in the wild type group was 1854 pmol / 8 × 10^8 erythrocytes and the mean in the heterozygote group was 319.7 pmol / 8 ×
10⁸ erythrocytes. Despite the large differences, the two-sided $P$-value was 0.22, again not showing a statistically significant difference between these two sample population samples.

**Figure 52 6MMP levels in patients with ‘wild type’ vs heterozygous mutations of TPMT**

**Mean thiopurine dosages in wild type vs. heterozygotes**

The dosage expressed in mg/kg azathioprine dose equivalents was 1.635 mg/kg in heterozygotes versus 1.576 mg/kg in normal 'wild type' homozygotes. This was surprising, as it would be expected that heterozygotes would have lower dosage equivalents given the increased 6-TGN levels with lower TPMT activity. These two groups were compared using a two-sample t-test (assuming equal variances). The two-sided $P$-value was 0.8553; a statistically significant difference was not found in these two sample groups.

**Table 37 Thiopurine drug dosage equivalents in heterozygotes compared to wild types**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean dose (mg/ kg)</th>
<th>Numbers of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygotes</td>
<td>1.635</td>
<td>4</td>
</tr>
<tr>
<td>Wild type</td>
<td>1.576</td>
<td>33</td>
</tr>
</tbody>
</table>

When 6-TGN levels were classified according to whether they were above, within or below the putative therapeutic range (235-450 pmol / 8 × 10⁸ erythrocytes), there were fairly similar dose equivalents in the groups of patients with low 6-TGN levels and those with normal 6-TGN levels. These two groups were compared with a two-sample t-test
(assuming equal variances). The difference of means was only -0.036 and the two sided $P$-value was 0.8834, no statistically significant difference was noted between these two groups. Similarly, a two-sample $t$-test (assuming equal variances) was applied to the normal and high 6-TGN groups. The difference of means was 0.34 and the two-sided $P$-value was 0.356. Again, no statistically significant difference was noted between these two groups however only four patients were in the high 6-TGN group.

Table 38 Thiopurine dosage stratified by 6-TGN levels

<table>
<thead>
<tr>
<th>Levels of 6-TGN</th>
<th>Mean dosage in mg/kg (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low 6-TGN</td>
<td>1.502 (16)</td>
</tr>
<tr>
<td>Normal 6-TGN</td>
<td>1.538 (11)</td>
</tr>
<tr>
<td>High 6-TGN</td>
<td>1.879 (4)</td>
</tr>
</tbody>
</table>

**Thiopurine Metabolites and Clinical Activity**

The Harvey Bradshaw Index (HBI) was used to assess the clinical severity for patients with Crohn's disease. An alternative approach would have been to use the Crohn's disease activity index (CDAI), which is now in more common use in clinical trials. As seen in the scatterplot in Figure 53, there is no obvious correlation between the HBI and 6-TGN levels in this population sample. The correlation coefficient was calculated as -0.223 suggesting a mild negative correlation at best.
Figure 53 Scatterplot of Harvey-Bradshaw Index and 6-TGN levels in patients with Crohn's disease

For patients with ulcerative colitis, the Simple Clinical Colitis Activity Index (SCCAI) was used to measure disease activity. SCCAI scores are compared with 6-thioguanine levels in Figure 54. As can be seen there is again minimal correlation between the two parameters, emphasised by the correlation coefficient calculated as -0.042.

Figure 54 Scatterplot of SCCAI compared to 6-TGN levels in patients with ulcerative colitis
**Thiopurine metabolite levels and biochemical / haematological markers**

Figure 55 examines a scatterplot of 6-thioguanine levels compared to mean cell volume (MCV) levels. Conventional wisdom related to thiopurines suggests that an elevated MCV suggests therapeutic levels of thiopurine. However as this scatterplot suggests, MCV levels are relatively unaffected by 6-TGN levels, and the correlation coefficient is only 0.223 suggesting a mild correlation at best.

![Figure 55 Scatter plot of mean cell volume (MCV, y axis) vs. 6-thioguanine levels (6TGN, x axis).](image)

A comparison of 6-MMP levels and alanine transaminase levels (ALT) is seen in Figure 56. This scatterplot suggests a mild correlation between the two parameters, and the correlation coefficient of 0.3558 is consistent with this. Of the two patients with 6-MMP values over 7500 pmol / 8 × 10⁸ erythrocytes, one patient has an elevated ALT however the other has an ALT within the normal range. This sample provides supportive evidence that ALT abnormalities in patients on thiopurine medications may relate to 6-MMP levels, however it does not support the therapeutic range of less than 7500 pmol / 8 × 10⁸ erythrocytes in this sample. This may relate to the small sample size of patients with elevated ALT levels in particular. It may well be that prior to study entry, clinicians who noted an elevated ALT after treatment with thiopurines subsequently reduced the dose and the ALT normalised.
DISCUSSION

This study examined thiopurine metabolites and *Thiopurine methyltransferase (TPMT)* genotype in patients already on a stable dose of thiopurine medications to see if it resulted in information to change clinical management. In addition, in this study, no association was found between 6-thioguanine (6-TGN) levels and disease activity. This result differs from previous studies, some of which have suggested those patients with 6-TGN levels below 235 pmol / 8 × 10⁸ erythrocytes are less likely to be in clinical remission. This result argues against the validity of the lower end of the provisional ‘therapeutic range’ for 6TGN; however this study may have been limited by small sample size. In this thesis, only results collected by the author are included, however this study formed part of collaboration with the Murdoch Childrens Research Institute and Box Hill Hospital. Using a larger sample size of 132 patients, there was still no association between 6-TGN and clinical activity detected. One possibility in this study is that patients with ongoing disease activity may have already had their dose adjusted by their clinician prior to study entry (without knowledge of thiopurine metabolite results), or even changed to an alternative treatment regimen. This may have reduced the pool of patients with poorly controlled disease activity in this study.

