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Risk Assessment in Acute Ulcerative Colitis

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Faculty of Medicine

Abstract

An episode of acute severe ulcerative colitis (UC) represents an important watershed moment during the course of the disease with a heightened risk of colectomy during and following these episodes. As such, the prompt identification and early implementation of the appropriate treatment is paramount to obtaining the best clinical outcomes for these unwell patients.

A prospective database of 349 consecutive presentations of Truelove and Witts criteria qualifying moderate and severe UC was collated at a single referral centre.

This resource was interrogated to identify the C-reactive protein (CRP) level that corresponds with the erythrocyte sedimentation rate (ESR) that is widely accepted as a marker of severe disease activity. A CRP threshold of ≥12mg/L was found to be an inclusive and sensitive cut off that when incorporated into the Truelove and Witts criteria, replacing the traditional ESR >30mm/h criterion, had similar performance characteristics when applied to the assessment of UC disease activity.

On the level of the individual patient, the serial CRP concentration is one of the few informative and objective measures of disease acuity and treatment response that will alter management during the hospital admission. This study provides the previously lacking reassurance that baseline immunomodulator usage status need *not* be taken into account when interpreting the ESR or CRP levels on presentation in established UC patients not on biologic therapy.

As many published IBD severity and prognostic indices in contemporary use incorporate either the ESR or CRP as objective markers of disease acuity, this study has high translatability to patients with a secure diagnosis of UC not in their initial flare.

Seventeen clinical, laboratory and endoscopic variables present at the time of hospital presentation were assessed for their ability to differentiate intravenous corticosteroid therapy responders from non-responders.

A risk score based on a logistic model including the admission indices of oral corticosteroid failure, bowel frequency, CRP, albumin and either disease duration or Mayo endoscopic

subscore (MES) was trained on a set of 349 presentations of acute UC. This individualised risk score has the potential to inform clinicians as to the timing of treatment escalation in acute UC.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

No publications included.

Submitted manuscripts included in this thesis

No manuscripts submitted for publication.

Other publications during candidature

Conference abstract (see Appendix B for full text):

Croft AR, Lord A, Radford-Smith GL Su1827 - Markers of Systemic Inflammation in Moderate-Severe Ulcerative Colitis: What Level of C-Reactive Protein Constitutes Acute Severe Ulcerative Colitis? *Gastroenterology* May 2018, Volume 154, Issue 6, Supplement 1, Page S-598

Contributions by others to the thesis

This thesis is based on a large body of work which precedes it. The data is drawn from a prospective inflammatory bowel disease database set up by Associate Professor Graham Radford-Smith in 1996. It has been accumulated over the years by members of the inflammatory bowel disease team, including the author from the year 2007.

Co-supervisor Dr Anton Lord provided statistical training and assisted with writing code for the more complex statistical analysis including the alternative analytical approaches undertaken in Chapter 3.

During the course of this degree the author completed a three day introductory course to *R* programming provided by QFAB Bioinformatics.

Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research Involving Human or Animal Subjects

The approving ethics committee for this research was the Royal Brisbane and Women's Hospital Human Research Ethics Committee.

The HREC Ref No was HREC/14/QRBW/323. Please see Appendix C for a copy of the research ethics approval letter.

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I would like to thank my supervisor Associate Professor Graham Radford-Smith for his inspirational dedication to research in the field of gastrointestinal diseases. His mentorship has fostered and focussed my abilities both as a researcher and as a clinician.

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To the patients of the IBD programme and Gastroenterology Department at the Royal Brisbane and Women's Hospital I owe a debt of gratitude for your ongoing and productive involvement in research.

Finally, I would like to thank my wife Celeste for her support and determination during the challenge that is undertaking a higher degree by research.

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Keywords

acute severe ulcerative colitis, C-reactive protein, colectomy, corticosteroid, erythrocyte sedimentation rate, immunomodulator, inflammation, risk prediction

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 110307: Gastroenterology and Hepatology Clinical sciences, 100%

Fields of Research (FoR) Classification

FoR code: 1103 Clinical sciences, 100%

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List of Figures & Tables

List of Abbreviations used in the thesis

AUC/AUROC: Area under the (receiver operator) curve

CAR: C-reactive protein to albumin ratio

CD: Crohn's disease

CRP: C-reactive protein

ECCO: European Crohn's and Colitis Organisation

ESR: Erythrocyte sedimentation rate

IBD: Inflammatory bowel disease

IQR: Interquartile range

MELD: Model for end-stage liver disease

MES: Mayo endoscopic subscore

MCV: Mean corpuscular volume

NPV: Negative predictive value

PPV: Positive predictive value

RMSD: Root-mean-square difference

SLE: Systemic lupus erythematosus

TNF-α: Tumour necrosis factor alpha

TWC: Truelove and Witts Criteria for acute ulcerative colitis

UC: Ulcerative colitis

UCEIS: Ulcerative colitis endoscopic index of severity

UK: United Kingdom

Chapter 1

The evaluation of C-reactive protein as a marker of disease severity in acute severe ulcerative colitis

1.1 Literature review

Introduction

An episode of acute severe ulcerative colitis (UC) complicates the disease course of a quarter of all patients with this diagnosis.[1] These exacerbations of disease activity represent an important watershed moment during the course of the disease. Studies with long follow up periods have shown that the risk of colectomy is elevated for 18 months to two years following an acute episode.[2, 3] While this risk is modulated by many parameters, a key variable that was identified as early as 1955 is that of disease activity and systemic toxicity at the time of treatment initiation.[4] A snapshot of disease activity at this juncture also carries with it significant prognostic power over the more immediate clinical outcomes at the time of discharge.[1] Therefore, the early and accurate identification of severe UC is imperative for timely commencement of the appropriate treatment to minimise the risk of colectomy in both the short and long term.

The Truelove and Witts criteria for ulcerative colitis.

There are a range of disease severity indices that are currently in use in both clinical and research settings.[5, 6] The original Truelove and Witts criteria (TWC) disease activity index was used for the final report on one of the first randomised controlled trials in the then nascent field of gastroenterology.[4] Its subject was the comparison of the effect of intravenous corticosteroids with placebo in the setting of mild-severe UC exacerbations.

In addition to the mandatory criteria of six or more bloody liquid stools within 24 hours there is a requirement for at least one of: tachycardia (heart rate >90beats per minute), fever (temperature >37.8°C), anaemia (haemoglobin <75% of the normal range, taken at 105 mg/L) or systemic inflammation (erythrocyte sedimentation rate (ESR) of >30mm/h). Mild disease episodes are assessed as ≤4 stools per day without any of the above additional criteria. Moderately severe cases are those that fall between mild and severe criteria. There is no mention in this publication or any supplementary material that indicate how these indices were derived or validated in a validation cohort. Indeed, the original authors used clinical experience to guide selection of the criteria and their binary cut off thresholds.

Despite this the TWC has a number of strengths that have contributed to its longevity. The variables in this index are all objective except for the reliance on patient reporting of stool frequency and blood content in the 24 hours preceding presentation. The special investigations are affordable, routinely performed and well within the repertoire of most accredited laboratories. Limitations of

the criteria include the fact that the TWC allocates patients into severity categories in a non-linear fashion using cut off values. For instance a stool frequency of six or 20 bloody stools per day are effectively given the same weight.

Additionally, ESR has been supplanted by the C-reactive protein (CRP) as the marker of choice for the assessment of acute systemic inflammation. When the ESR is not assessed soon after presentation the criteria are incomplete and can be difficult to apply in circumstances when no other criteria in addition to the mandatory stool frequency criteria are fulfilled. This situation contributes to the difficulty in assessing these patients via telephone when they are referred for consideration of transfer from secondary referring hospitals.

Erythrocyte sedimentation rate

This test was developed in 1897 and is the measurement of the vertical descent of erythrocytes in a column of plasma over one hour. The result of the test reflects the balance between prosedimentation factors including those promoting rouleaux formation and anti-sedimentation factors. The zeta potential is the term for the electrostatic force repelling erythrocytes from each other. This is due to negatively charged sialic acid residues present on the erythrocyte membrane. In the setting of inflammation, the increase in fibrinogen and gamma globulins in the plasma reduces the zeta potential contributing to increased rouleaux formation.