No correlation was noted between 6-TGN levels and lymphocyte levels, arguing against the clinical dogma that lymphopaenia in patients suggest therapeutic levels of a thiopurine
medication. Similarly, no correlation between 6-TGN levels and red blood cell mean cell volume (MCV) was noted, also arguing against dogma that macrocytosis (high MCV) is suggestive of therapeutic thiopurine levels. Given this, on the basis of this study lymphopaenia and macrocytosis should not be used for clinical decision making with thiopurine medications. If they provide any information, the presence of macrocytosis or lymphopaenia may be suggestive that the patient is taking the medication only. There was minimal correlation between 6-TGN levels and 6-MMP levels (correlation coefficient 0.074), suggesting that levels of the two metabolites are not related.

With respect to the TPMT genotyping results, 7.69% of patients had a heterozygous mutation (or 9.52% if the three patients excluded from analysis are included; these three patients had also undergone genotyping). This is broadly consistent with the published rate of 10% heterozygous mutations for TPMT.474,494 6-TGN levels were compared in patients with wild type genotype compared to heterozygous genotype, and the difference in 6-TGN levels between the two groups (259.8 in wild type vs. 328.3 in heterozygotes) was not statistically significant, however this may have been a reflection of the small number of heterozygotes. Similarly, although the mean 6-MMP levels were quite different between the two groups (1854 pmol / 8 × 10^8 erythrocytes in wild type vs. 312.7 in heterozygotes), the difference was surprisingly not statistically significant. In summary, in this study no difference in 6-TGN or 6-MMP levels were seen in TPMT heterozygotes compared to wild type.

No difference in thiopurine drug doses (in azathioprine equivalents) was seen between heterozygotes and wild type patients. This is surprising as lower doses would be expected to be prescribed in heterozygotes due to less TPMT activity causing higher 6-TGN levels. Again this may reflect the small number of heterozygote patients. One particular study suggested that individuals without mutations of the TPMT gene ended up with a dose that was twofold higher than heterozygotes after nine months of dose titration.491 When patients were classified according to 6-TGN levels, in particular whether they were below, within or above the 'therapeutic range' of 235-450 pmol / 8 × 10^8 erythrocytes, the mean dosage was higher in those with 'high' 6-TGN levels compared to those within the 'normal' range (1.879 vs. 1.538), however this difference was not statistically significant, possibly reflecting the small number of patients with high 6-TGN levels. Mean dosage in those with 'low' 6-TGN levels was in fact similar to those with 'normal' 6-TGN levels, and no statistically significant difference was noted between these two groups.
In terms of improving quality and safety, the approach of using thiopurine metabolites according to the clinical situation is a reasonable one. For example, measurement of metabolites in patients who are not in clinical remission, and in patients who have developed abnormal liver function tests. In the former situation this may help to differentiate between non-adherence, low thiopurine dose, thiopurine resistance or thiopurine refractory patients (Table 39). In patients with abnormal liver function tests, if 6-MMP levels are high and 6-TGN levels are low this patient may be 'shunting' into 6-MMP and may be a candidate for careful coadministration of small doses of allopurinol, in conjunction with a reduced dose of thiopurine. Allopurinol inhibits xanthine oxidase, and in this particular situation can facilitate 6-TGN levels within the therapeutic range, with 6-MMP levels often plummeting to well lower than 7500 pmol / 8x10^8 erythrocytes. The mechanism of this is not well understood.

There are a number of reasons as to why the study results did not prove the stated hypothesis. The main reason as mentioned previously is the study design involving patients already on a stable dose of thiopurine meant that clinicians may have already adjusted their thiopurine dose with the aim of achieving remission, and those patients experiencing dose-related toxicity may have had their thiopurine stopped and as such not been eligible for the study inclusion criteria. The nature of IBD is that of a relapsing and remitting disorder, and patients in this study were captured as a 'snapshot' in time; a patient presently in remission could conceivably have increased disease activity at a later date. Some patients may go into clinical remission spontaneuously, despite the use of a thiopurine and as such they may have 6-TGN levels that are artificially low. Conversely, some patients may be refractory to thiopurines and may have had their dose significantly increased already, reflecting high 6-TGN levels and increased disease activity. In addition, patients who were non-adherent with their medication may have diluted the results of this study. A prospective study of these of these diagnostic modalities may have revealed a more accurate picture of the effect of these tests on clinical practice.
Table 39 Interpretation of thiopurine metabolite results in patients with ongoing disease activity (adapted from Dassopoulos et al, 2012)

<table>
<thead>
<tr>
<th>6TGN</th>
<th>6MMPR</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>Underdosing or non-adherence</td>
<td>Check adherence, increase dose</td>
</tr>
<tr>
<td>Low</td>
<td>Normal or high</td>
<td>Underdosed due to preferential 6MMP production</td>
<td>Consider adding allopurinol or switch therapy</td>
</tr>
<tr>
<td>Normal</td>
<td>Low, normal or high</td>
<td>Appropriate dose</td>
<td>Switch therapy</td>
</tr>
<tr>
<td>High</td>
<td>Low, normal or high</td>
<td>Dose too high</td>
<td>Switch therapy</td>
</tr>
</tbody>
</table>

Routine measurement of TPMT genotype prior to commencement of a thiopurine medication is a reasonable strategy for patients with inflammatory bowel disease, in order to predict target dosing and to screen for patients with homozygous or compound heterozygote mutations. Patients with homozygous mutations should not be started on a thiopurine medication, and those with heterozygous mutations can conventionally be started on 50% of the ‘standard dose’. Genotyping has the advantage of being more reproducible, though has the disadvantages of missing less common genetic mutations of TPMT, as well as non-genetic causes of TPMT deficiency. In addition, those patients with excessively high TPMT activity who may require higher doses of thiopurine would not be picked up using genotyping. Phenotyping has the advantage of being a more ‘functional’ measure of TPMT activity, though has the disadvantages of being less reproducible (between laboratories for example), and may be affected by patient factors such as recent blood transfusion.

There is significant heterogeneity between gastroenterologists in terms of the approaches to starting a thiopurine drug (see Table 40). Each approach has advantages and disadvantages. One disadvantage to initially starting at the ‘target dose’ (such as 2.0-2.5 mg/kg) is that patients may be more likely to be intolerant of the medication due to nausea or other ‘minor’ adverse effects. An alternative approach is to start at a lower dose such as 50mg or 100mg and slowly titrate the dose up to the ‘target’ dose, even if TPMT genotype is known.
<table>
<thead>
<tr>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed dosing e.g. 150mg for all patients</td>
</tr>
<tr>
<td>Weight based dosing e.g. 2mg/kg</td>
</tr>
<tr>
<td>Starting a low dose and slowly increasing to 'target dose'</td>
</tr>
<tr>
<td>TPMT genotyping or phenotyping prior to commencement of treatment</td>
</tr>
</tbody>
</table>

**Table 40: Approaches to starting a thiopurine drug**

There are a range of barriers previously described as being associated with the clinical uptake of pharmacogenetic tests such as *TPMT* genotyping. This includes perception of cost-effectiveness of the test, lack of confidence interpreting the results of pharmacogenetic tests, and even ethical concerns regarding use of DNA. In addition, constraints include the required educational strategies and laboratory equipment infrastructure can influence uptake. This project has introduced the concept of *TPMT* testing and measurement of thiopurine metabolites into a major metropolitan teaching hospital in Melbourne, with the hope of improving quality and safety relating to thiopurine usage. Contemporary enquiry confirms that this has been effective; including the development of a state funded pilot service of pharmacogenetic testing for *TPMT*, which is currently being piloted at The Royal Melbourne Hospital, signaling a translational consequence of this thesis.
CHAPTER 7: CONCLUDING CHAPTER

INTRODUCTION

The studies reported in this thesis had a double objective: to explore the role of the microbiota in the pathogenesis of inflammatory bowel disease (IBD), and to examine ways of improving quality and safety relating to the pharmacological treatment of IBD. These are important areas for further study in IBD because they are likely to influence the treatment of patients with IBD, and therefore clinical outcomes and quality of life. The role of the microbiota in the pathogenesis of IBD includes the “single-pathogen” hypothesis, and the hypothesis that alterations in the normal commensal microbiota in the gastrointestinal tract contribute to pathogenesis. The single organism hypothesis was examined by testing mucosal samples for *Mycobacterium avium subsp. paratuberculosis* (MAP) using a polymerase chain reaction to the IS900 gene specific to MAP. The dysbiosis hypothesis was examined in a metagenomic approach using a custom microarray based on 16s ribosomal RNA phylogenetic based oligonucleotide probes. A sub-study involving genotyping common germline single nucleotide polymorphisms associated with IBD was also performed.

Quality and safety in IBD was initially assessed by a retrospective audit of the use of infliximab in a tertiary level hospital covering the period of 2002 - 2010. The role of screening for latent tuberculosis infection (LTBI) and hepatitis B were examined in particular, as well as the incidence of infliximab infusion reactions. A survey of gastroenterologists in Australia relating to screening for latent infections and vaccination of patients with IBD was performed, showing significant heterogeneity in clinical practice. A multicentre study assessing the use of thiopurine metabolites and *thiopurine methyltransferase (TPMT)* genotyping in patients already established on thiopurine medications was performed. The development of a clinical decision support system (CDSS) for clinicians relating to screening for latent infections prior to the use of anti-TNFα agents in IBD was examined. The uptake of this CDSS by clinicians was unfortunately poor, and the barriers to the uptake of this CDSS and potential alternative approaches are discussed. The role of the IBD clinical nurse specialist (CNS) in maintaining quality and safety standards in the treatment of IBD was examined using qualitative methodology by interviewing of nurses who work in these positions.
MICROBIOTA STUDIES