Due to its very nature the ESR is affected by many parameters (Table 1.1). In patients presenting with severe UC the most important are anaemia, which is often microcytic, and a low plasma protein concentration. ESR is inversely proportional to haematocrit and is an exponential function of the plasma colloid concentration.[7]

Table 1.1 Factors known to influence ESR

Factors that increase ESR	Factors that decrease ESR
Anaemia	Microcytosis
Inflammation	Hypoproteinaemia
Female gender	Spherocytosis or acanthocytosis
Increasing age	Polycythemia
Macrocytosis	Hypofibrinogenaemia
Pregnancy	Hypogammaglobulinaemia
Malignancy	Vibration during testing
Tilted tube	Clotted sample or inadequate mixing

Adapted from: https://www.slideshare.net/RaviJain7/esr-9713905

C-reactive protein

First characterised in the early 1930s, CRP was found to react with the somatic C-antigen of *Streptococcus pneumoniae*.[8] Initially thought to be pathogenic, this annular, pentameric protein was later proven to be natively synthesised in the liver. It is released in response to proinflammatory cytokines including IL-6, IL-1β and TNFα released from macrophages and T-cells as part of the acute phase response. It plays an important part of the innate immune system by binding to phosphocholine on the surface of microorganisms and inducing activation of the classical complement pathway.

As UC is primarily a disease of the colonic mucosa, active disease results in relatively lower production of acute phase cytokines compared to those seen in the transmural inflammation characteristic of active Crohn's disease (CD).[9] This can commonly result in the return of CRP tests within the normal range in the setting of clinically active UC.

For a diagnosis of acute severe UC the ECCO guidelines indicate that the ESR or CRP are equivalent at a cut off value of >30mm/h or >30mg/L respectively to qualify as an additional TWC.[10] The UK IBD audit used the hurdle of a CRP >10mg/L for qualifying for acute severe UC.[11] Neither of these cut offs have been presented with data detailing their derivation.

Comparison of inflammatory markers in ulcerative colitis

The most thorough and contemporaneous study in ulcerative colitis patients was performed in the paediatric setting by Turner *et al*.[12] These investigators used retrospective data to determine levels of ESR and CRP corresponding to clinical activity, defined as remission, mild, moderate and severe

disease, by comparing them to the previously validated paediatric UC activity index (PUCAI) score, endoscopic activity and other indices of UC activity (Figure 1.1). Despite the lack of a direct comparison between the ESR and CRP values, this study was informative.

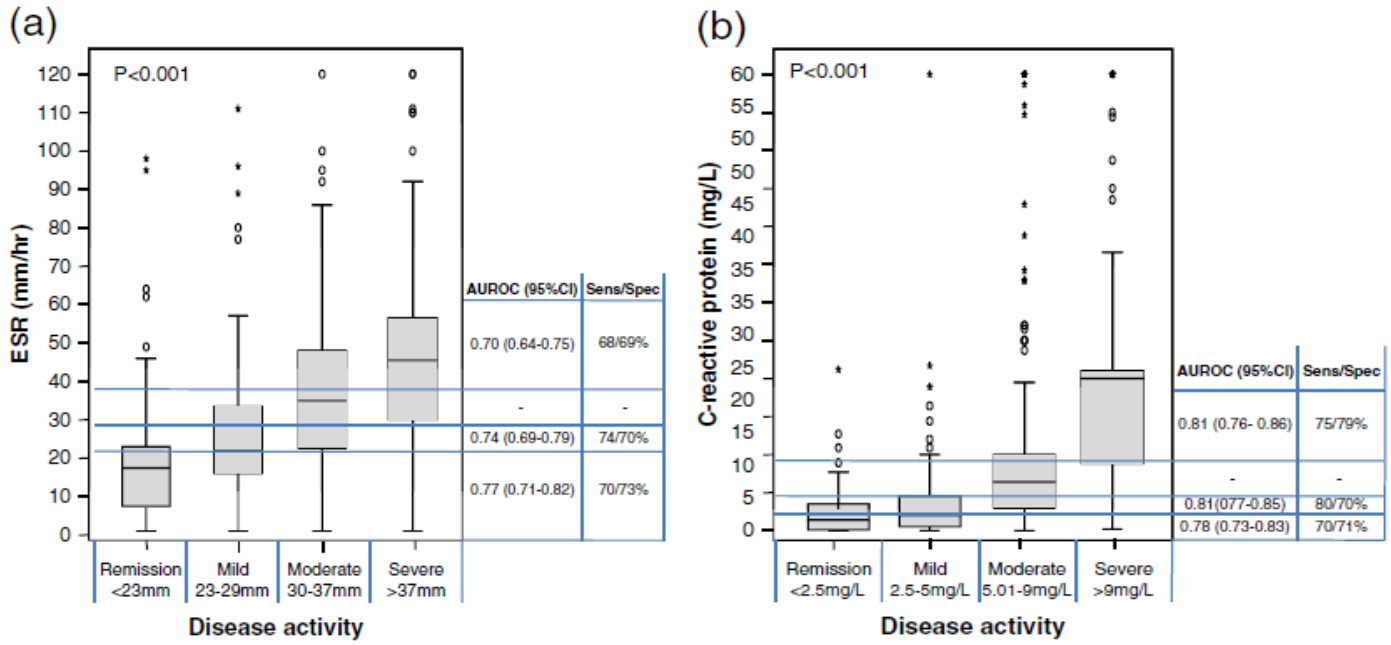

Figure 1.1 ESR and CRP compared to disease activity by PUCAI score[12]

Overall CRP was found to have a slightly higher AUROC and mean Spearman's correlation coefficient than ESR when it was compared to a number of activity indices (Table 1.2). CRP was also found to be better at discriminating severe from moderate disease with a threshold of >9mg/L for severe disease compared to an ESR of >37mm/h. At the other end of the disease activity spectrum ESR was found to have more discriminating power between remission and mild disease than CRP. These results contrast findings in Crohn's disease where CRP is markedly superior as a marker of disease activity.[13, 14]

In the longitudinal component of this study, when one of ESR or CRP were found to be associated with disease activity, serial measurement of the disease activity-associated test in isolation was able to accurately reflect disease activity in 85% of subsequent encounters. Higher readings of both ESR and CRP were found in extensive (Montreal classification E3) versus left-sided disease (Montreal classification E1-E2).

Table 1.2 Spearman's correlation of blood indices with constructs of UC disease activity[12]

Table 4 Spearman's correlation of common blood results with constructs of disease activity in pediatric ulcerative colitis (*P<0.05).

	ESR	CRP	Albumin	WBC	Hb	ANC	PLT
Colonoscopic score [a]	0.41*	0.55*	−0.56*	0.48*	−0.26*	0.34*	0.27*
PGA [b]	0.46*	0.61*	−0.66*	0.45*	−0.39*	0.45*	0.31*
Mayo score[36 a]	0.41*	0.43*	−0.57*	0.26	−0.21	0.10	0.23
PUCAI score[4]	0.46*	0.61*	−0.68*	0.50*	−0.43*	0.46*	0.35*
Lichtiger index[37]	0.39*	0.34*	−0.48*	0.28	−0.18	0.10	0.14
Average	0.43	0.51	−0.59	0.39	−0.29	0.29	0.26

PGA, physicians' global assessment; PUCAI, pediatric ulcerative colitis activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; Hb, hemoglobin.

[a] Available on the colonoscopy cohort only (n=76); colonoscopic score was performed according to Beattie et al.[7] in each colonic segment, as previously described.[4]

[b] Scored on a 100 mm visual analog scale.

In a 1982 study of 50 patients at Hammersmith Hospital, London nine patients were considered to have severe disease as per the Truelove and Witts criteria. Only 4/9 were admitted indicating either a high admission threshold or that some moderately severe cases were present in the series. In these severe patients the CRP median was 12mg/L (range, 2-33). ESR median 28mm/h (range, 10-59). A smaller study performed in India found that a CRP of ≥12mg/L was found in 14/17 patients with severe UC.

ESR and CRP were both reported in a retrospective study of 67 severe UC patients in Italy.[15] ESR and CRP levels at study entry were similar between the two groups separated by response (52 ± 29mm/h, 48.3 ± 47.5mg/L) or non-response to intravenous steroid therapy (80 ± 32mm/h, 97.3 ± 110mg/L), respectively.

There were no direct comparative analyses between ESR and CRP results in any of these early series.