Analysis for MAP

101 mucosal samples were obtained from colonoscopic biopsies from patients with Crohn's disease (CD), ulcerative colitis (UC) or control patients. There was a low rate of MAP positivity in samples using a polymerase chain reaction test to the IS900 gene specific to MAP; only eight samples were positive for MAP and one result was indeterminate. There was no association detected between the presence of MAP and whether the patient had either CD or UC, and also no association between MAP and the presence of inflammation in biopsies. The rate of MAP positive samples in this study was significantly lower than previously reported in many different studies. These results argue against MAP playing a pathogenic role in IBD. It also does not support the hypothesis that the presence of MAP is related to inflammation.

As previously mentioned, there are a number of potential reasons for the low rate of MAP positivity compared to existing studies, including different methods of sample collection, specimen handling, and analysis methodology including DNA extraction and PCR analysis. The lower rate of MAP positivity may have reflected improved study methodology compared to other studies in which false positives could potentially be introduced due to methodological issues. Given the low rate of MAP positivity it is unlikely that a larger sample size would have detected a biologically significant difference between the presence of MAP and the IBD status of the patient or the presence of inflammation.

A potential limitation of this study is that the control group consisted of patients recruited from scheduled colonoscopy lists who were having a procedure to investigate chronic diarrhoea or change in bowel habit, with normal macroscopic and histopathology results at colonoscopy. Acute infections had been excluded in this group on clinical grounds. Some of these patients presumably had irritable bowel syndrome or other causes of diarrhoea, and could have had colonic microbiota that differed from the microbiotic of the healthy general population. It is very difficult to recruit healthy volunteers for an elective colonoscopy for the purpose of a control group due to the unpleasantness and potential risks of the procedure, albeit low. An alternative approach may have been to recruit patients undergoing colonoscopic screening for previous colonic polyps, although it is possible that even this group of patients may have differences in their gastrointestinal
microbiota compared to the normal population. At the time of study conception the data to support an altered microbiota in irritable bowel syndrome was inconclusive, however since then the evidence in the literature suggests that alterations in the microbiota, and possibly even alterations in host-microbial interactions may play a role in the pathogenesis of IBS, analogous to their role in IBD.\textsuperscript{594,595}

The negative results from the multi-centre study of clofazimine, clarithromycin and rifabutin in Australian patients with IBD which was published after this study was initiated is also evidence against MAP playing a pathogenic role in IBD, though even this study had limitations including patients not being tested for MAP, and inclusion of patients with advanced disease who would be unlikely to reverse their disease activity even if MAP was eradicated.

**Microarray analysis**

The microbiota in twenty samples from four different groups was studied using a custom phylogenetic microarray based on oligonucleotide 16s ribosomal RNA probes. The four groups were Crohn’s disease inflamed, Crohn’s disease non-inflamed, ulcerative colitis, and control patients. The results showed clear differences in the microbiota between samples from patients with either CD or UC compared to controls. No difference was detected between inflamed and non-inflamed samples from patients with CD, arguing against the hypothesis that inflammation is the cause of dysbiosis in CD.

A number of specific organisms were found to be associated with either CD or UC. These included *Shigella flexneri, Dorea longicatena*, and *Xenorhabdus bovienii*, which were associated with CD but not UC. *Faecalibacterium prausnitzii* was lower in abundance in CD compared to controls, as described in a number of previous studies published after the commencement of this study.\textsuperscript{250,252} *F. prausnitzii* is thought to have anti-inflammatory properties, possibly mediated by butyrate production. *Yersinia pestis* and *Eubacterium rectale* were associated with ulcerative colitis.

Differences in the microbiota between IBD and control patients have previously been documented using a number of different techniques including temporal temperature gradient gel electrophoresis, denaturing gradient gel electrophoresis, real-time PCR, and metagenomic approaches. The specific organisms identified in this study found to be associated with either CD or UC question the ‘single pathogen’ theory and suggest that
there is a broader dysbiosis in IBD. Further studies are required to confirm this however this conclusion is consistent with current thinking in relation to altered microbiota in IBD. Proof of a broader dysbiosis in IBD does not necessarily link this to the pathogenesis of IBD, since such changes could be secondary to other changes in IBD or to treatment. Altered microbiota may also contribute to exacerbations and remissions of IBD and therefore further studies of colonic microbiota are indicated.

A subset of thirty-one patients underwent genotyping for common SNPs associated with IBD, in particular with CD. Testing was performed for the three mutations leading to the insertion and amino acid changes of NOD2 / CARD15 associated with CD, the 3020insC (1007 frameshift), R702W and G908R mutations. Testing for the mutations leading to the amino acid changes Arg381Gln of IL23R, Asp299Gly of TLR4, and T300A of ATG16L1 were also done. In addition, mutations encoding they were tested for mutations encoding the +32656 deletion variant of a complex insertion*2 / deletion*1 polymorphism mutation of NOD1, the G113A mutation of DLG5, and the L503F mutation of OCTN1. The association between IBD and some of these SNPs had not been subsequently replicated in independent studies and on reflection were less plausible candidates to study.

Given the small number of patients eventually recruited into this genotyping sub-study, these results are provided as descriptive only. On reflection the sample size was far too small to ensure that the results of such a comparison were adequately powered. A small sample size may have been adequate only to exclude an exclusive relationship between genotype and MAP leading to disease, though not to exclude important less dominant relationships such as those found in polygenic diseases. Genotyping results were correlated with phenotype (UC vs. CD) and MAP status. These results were subsequently compared to the expected prevalence of these mutations in the general population and no difference was detected, as expected given the small numbers being assessed. There were no technical problems with the genotyping in this study, and this sub-study gives a framework for a potential future study assessing correlation between genotype and the presence of MAP. Such a study would need to have careful statistical analysis in order to ensure it was adequately powered. Given that this study found low rates of MAP in general, the feasibility and significance of such as study is questionable, and possibly not worth pursuing at least for an association with MAP. The approach remains feasible for interactions of genotypes with other organisms or dysbiotic characteristics.
In summary, these studies have attempted to test the hypothesis that alterations in the normal commensal microbiota in the gastrointestinal tract contribute to pathogenesis. Although the studies are not conclusive in relation to the hypothesis, they show that altered microbiota is a feature of IBD, consistent with the findings of other investigators (with some differences), and therefore further investigation is indicated because of the possible relevance of this to pathogenesis of IBD including exacerbations and remissions. Such studies also may have relevance in terms of prevention and treatment of IBD.

QUALITY AND SAFETY IN IBD

Vaccination survey

This survey recruited 44 Australian gastroenterologists using a newsletter sent by the Gastroenterology Society of Australia (GESA) to members of the GESA IBD special interest group ('IBD-A'). The survey asked questions related to the use of vaccination in patients with IBD, and also screening for latent infections such as latent tuberculosis infection (LTBI) and hepatitis B (HBV). The “standard of care” supports vaccination and screening for all patients who are being considered for anti-TNFα treatment because such treatment can result in relapse of latent infection with potentially serious clinical consequences. At the time of the survey, the results showed a gross heterogeneity in practice towards vaccination and screening of these patients. About two thirds of respondents screened patients for LTBI prior to the use of infliximab or adalimumab, though the methods varied greatly using modalities including chest radiographs, the tuberculin skin test, and interferon gamma release assay (IGRA) blood tests. A small number of respondents screened patients for LTBI prior to the use of immunomodulators, corticosteroids, or screened all patients with IBD; however there is not a strong evidence base to support this approach at present. Existing guidelines suggest screening all patients for LTBI prior to the use of anti-TNF agents. As the survey was done at a time when clinical guidelines relating to these aspects of care were just emerging overseas, heterogeneity of practice is understandable.

Approximately 42% of respondents screened patients for HBV prior to the use of anti-TNF agents, and a similarly a small number screened patients prior to the use of immunomodulators, corticosteroids, or screened all patients with IBD. Surprisingly, only 59% screened patients using hepatitis B surface antigen (HBsAg), 58% tested for surface
antibody (HBsAb) and 54% tested for core antibody (HBCAb). These studies show that a more consistent approach is needed. All patients should be tested for HBsAg and HBCAb (given biological treatment can reactivate seronegative disease), and it is reasonable to also test for HBsAb to assess for pre-existing immunity. Again, the timing of the questionnaire in relationship to emergence of evidence and guidelines accounts for a lot of the heterogeneity. A contemporary survey would be expected to align with guidelines a lot better now.

The use of vaccination was also heterogeneous with approximately one third of respondents vaccinating patients for HBV and influenza, and over half vaccinating for Pneumococcus. When this was stratified for patients having treatment with anti-TNF agents, immunomodulators or corticosteroids, or for vaccination, the percentages were low in each category suggesting lack of consensus on practice in this area at the time. This is not surprising given the lack of consensus guidelines for vaccination in IBD. Guidelines from the European Crohn’s and Colitis Organisation (ECCO) allow for a very broad range of options for testing and vaccination, and the evidence base to support some of these recommendations is marginal with several recommendations made on the basis of ‘expert opinion’. The timing of vaccination was also variable, with about one third of respondents preferring vaccination well before the use of corticosteroids, immunomodulators or biological agents. There is some merit in this approach as the use of these agents may reduce the effectiveness of vaccination, though it may potentially mean overuse of vaccination in low risk patients. The majority of respondents were of the view that vaccination was the responsibility of the gastroenterologist and general practitioner in collaboration, an approach that seems both sensible and practical.

As a consequence of this survey, existing guidelines for screening for latent tuberculosis were reviewed and new provisional guidelines were suggested based on the use of an IGRA and chest radiograph, and these guidelines were published in the Medical Journal of Australia (this is enclosed as an attachment). It is likely that clinical practice has improved with respect to LTBI, and hepatitis B screening, and vaccinations since this survey was performed in 2008. There has been focused attention on these aspects of clinical care since the current questionnaire was administered, partly as a result of publications from this thesis and other educational activity within the IBD community.
Audit of anti-TNFα agent use in a tertiary hospital

This audit examined the use of infliximab by gastroenterologists at a tertiary hospital in Australia over a period 2002 to 2010. A total of 219 infusions given to 53 patients were included in this study. Many of the infliximab infusions included in this audit were given as episodic therapy, as the majority predated the approval of infliximab on the Pharmaceutical Benefits Scheme (PBS) in Australia for moderate to severe Crohn’s disease. Episodic treatment was often given, as individual doses had to be applied for under the hospital drugs and therapeutics program. Cost constraints to clinical decision making were severe prior to Medicare funding of anti TNF agents.