Inflammatory markers in Crohn's disease

Early work by Tromm *et al* evaluated several inflammatory markers including ESR and CRP and correlated their levels with endoscopic appearance separating the participants on the basis of mild or severe ulceration.[16] With hindsight, the high rate of 37% of both groups having disease limited to the large bowel indicates a likely admixture of CD and UC. Unsurprisingly, higher readings of both inflammatory markers were found in the severe versus the mild ulceration and control groups. In a direct comparison between ESR and CRP the correlation coefficient was r=0.29. When the inflammatory markers were compared to the Crohn's disease activity index (CDAI) the coefficients were r=0.24 and r=0.37 respectively for ESR and CRP.

A randomised controlled trial of mesalazine for the prevention of CD flares following a course of oral corticosteroids generated data that enabled the investigators to construct a simple risk prediction model for CD flares occurring in the six week period following the collection of serially collected inflammatory markers.[17] The median CRP at the commencement of the study was relatively high at 15.2mg/L, ESR 18mm/h. This median CRP level is consistent with a degree of persistent disease activity at enrolment.

Of the 71 enrolled patients, 38 had a relapse during the 54 week study period. The biological prediction score was defined as either CRP >20mg/L and/or ESR >15mm/h. A positive prediction score conferred an eight times relative risk of CD flare compared to those patients with a negative risk score. A strength of this score is its negative predictive value of 97%. Unlike the previous study there was no direct comparison made between ESR and CRP.

ESR and CRP in other inflammatory diseases

In a study of over 700 rheumatoid arthritis patients the ESR and CRP were compared directly to an accepted index of disease activity.[18] As in most IBD studies there was no direct comparison between ESR and CRP.

CRP was found to be only slightly better as a measurement of inflammation while ESR, because it is affected by non-acute phase factors such as immunoglobulins and rheumatoid factor, may be a better measure of general disease severity.

In summary, there is a high degree of clinical relevance to the accurate and prompt identification of acute severe episodes of UC. ESR and CRP are both useful inflammatory markers that can assist in this differentiation. Key knowledge gaps include an evidence-based CRP cut off for the diagnosis of severe UC in adults to guide patient disposition and acute management.

1.2 Aims

- To determine the concentration of CRP corresponding to an ESR>30mm/h in adult patients presenting with acute severe colitis as defined by the Truelove and Witts criteria.

- To compare clinical outcomes between groups stratified for disease severity on the basis of number of Truelove and Witts criteria satisfied at presentation using either ESR or a newly derived CRP criterion.

1.3 Hypothesis

The equivalent CRP cut-off to an ESR of 30mm/h in severe UC will be between the already proposed CRP cut offs of >10mg/L and >30mg/L.

1.4 Methods

Patients

Individuals who presented to a single tertiary centre with acute severe UC by TWC during the study period were eligible for study inclusion. Initial presentations of UC were included as were those generated by known UC patients. The diagnosis of UC was confirmed by histology together with endoscopic, radiological and clinical correlation. Patients were required to have had a paired ESR and CRP result from within 24 hours of presentation to be included. Written informed consent was obtained from all patients included in this study and the study protocol approved by the Royal Brisbane and Women's Hospital medical ethics committee.

Patients on immunomodulators, oral corticosteroid and/or 5-aminosalicylates at the time of admission were included in the study. Patients were considered to be taking an immunomoudulator if they had been on a thiopurine for four months or more prior to presentation. Treatment adherence was monitored with thiopurine metabolite levels when these became available For the purpose of maintaining consistency across the cohort, those patients on any biologic therapy at the time of admission were excluded.

Laboratory tests

ESR tests were performed on the Ves-matic Junior 20 (Diesse Diagnostica Senese, Monteriggioni, Italy). CRP was assessed on the Roche-Hitachi 747 Analyzer (Roche Diagnostics, Mannheim, Germany, 1996-2008) and the Beckman-Coulter DxC 800 Analyzer, (Beckman-Coulter, Fullerton, USA, 2008-2017) platforms.

Statistics

R version 3.4.0 "*You Stupid Darkness*" (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

Each unique CRP level reported in the dataset was tested as a potential cut-off point to approximate an ESR of >30mm/h. For each tested cut-off, a confusion matrix was generated between ESR >30mm/h and the CRP cut-off. The positive predictive value (PPV) was then used to establish the lowest CRP cut off with a PPV >85%.

Chi-squared and Fisher's exact tests were used to compare corticosteroid therapy failure and colectomy rates between ESR>30 and CRP≥12 allocated groups.

Mann-Whitney U-tests were used to compare presentation faecal calprotectin and Mayo endoscopic subscores (MES) between groups separated by the inflammatory marker criteria used and stratified by the number of TWC satisfied at presentation.

See the Statistics subheading of the Methods section in Appendix A for a full description of the statistical methods undertaken in an attempt to obtain an expression that would accurately predict an ESR result based on the CRP adjusted for clinical variables available at the time of presentation. This analysis was performed in a larger (n=270) and more heterogeneous patient series.

1.5 Results

During the study period 349 presentations of moderate-severe UC were recorded. Of these, 204 presentations were considered to be severe by TWC and initially presented directly to the Royal Brisbane and Women's Hospital. Of these presentations, 163/204 had paired ESR and CRP levels recorded within 24 hours presentation (Table 1.3). These presentations were generated by 153 individuals. There were 41 patients who presented to the Royal Brisbane and Women's Hospital with severe UC who did not have an ESR recorded within the first 24 hours of admission (ESR negative group). There were no deaths of hospitalised patients as a consequence of UC, surgery or any subsequent complications.

Table 1.3. Acute severe UC patient characteristics on admission

	Study participants (n=163)	**ESR negative group (n=41)**	**p-value**
Gender (male)	85 (52%)	19 (46%)	0.51
Age at admission in years median (IQR)	33 (25-43)	35 (25-53)	0.45
Disease duration in years median (IQR)	2 (1.2-9.2)	1.5 (0-4)	0.004
First UC presentation Relapse	23 (14%) 140 (86%)	15 (37%) 26 (63%)	0.00095
Disease extent (Montreal classification) Proctitis (E1) Left-sided (E2) Extensive (E3)	 2 (1%) 55 (34%) 106 (65%)	 0 (0%) 19 (46%) 22 (54%)	0.85*
Immunomodulator on admission No Yes	 123 (75%) 40 (25%)	 27 (66%) 14 (34%)	0.21
Bowel frequency median (IQR)	10 (8-15)	10 (7-11)	0.081
CRP mg/L median (IQR)	58 (28-109)	60 (24-140)	0.74
Albumin g/L mean (Standard deviation)	32 (6)	30 (6)	0.14

UC: ulcerative colitis; TWC: Truelove and Witts criteria; CRP: C-reactive protein; IQR: interquartile range. *E1 and E2 disease was pooled for this analysis.

There was a higher rate of first presentations of UC in the ESR negative cohort (37%) compared to those with ESR recorded (14%; p=0.00095). This result contributed to the reduced UC disease duration prior to the severe UC episode in this cohort (median 1.5 versus 2 years, p=0.004).

In the paired ESR and CRP group there was a male predominance of 52% with a median age of 33 years at admission. Fourteen percent of cases were index (first) presentations of UC. The median time elapsed between UC diagnosis and admission was two years (range 0-45 years). Two thirds (65%) of cases had disease proximal to the splenic flexure. One quarter (25%) of cases were receiving systemic immunosuppression at the time of presentation.

Determining and applying the CRP cut off for the Royal Brisbane and Women's Hospital severe colitis n=163 cohort

Compared to the traditional ESR cut off of >30mm/h, the lowest CRP value that produces a PPV of 85% was ≥12mg/L. This threshold captures 95% of cases with an ESR >30mm/h. The area under the curve is 0.82. A cut off of 31mg/L generated a PPV of 87% (Table 1.5); this captured 81% of cases. The PPV is consistently above 80% for CRP cut off values from 20-200mg/L (Figure 1.2). The level of accuracy decreased markedly above a cut off of ≥12mg/L (Figure 1.3).