The major findings were that screening for LTBI was performed in approximately 75% of patients, however there was one case of pulmonary tuberculosis in a patient born in Chile in whom screening for LTBI was ordered by clinicians, though unfortunately not performed prior to infliximab administration. Screening for hepatitis B and hepatitis C was performed infrequently (11.3%). Infusion reactions were common, occurring in approximately 15% of infusions, higher than previously reported. A small number of severe infusion reactions were recorded (3 out of 53), including the unusual reactions of Stevens-Johnson syndrome and the development of bilateral parotidomegaly in another case. The latter case was described in a publication submitted to the journal *Inflammatory Bowel Diseases*, and is enclosed as an attachment.

The higher than expected rate of infusion reactions may well reflect the widespread use of episodic treatment of patients in this audit, which leads to a higher risk of infusion reactions not probably due to the development of antibodies to infliximab (ATIs). The development of ATIs can be reduced by scheduled maintenance treatment, and is likely reduced by concomitant use of immunomodulators, which was variable in this group of patients. The reduced development of ATIs is likely to lead to fewer patients losing efficacy to infliximab, however this parameter could not be captured in this study. The use of premedication including hydrocortisone and antihistamines was also variably used, though the evidence base to support their use in reducing infusion reactions is slim. In this study, the use of promethazine was associated with a higher rate of infusion reactions, the use of hydrocortisone with a lower rate of reactions, and cetirizine was not associated with any reactions. There are possible confounders: it is possible that patients on maintenance therapy were more likely to be given hydrocortisone and cetirizine, as the use of
promethazine had in general been replaced with these agents for scheduled outpatient infusions.

On the basis of this audit, new departmental guidelines were written regarding the management of infusion reactions, the use of pre-medication prior to infusions, and screening for latent infections prior to the use of anti-TNF agents. This audit could have been strengthened if it had been performed prospectively, and the measurement of either ATIs or infliximab trough levels could have been correlated with the development of infusion reactions and clinical efficacy to provide more insight into this process.

**Development of a clinical decision support system (CDSS)**

The survey of gastroenterologists in Australia, and the audit of the use of infliximab in a tertiary hospital led to the clear recognition that screening for latent infections including LTBI and HBV in patients with IBD was being performed suboptimally, with potential for significant adverse events as a consequence. In terms of strategies to improve the uptake of screening in a tertiary hospital, initially new guidelines were written however uptake of these guidelines was variable. An alternative approach was sought, and the development of a clinical decision support system (CDSS) based on the existing Guidance framework previously used mainly for antibiotic stewardship was pursued.

The CDSS for infliximab asked the user questions about risk factors for LTBI and HBV, and suggested modalities of screening for these latent infections. Once the investigations were completed the system gave clinical guidance on how to interpret the results based on existing guidelines. Later, further modules were added to record information regarding patient phenotype and medications for potential research purposes.

The uptake of the use of this CDSS was regrettably low, and a number of factors are likely to have contributed to this. On reflection a more appropriate approach would be to interview all stakeholders including prescribing physicians and pharmacists to ascertain their views regarding screening of patients with IBD and potential barriers to treatment. If the development of a CDSS was thought to be a useful way forward by the majority of stakeholders, they should have been involved with the design of the CDSS modules and ongoing involvement and feedback should have been obtained.
Thiopurine methyltransferase (TPMT) genotyping and measurement of thiopurine metabolites

The second part of the investigations presented in this thesis related to aspects of safety and quality standards in the management of IBD patients by the author and also by an expert group of Australian gastroenterologists. This study analysed a subset of 42 patients recruited by the author from a single centre, forming part of a multi-centre study assessing patients with IBD on an established dose of either azathioprine or 6-mercaptopurine. Results of the multi-centre study were published in the journal *Pharmacogenetics* and this paper is included as an attachment. Clinical information was collected for each patient, including disease activity (using the Harvey-Bradshaw index in the case of Crohn’s disease, or the simple clinical colitis activity index (SCCAI) in the case of ulcerative colitis), disease phenotype and routine laboratory test results. Patients underwent testing with genotyping for common mutations of the *Thiopurine methyltransferase* (TPMT) gene, which encodes for an enzyme with the same name that is pivotal in the metabolism of these thiopurine drugs. In addition, patients were tested for the thiopurine metabolites 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP). 6-TGN is comprised of a group of metabolites believed to be the active metabolites which exert their effect directly on lymphocytes.\(^{46}\,^{47}\) 6-MMP is a byproduct of metabolism which has previously been associated with the development of abnormal liver function tests and is regarded as a marker of potential toxicity.\(^{48}\)