Table 1.4 CRP cut off positive predictive values for a paired ESR of >30mm/h

CRP cut off (≥mg/L)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
12	85	63	95	35	82
18	86	50	88	44	79
25	86	44	84	47	77
31	87	43	81	56	75

CRP: C-reactive protein; PPV: positive predictive value for CRP cut off having a paired ESR >30mm/h; NPV: negative predictive value for CRP cut off having a paired ESR >30mm/h

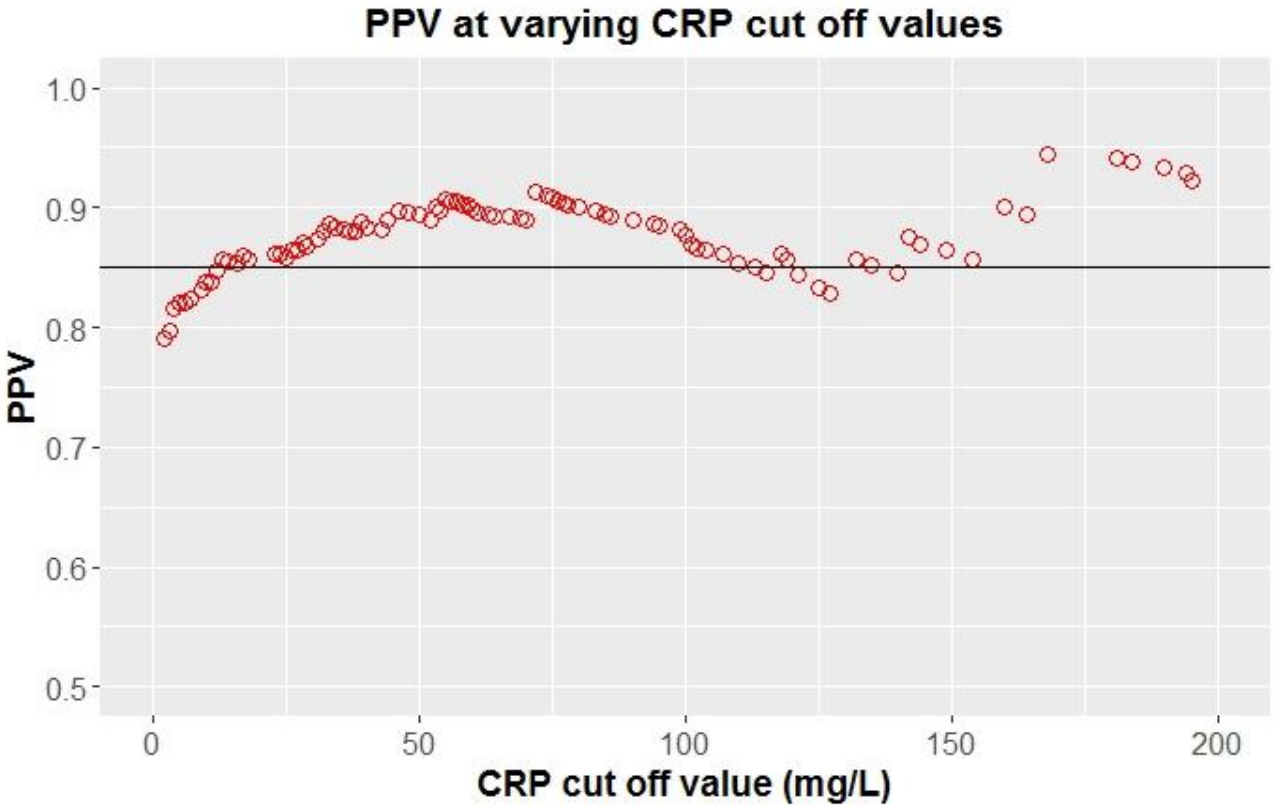

Figure 1.2 Positive predictive value for a matched ESR >30mm/h for a paired CRP cut off

The application of the new CRP≥12mg/L cut off to the data altered the total number of TWC fulfilled for 29/163 (18%) of patients (Figure 1.3). Conversely, 134 or 82% of presentations remained unchanged in their number of TWC satisfied on admission. The maximum total number of TWC fulfilled was five. This was assessed as a combination of the single mandatory stool frequency criteria with up to four additional criteria.

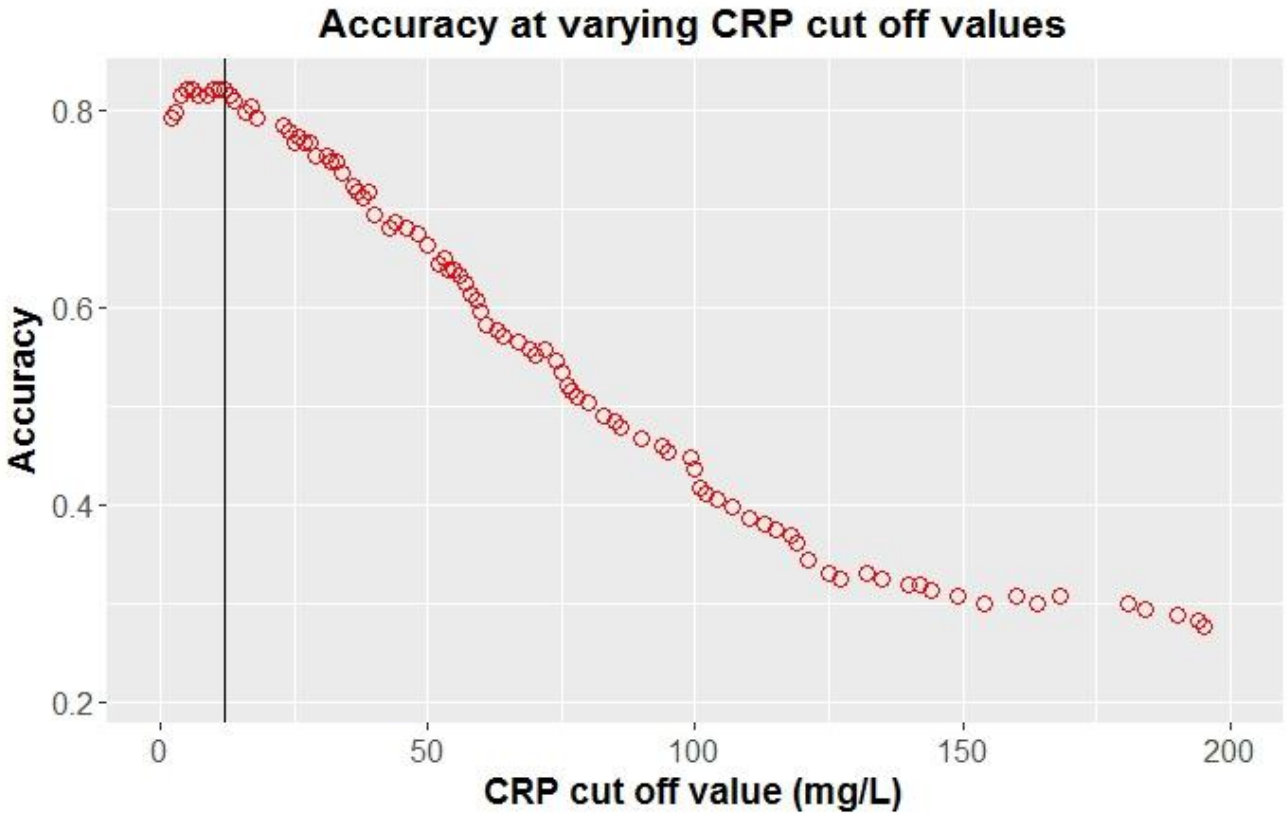

Figure 1.3 CRP accuracy at varying CRP cut off values.