9.52% of patients had a heterozygous mutation of TPMT, consistent with published prevalence of approximately 10% in the Caucasian community (there are significant differences in the incidence of mutations in different racial groups). As expected given the absence of severe myelosuppression in series, no patient had a homozygous mutation of TPMT given homozygous mutations normally cause severe myelosuppression when thiopurine drugs are prescribed. The mean adjusted dose of thiopurine was slightly higher in heterozygotes compared to normal ‘wild type’ homozygotes; 1.635 mg/kg compared to 1.576 mg/kg. This was a surprising result as previous studies have suggested that TPMT heterozygotes normally are less tolerant of higher doses of thiopurines compared to wild type genotype subjects. However this may be due to lack of awareness by the treating clinician of the genotype, or the presence of a threshold for adverse effects such that only a proportion of heterozygotes will develop clinically significant side effects. Nevertheless, some authors suggest that patients with a heterozygous mutation should be started at half
the normal dose and it is generally accepted that heterozygotes should be monitored very carefully for potential adverse effects.\textsuperscript{491}

6-TGN results for each patient were categorised according to whether they were below, within or above the suggested therapeutic range of 150 - 450 pmol / 8x10\(^8\) RBC, based on previous studies showing reduced efficacy below 150 pmol / 8x10\(^8\) RBC, and myelotoxicity above 450 pmol / 8x10\(^8\) RBC. When these were correlated with thiopurine drug dose in mg/kg, the differences between the three groups were not statistically significant. This is again surprising, as one would expect a correlation between adjusted drug dose and 6-TGN levels. This underscores the fact that multiple enzymes are involved in the metabolism of thiopurine drugs in addition to \textit{TPMT}. Another explanation may be that the sample size was relatively small.

There was a weak correlation (\(r = -0.223\)) between 6-TGN levels and clinical activity in CD and UC, whether measured by Harvey-Bradshaw index or the SCCAI. Again, this is a surprising result as previous studies have suggested those patients with 6-TGN levels below 150 pmol / 8x10\(^8\) RBC as a group have higher clinical activity compared to those with levels above this. When 6-TGN levels were correlated with specific laboratory parameters, there was no correlation between 6-TGN levels and mean cell volume (MCV), arguing against the conventional dogma that patients with therapeutic thiopurine doses have a high MCV. When 6-MMP levels were compared to alanine transaminase (ALT) levels, there was again only a weak correlation (\(r = 0.3558\)) between the two. Previous studies have suggested that patients with 6-MMP levels of over 5700 pmol / 8x10\(^8\) RBC are more likely to develop hepatotoxicity; however this was not reflected in this study, perhaps due to the small sample size. This may relate to the fact that only a few patients had ALT levels above the reference range, and only two patients had ALT levels over 100 units. It is worth noting that of the two patients with 6-MMP levels over 7500 pmol / 8x10\(^8\) RBC, one had an ALT over 100 whereas the other had an ALT within the normal range. In this study population, it may be that patients with elevated ALT levels either already had their dose of thiopurine reduced as a consequence, or the medication may have been ceased without the investigator's knowledge. As a consequence these patients may not have been captured in this study. Other causes of elevated ALT could also have been at play (for example, non-alcoholic fatty liver disease).

Thus, this study does not support a number of previous findings including association between \textit{TPMT} genotype and thiopurine dosage, and even association between drug
dosage and 6-TGN levels. No correlation was seen with 6-TGN level and clinical markers of disease activity, or mean cell volume. 6-MMP levels did not correlate with liver function test abnormalities. Although these results argue against the use of both TPMT genotyping and measurement of thiopurine metabolites in routine clinical practice, there is the caveat that due to study design, patients with poorly controlled disease activity or previous adverse events such as intolerance to thiopurines or abnormal laboratory markers may have already had their dosage adjusted or the medication ceased.

**CONCLUSION**

The contribution of the gastrointestinal microbiota to the pathogenesis of IBD may involve a single pathogenic organism or alterations in the composition of the normal microbiota termed ‘dysbiosis’. This thesis does not support a role for the organism *Mycobacterium avium* subsp. *paratuberculosis* (MAP) or other single microorganisms in the pathogenesis of IBD. A metagenomic approach using an oligonucleotide microarray suggests that the composition of both CD and UC differs from control patients, and that inflammation is not likely to be playing a confounding role. A number of specific associations were found with either Crohn’s disease or ulcerative colitis (including decreased low abundance of *F. prausnitzii* as described previously). The role of these organisms needs to be further delineated using a larger sample size with more specific testing modalities such as a polymerase chain reaction probe specific for each organism. Overall, the findings in this thesis support an association between IBD and alterations in the normal microbiota but do not answer the important question of whether these changes contribute to the pathogenesis of IBD. This will require further studies to examine the relationship between colonic microbiota and disease onset, severity, and clinical activity. Such studies are beyond the scope of this thesis. Nevertheless these studies are important because they could lead to new therapeutic modalities and possibly also to prevention of clinical relapses or even prevention of disease in genetically susceptible individuals.