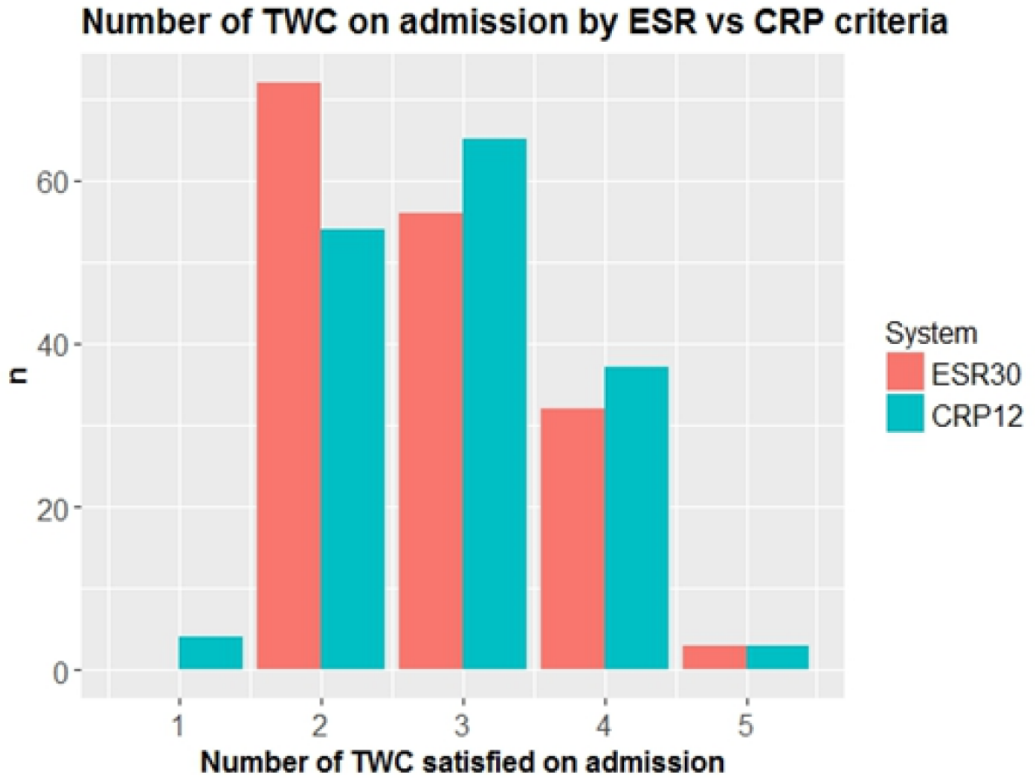

Figure 1.4 Number of Truelove and Witts criteria present at admission by ESR and CRP criteria. The four patients downgraded from TWC severe to moderate colitis when the CRP≥12mg/L criterion was applied in place of the ESR>30mm/h criterion only fulfil the bowel frequency criterion of the TWC and are represented by the green bar at the x-axis value of 1.

Twenty-two patients increased their total number of fulfilled TWC by one. Conversely seven patients had a decrease of total TWC by one. Overall, there was a net increase in the number of TWC satisfied in 15 (9%) patients when the CRP≥12mg/L criteria was applied. Four patients were downgraded from severe to moderate colitis. One of these patients underwent colectomy prior to discharge.

Clinical outcomes

Following the application of the CRP≥12mg/L cut off there were no statistically significant changes to the rate of corticosteroid therapy failure in any single group separated by the number of TWC met at admission (TWC 2/5, 3/5 or ≥4/5). Those patients who satisfied 4 or 5 TWC on presentation were combined to provide sufficient numbers for a meaningful analysis (Figure 1.5). When the different inflammatory marker systems were compared the p-values for the corticosteroid failure rate between TWC categories 2/5, 3/5 and ≥4/5 combined were 1, 0.71 and 0.88, respectively.

There were no statistically significant differences by Chi-squared test in the colectomy-by-discharge rate by number of TWC satisfied on admission between the ESR>30 and CRP≥12 criteria (Figure 1.6). TWC categories 4 and 5 were combined to provide sufficient numbers for the analysis. The p-values for TWC categories 2, 3 and ≥4/5 were 1, 1 and 0.96, respectively.

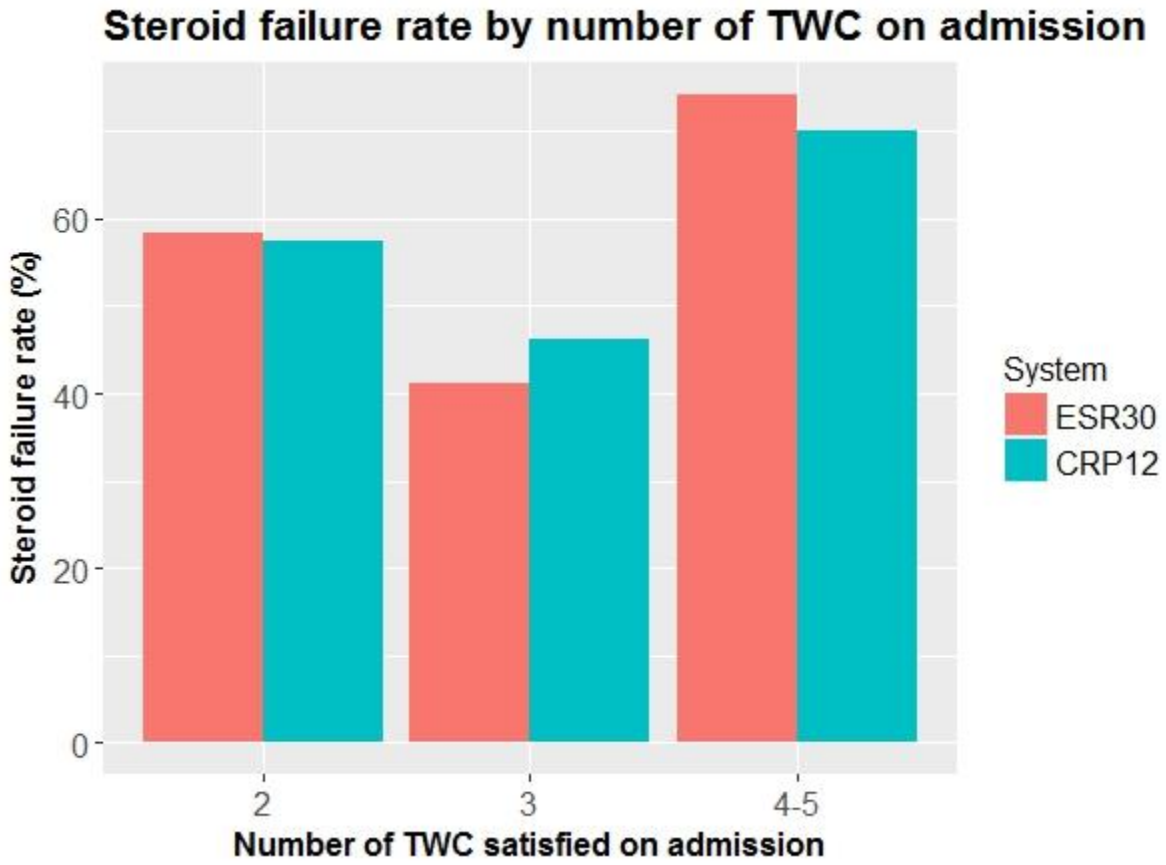

Figure 1.5 Corticosteroid failure rate by number of TWC and inflammatory marker allocation system

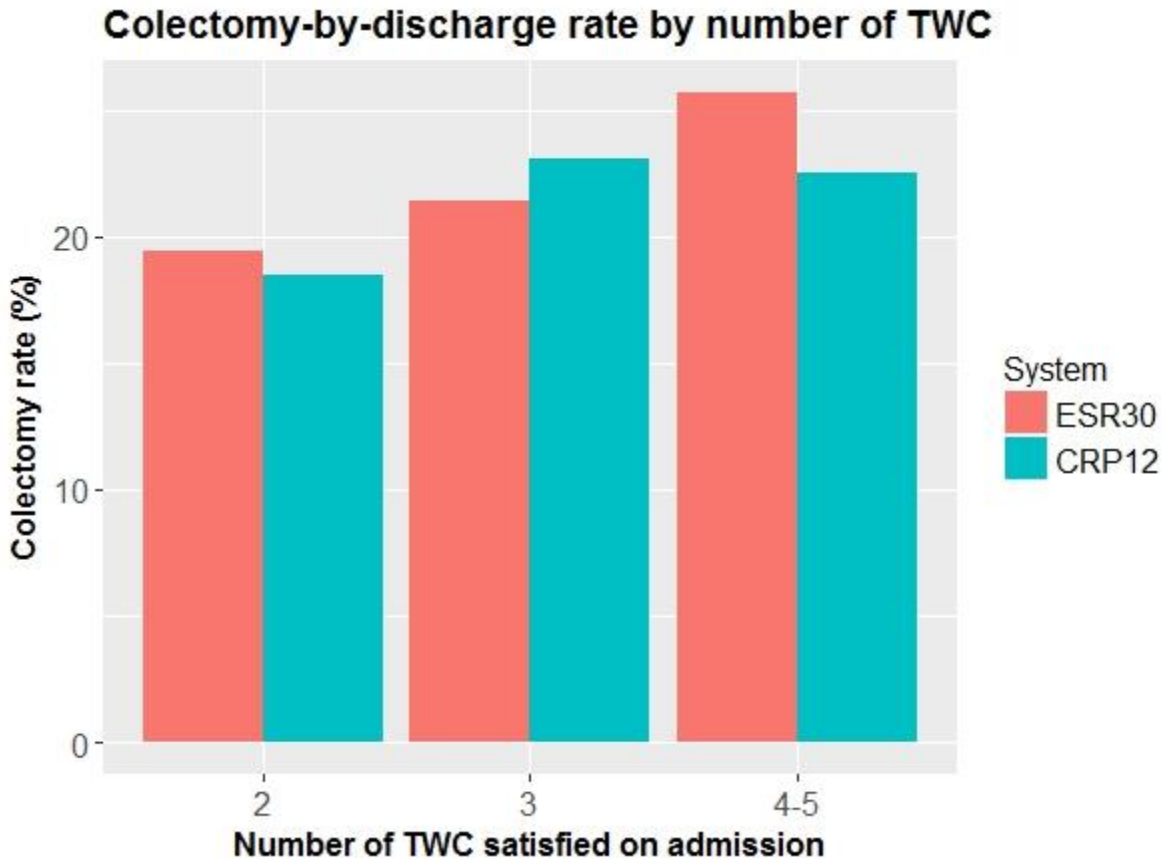

Figure 1.6 Colectomy-by-discharge rate by number of TWC fulfilled at admission and inflammatory marker allocation system

Other markers of disease severity on admission to hospital

Faecal calprotectin

Faecal calprotectin results were available for 57 patients with ESR criteria and the 55 patients with CRP criteria qualifying severe colitis (Figure 1.7). The median calprotectin in the TWC 2/5 group was 1750µg/g (n=22, IQR 870-4325µg/g) and 2550µg/g (n=16, IQR 1135-4400µg/g) for ESR>30 and CRP≥12 criteria, respectively. The median calprotectin in the TWC 3/5 group was 1800µg/g (n=21, IQR 530-3900µg/g) and 1600µg/g (n=25, IQR 530-4600µg/g) for ESR>30 and CRP≥12 criteria, respectively. The median calprotectin in the TWC ≥4/5 group was 2700µg/g (n=14, IQR 1475-3950µg/g) and 2700µg/g (n=14, IQR 1475-3950µg/g) for ESR>30 and CRP≥12 criteria, respectively.

There were no statistically significant differences between ESR>30 and CRP≥12 allocated groups by TWC satisfied at admission. The p-values were 0.68, 0.86 and 0.72 for TWC 2/5, TWC 3/5 and TWC ≥4/5, respectively.

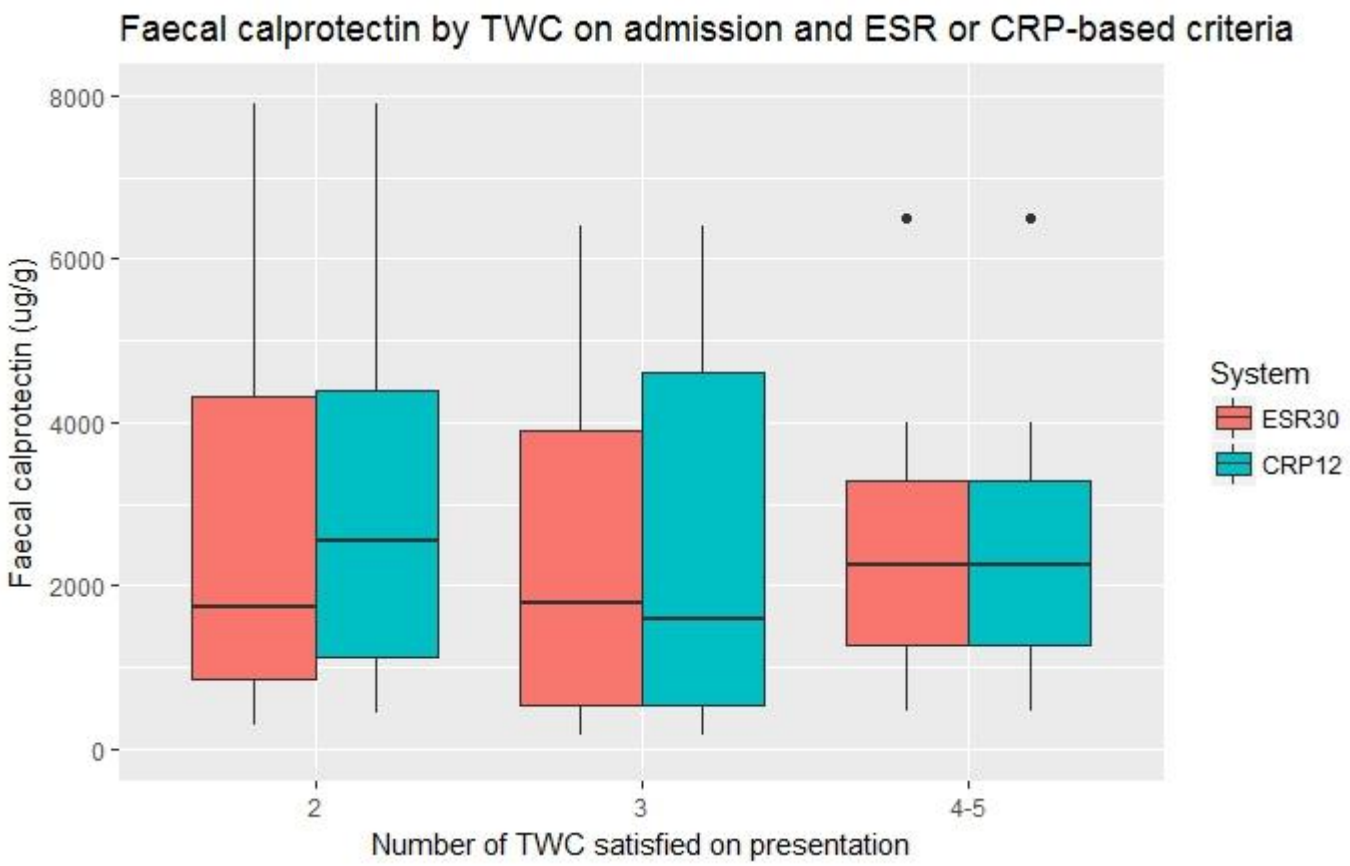

Figure 1.7 Faecal calprotectin at presentation by TWC activity on presentation and inflammatory marker allocation system. (ESR>30 n=57, CRP≥12 n=55; four data points not visualised due to y-axis limitation of 8000µg/g).

Mayo endoscopic subscore

Mayo endoscopic subscore results were available for 118 TWC severe UC patients by ESR>30 criteria (n=2, 8, 53, 55 for MES 0-3, respectively) and 114 with TWC severe UC patients by CRP≥12 criteria (n=2, 8, 51, 53 for MES 0-3, respectively; Figure 1.8). Grouped by the number of TWC present at admission there were n=TWC 2/5: 56, 39; TWC 3/5: 37, 49; TWC ≥4/5: 25, 26; by ESR>30 and CRP≥12 criteria, respectively.

There were no statistically significant differences between ESR>30 and CRP≥12 allocated groups by TWC satisfied at admission. (TWC ≥4/5 groups combined for this analysis). The p-values were 0.57, 0.70 and 0.66 for TWC 2/5, TWC 3/5 and TWC ≥4/5, respectively.