The use of a survey of gastroenterologists in Australia and an audit of the use of infliximab in a tertiary hospital suggest that, at least at the time of these audits and questionnaires, screening for latent infections was performed suboptimally, and the methods used to screen varied significantly. In addition, vaccination of patients with IBD to reduce the rate of infections in this group of patients commonly treated with immunosuppressive medications was also suboptimally performed, and suffered from a lack of consistent national and international guidelines. The development of formal guidelines, possibly
through the vehicle of the professional body GESA is a potential way forward to ensure more consistency in practice. Clear guidelines for clinicians based on higher levels of evidence (than expert opinion) will lead to better outcomes for patients and may also be more cost-effective for management of IBD patients.

The use of pharmacogenetic testing with $TPMT$ genotyping and the use of thiopurine metabolites has grown in popularity with the aim of improving the quality and safety of treatment. In this thesis, the results do not support the use of these investigations in patients with stable doses of thiopurines; however the study design may have excluded patients who could have benefitted the most from these investigations. The lack of correlation with the active metabolite 6-TGN and disease activity and drug dosage was very surprising and not consistent with previous literature. In addition the lack of correlation with 6-MMP and liver function tests was also not consistent with previous literature. Thus, the findings of the investigations reported in this thesis question whether these tests have a useful and cost-effective role in clinical practice. Further studies are needed to resolve this. Outside the parameters of this study, the ideal use of thiopurine metabolites may be in patients who have poorly controlled disease activity or in patients who have developed abnormal liver function tests on a thiopurine drug. This may provide surrogate information suggestive of poor adherence, sub-therapeutic dosage, or refractory disease thus assisting in decisions about the dose of medication or indeed whether they should be ceased. High 6-MMP levels may suggest drug hepatotoxicity, whilst low levels may suggest that an alternative cause for the abnormal liver tests should be sought.

The use of a clinical decision support system (CDSS) is an approach with considerable promise, given the increasing complexity of IBD treatments and the increasing use of information technology and electronic health records. In this thesis, a CDSS was carefully developed, however the uptake was poor, probably because key stakeholders were not consulted prior to the development of the CDSS or on an ongoing basis. This alternative framework would be more appropriate if development of a CDSS is considered in the future. The implementation of an IBD clinical nurse specialist (CNS) is pivotal in a busy gastroenterology service where many patients are on anti-TNF treatments, and provides an important way of managing the complex requirements of screening for latent infections, organising ongoing treatment logistics such as meeting Pharmaceutical Benefits Scheme regulatory requirements, and monitoring side effects of pharmacological treatment.
The studies reported in this thesis show that changes in colonic microbiota are a feature of IBD patients. These changes are worthy of further study because they may lead to a better understanding of the pathogenesis of IBD and to novel therapeutic approaches. The studies also highlight the lack of consensus in pharmacological management of IBD patients and in particular in screening potentially immunosuppressed patients for preventable diseases. The studies emphasise the need for agreement on, and dissemination of, clinical guidelines for clinicians. Such guidelines should also be made available to general practitioners and to the patients themselves.

The future of IBD treatment will depend on a better understanding of the pathogenesis of these common diseases, achieving an evidence-based consensus in the use of potentially toxic medications, and maintaining safety and quality standards in the overall management of patients with these diseases. The present study has explored particular aspects of each of these areas and uncovered opportunities for further investigation.
REFERENCES


52. Simms LA, Doecke JD, Walsh MD, Huang N, Fowler EV, Radford-Smith GL. Reduced α-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. Gut 2008;57:903-10.


139. Zoetendal EG, Akkermans ADL, De Vos WM. Temperature Gradient Gel Electrophoresis Analysis of 16S rRNA from Human Fecal Samples Reveals Stable and Host-Specific Communities of Active Bacteria. Applied and Environmental Microbiology 1998;64:3854-9.


221. Verschoor CP, Pant SD, You Q, Schenkel FS, Kelton DF, Karrow NA. Polymorphisms in the gene encoding bovine interleukin-10 receptor alpha are associated with Mycobacterium avium ssp. paratuberculosis infection status. BMC genetics 2010;11:23.


424. Carroll MB, Bond MI. Use of Tumor Necrosis Factor-α Inhibitors in Patients with Chronic Hepatitis B Infection. Seminars in arthritis and rheumatism 2008;38:208-17.


431. Benjamin A. Kupronis CLRJCGW. Invasive Pneumococcal Disease in Older Adults Residing in Long-Term Care Facilities and in the Community. Journal of the American Geriatrics Society 2003;51:1520-5.


Chande N, Tsoulis DJ, MacDonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. Cochrane database of systematic reviews 2013;4:CD000545.


Sorrentino D. Role of Biologics and Other Therapies in Stricturing Crohn's Disease: What Have We Learnt So Far? Digestion 2008;77:38-47.


Han PD, Cohen RD. Managing Immunogenic Responses to Infliximab: Treatment Implications for Patients with Crohn's Disease. Drugs 2004;64:1767-77.


595.  Collins SM. A role for the gut microbiota in IBS. Nat Rev Gastroenterol Hepatol 2014;advance online publication.
APPENDIX


3. Bilateral Parotidomegaly Following Anaphylaxis to Infliximab. *Inflammatory Bowel Diseases*, 2009

Title:
Understanding the role of infections in the pathogenesis of inflammatory bowel disease, and improving the quality and safety of treatment

Date:
2014

Persistent Link:
http://hdl.handle.net/11343/48440

File Description:
Part 3