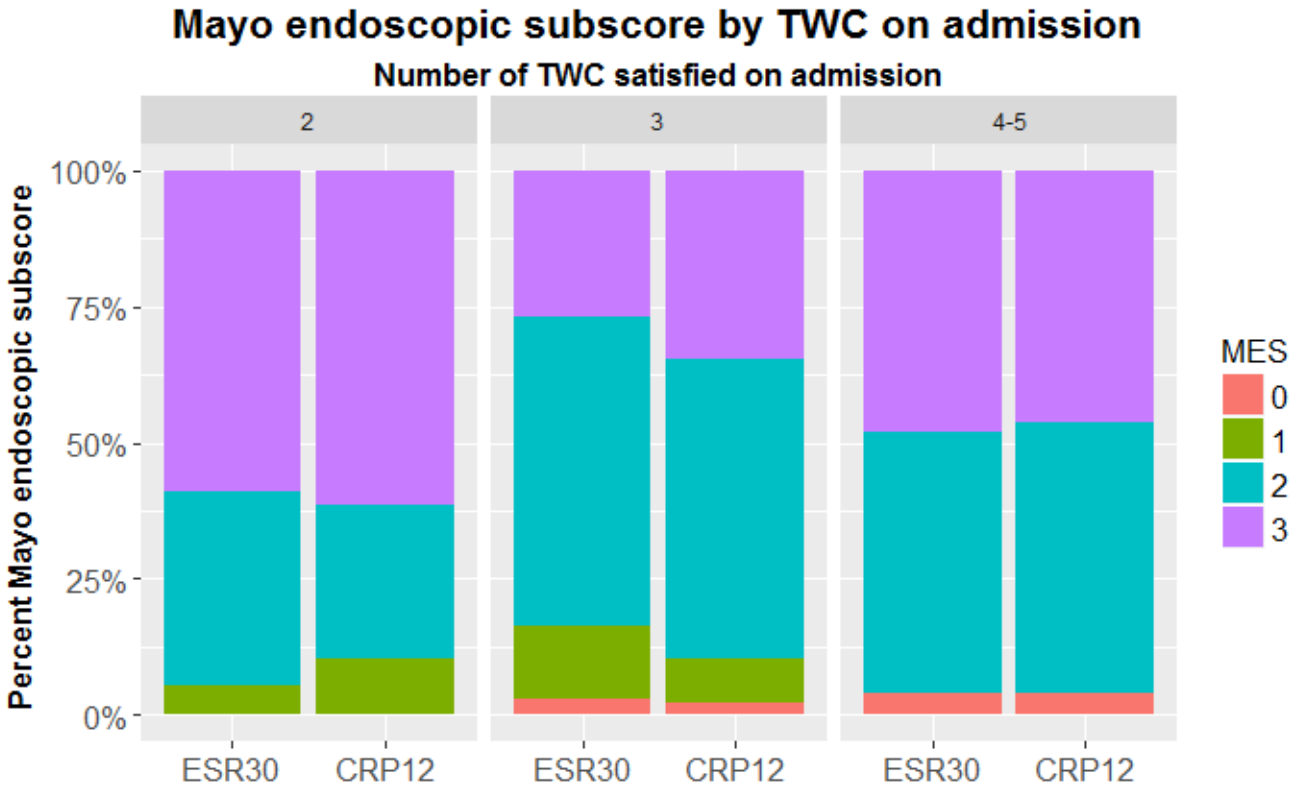

Figure 1.8 Mayo endoscopic subscore at presentation by TWC fulfilled and inflammatory marker allocation system

1.6 Discussion

The primary finding of this observational study is the establishment of an evidence-based CRP equivalent to the previously used ESR>30mm/h cut off proposed by Truelove and Witts in 1955 to be used as an additional criterion for the classification of UC disease severity. This derivation was performed in a real-world data set of 163 presentations of severe UC as assessed according to the historical TWC. As encountered in clinical practice, there was a wide spectrum of both UC disease activity and systemic toxicity observed in the presenting patients.

The proposed CRP cut off of ≥12mg/L is an inclusive and conservative additional Truelove and Witts criterion capturing 95% of patients with a paired ESR of >30mm/h. When compared to the ESR-based criterion it resulted in a net increase in the number of TWC satisfied in 9% of presentations.

When the important clinical outcomes of corticosteroid therapy failure and inpatient colectomy were considered, no statistical differences were observed between the ESR>30 and CRP≥12 allocated groups. From this result it can be inferred that the 18% of patients that underwent a change in their TWC allocation underwent a balanced redistribution along the TWC severity continuum producing a distribution of patients with clinical characteristics analogous to those produced by the traditional ESR criterion.

The fact that one patient who was reclassified from severe to moderate UC underwent inpatient colectomy is of interest but is not an unexpected outcome, nor is it an outcome reaching statistical significance. Operative management and the initiation of biologic therapy are not uncommon clinical outcomes in this clinically important but frequently overlooked group with TWC moderate disease who have accumulated a significant inflammatory burden. There is a paucity of data specifically relating to the moderate severity group because many studies of acute UC requiring hospital admission combine patients with moderate and severe levels of disease activity.[19-22]

The new CRP-based criterion did not produce any statistically significant differences in the absolute number of patients with either corticosteroid therapy failure or the closely related clinical outcome of rescue therapy administration in moderate and severe groups.

Consistency between the ESR>30 and CRP≥12 allocated groups is evidenced by the lack of statistically significant differences observed in validated markers of disease activity. These included

the presenting faecal calprotectin and the Mayo endoscopic subscore as assessed in 57 and 118 patients, respectively.

The group of 163 presentations are representative of the 204 presentations to the main study site during the period under evaluation. The statistically significant differences between the ESR negative (n=41) and main cohort (n=163) were limited to a higher rate of first presentations of UC and reduced disease duration in the former group. Key objective clinical parameters known to affect clinical outcomes in severe UC including bowel frequency, albumin, CRP and immunomodulator usage status were not found to be statistically disparate between these groups.

The establishment of a CRP cut off is of clinical importance given the relative accessibility of this test in contemporary clinical practice, the ongoing applicability of the TWC in clinical decision making, and the importance of the inflammatory marker criterion within this instrument. Of the 163 severe cases as per the original TWC, 129 (79%) had an ESR >30mm/h. For 47 (29%) of these cases an ESR of >30mm/h is the only additional Truelove and Witts criterion admitting them into the severe category.

Reluctance in utilising the TWC is borne of a number of previously described limitations including its unclear statistical origins, lack of successful validation and the abundance of other contemporaneous instruments.[5, 6] Despite these detractions Dinesen *et al* have shown that the TWC not only provides a tool for disease activity categorisation, but that together they also have prognostic value with regards to predicting the likelihood of inpatient colectomy.[1]

The comparison of our data with the Turner *et al.* study yields low validity. This paediatric series, while having a significant number of patients admitted for intravenous steroids (227/451), assessed disease activity after three days of intravenous corticosteroid therapy to obtain a more heterogeneous pool of results.[12] Additionally, disease activity was determined by a very different instrument that contains a paucity of objective measures of disease activity. Finally, baseline CRP values are likely to be higher with increasing age and body mass index encountered in adult series.[23, 24] This latter point was a key reason why the less specific CRP cut off of ≥10mg/L was not chosen.

The described CRP cut off of ≥12mg/L lies between the previously described cut offs of >10mg/L and >30mg/L reported in the UK IBD Audit and the ECCO e-guidelines, respectively.[10, 11]

In the 2004 study by Ho *et al* describing a score predicting failure of rescue therapy from day 0-3 data from a Scottish cohort of 167 TWC severe UC cases the mean ESR and median CRP were 44.8 (standard deviation 26.5) and 4.4 (IQR 2.1–13.3), respectively.[25] Whilst there was no comparison between the ESR and CRP reported, the CRP IQR values reflect the fact that the majority of these severe UC cases have only a mild elevation of CRP with some in the normal range. This finding is supported by the data presented in this chapter.

The PPV threshold of >85% was chosen to reflect the level of assurance most clinicians would accept for the CRP cut off to produce a similar disease activity grading to the traditional ESR-based criterion. This level of prediction is identical to the 85% risk of inpatient colectomy conferred by satisfying the Day 3 (Oxford) criteria for assessment of corticosteroid-refractory disease, a widely accepted indication for treatment escalation or surgery in this context.[26] The selected CRP cut off also generated the highest combined accuracy and PPV of all CRP values on presentation.

Some investigators have proposed using either or both of ESR and CRP in the assessment of acute IBD.[17] As the purpose of this study was to supersede the ESR assay in favour of the CRP, this approach was not taken.

For the purposes of this study we have kept within the framework of the original Truelove and Witts disease activity assessment criteria. The rate of colectomy-by-discharge plateaued after three TWC were satisfied. This suggests that the weighting of each of the additional TWC with regards to the degree of risk of colectomy they confer is likely to be unequal. This is in part due to the binary nature of the individual components of the TWC.

The ability to predict intravenous corticosteroid therapy failure was poor using either inflammatory marker platform. Those patients satisfying two TWC had higher response rates to corticosteroid therapy than patients satisfying three criteria. This may reflect the original choices of clinical criteria and cut offs by Sidney Truelove with the goal being the identification of patients at high risk of colectomy.

Faecal calprotectin

There were no statistically significant differences in the faecal calprotectin concentrations between ESR>30 and CRP≥12 allocated TWC severity groups (Figure 1.7). Factors contributing to these findings include the reduced patient numbers with available admission faecal calprotectin data

(n=57) and the lack of a standardised protocol for faecal specimen collection during the course of this study.

These data support the idea that this quantitative test is better suited to determining the presence of any significant inflammatory disease activity from remission rather than differentiating between moderate and severe disease activity states.[27]

Critique

Strengths of this investigation include the large number of patients included with severe UC. This series reflected a real-world cohort of consecutive inpatient acute UC cases. Objective measures of disease activity in addition to clinical outcomes were compared refuting significant differences between the ESR and CRP-defined groups.

Limitations of this study include the long study duration. Whilst the treatment of acute severe UC has evolved extensively over the last two decades, the initial three days of therapy has remained remarkably consistent since the mid-1970s. Other consistencies of this study include the limited pool of two treating IBD clinicians, the application of a heavily protocolised inpatient management algorithm and the exclusion of patients with recent biologic therapy.

Whilst this exclusion may initially seem to limit the external validity of the findings, it was an important feature of the study protocol. The experience in our centre is that symptomatic patients admitted with a secondary loss of response to biologic therapy have a tendency to take a corticosteroid responsive course. This group usually display lower levels of systemic toxicity at presentation. This is probably in part due to a continued partial response to biologic therapy and earlier presentation secondary to engagement with healthcare providers.

Study cohort limitation

For the purpose of increasing the robustness of the data for publication the cohort was limited to cases presenting to the Royal Brisbane and Women's Hospital only. This was to increase the consistency of the laboratory results by removing bias that could be introduced by the use of different inflammatory marker testing platforms at other facilities. The risk of recall bias of clinical data from peripheral sites was also limited by taking this approach. See Appendix A for the analysis of the unrestricted n=270 cohort including moderately severe cases and those cases transferred from other facilities.

As the cut off finding approach was used in preference to the previously trialled but insufficiently precise direct statistical relationship approach (see Appendix A), the importance of including the moderate UC cases was diminished.

As encountered in this study, cases of TWC moderate UC often present with inflammatory markers within the normal range. As the inclusion of these moderate UC cases will influence the rate of true positives and negatives and potentially be controversial to examiners and reviewers, this group was also removed from the study cohort.

Clinical relevance

This work may influence clinical practice by relegating ESR as a necessary admission blood test in acute presentations of UC. Utilising the CRP-based criteria will improve accessibility to the clinical information required for time critical decision making regarding patient disposition and acute management. The primary study finding also supports the paradigm that in inflammatory diseases that are known to be poor inducers of CRP production, a mildly elevated CRP between two and three times the upper limit of normal may be an indicator of a clinically significant inflammatory burden. Finally, the inclusive nature of the CRP criteria will enhance access to infliximab in healthcare settings where satisfying the TWC is mandatory for reimbursement.

In summary, a CRP threshold of $\geq$12mg/L was found to be an inclusive and sensitive cut off that when incorporated into the Truelove and Witts criteria, replacing the traditional ESR >30mm/h criterion, had similar performance characteristics when applied to the assessment of acute UC disease activity.

Chapter 2

The influence of systemic immunosuppression by non-biological agents on markers of systemic inflammation in acute ulcerative colitis

2.1 Literature review

The use of systemic immunosuppression as a corticosteroid sparing strategy is common in UC. In a recent clinical audit, rates of immunomodulator use in Australian UC patients were reported to be 40% (IQR 20-55%).[28]

There are a number of putative mechanisms by which immunosuppressants can affect inflammatory marker levels. As already alluded to in the previous chapter, immunosuppression with the thiopurines and methotrexate, can increase mean corpuscular volume (MCV). An increase in MCV is known to increase both erythrocyte agglutination and sedimentation rates.[7]

With respect to the most frequently prescribed thiopurine class, including azathioprine, 6-mercapturine and 6-thioguanine, the exact therapeutic mechanism(s) of action is still poorly understood despite over 50 years of study. Broadly, these agents and their metabolites work to attenuate pro-inflammatory processes through antimetabolite (6-methylmercaptopuirne) and the relatively quick-acting proapoptotic (6-thioguaninie nucleotide) processes.[29] With the latter process predominantly acting on activated T-lymphocytes, it is highly likely that there will be some influence on the important inducers of CRP production such as IL6, IL-1β and TNFα, known to be produced by this cell class.

Perhaps surprisingly, given the clinical importance of systemic inflammatory markers in disease activity assessment and prognostication, there are limited data specific to the IBD sphere available to answer this question. The prevailing expert opinion in this field is that non-biologic immunomodulator and anti-inflammatory agents at therapeutic doses do not directly influence CRP levels.[30-32]

The true pharmacodynamic situation may, in fact, be more nuanced. Calcineurin inhibitors and corticosteroids have been associated with a blunted CRP response in acute renal transplant rejection.[33] However, a prompt CRP response is preserved when bacterial infection occurs after transplant. Conversely, azathioprine treated patients routinely produce a CRP response to acute graft rejection.[34] The proposed mechanism for this selectivity is the specific blocking of IL-1 production by cyclosporine.

One of the few relevant studies in the IBD field found 11 (9%) patients treated with a thiopurine had persistently elevated ESR with a normal CRP in the setting of asymptomatic disease.[35]

However, the significance of the findings in this study of 120 paediatric subjects is limited by the fact the majority of participants were in clinical remission and were assigned a diagnosis of Crohn's disease (79%).

Another noteworthy observation from this study is that those individuals with discordant results (elevated ESR and normal CRP) had a higher mean MCV than the participants with concordant inflammatory marker levels. This observation reached statistical significance (p=0.0373). Whilst the numbers in the discordant group were small, these findings suggest an association between discordantly low CRP levels and a known thiopurine effect on erythrocytes in the setting of established immunomodulatory therapy.

Effect of established immunomodulator use on risk of colectomy

A key early piece of evidence is the large retrospective series presented by Moskovitz that reported an experience with cyclosporine for corticosteroid-refractory acute UC.[36] A disparity in the colectomy rate by immunomodulator status on admission was found with immunomodulator treatment rates being 46% in patients requiring colectomy-by-discharge versus 28% of those treated non-operatively (p=<0.01). As many as 88% of patients established on azathioprine at the time of presentation underwent a colectomy within 12 months of presentation.

Whilst other observational studies of colectomy-by-discharge rates following rescue therapy have not shown this effect, [20] the Moskovitz study and other concordant studies continue to influence the choice of rescue agent in clinical practice.[37]

More recent studies in the entire severe UC population from our centre with ciclosporin or infliximab rescue do not show this effect on colectomy risk for immunomodulator or oral corticosteroid exposure at the time of admission, OR 0.76 (0.37 - 1.49), 1.2 (0.65 - 2.19), respectively.[38]

Other inflammatory disorders

Much of the published work in this field has focussed on presentations of infection in patients with systemic lupus erythematosus (SLE) on immunosuppressant therapy. Unless complicated with serositis, active SLE, like UC, dermatomyositis and Sjögren's syndrome, is known to produce lower CRP levels than active Crohns or rheumatoid arthritis.[39] Relevant differences between SLE and UC include the much higher rate of both hepatic pathology and interferon-α production. There is evidence that both of these processes lead to a decreased rate of CRP production.[40] There was no

statistically significant relationship found between CRP level and the use of either immunosuppressants or corticosteroids in 193 patients with SLE subsequently found to be harbouring a bacterial infection.[41]

A retrospective Canadian study of 839 individuals investigating the causes of all presentations with CRP levels >100mg/L (55% infections, 2.5% IBD presentations) did observe a trend towards a reduction in CRP levels in the 21 patients on anti-TNFα and not other agents.[42] This observation was not reported to reach statistical significance as it was limited by overlapping medication exposure to different classes of immunosuppression.

In summary, the current expert opinion is that CRP is *not* affected by immunomodulator use in IBD patients. However, there is limited evidence in the IBD field and instances in other inflammatory states that refute this position. More data are needed to address this relevant clinical question.

2.2 Aim

To analyse acute UC presentation data to evaluate for any effect of systemic immunosuppression on inflammatory marker levels.

2.3 Hypothesis

In presentations of acute UC there will *not* be a statistically significant effect of systemic immunomodulator use on levels of commonly used markers of systemic inflammation.

2.4 Methods

Patients

To be eligible for study inclusion individuals had to have a confirmed clinical and histological diagnosis of UC of at least 90 days duration at the time of hospital presentation to a single tertiary centre with moderate-severe UC according to the TWC. A paired ESR and CRP collected within 24 hours of admission was also mandatory for inclusion. Written informed consent was obtained from all patients included in this study and the study protocol approved by the Royal Brisbane and Women's Hospital medical ethics committee.

Patients on immunomodulators, oral corticosteroid and/or 5-aminosalicylates at the time of admission were included in the study. Patients were considered to be taking an immunomodulator if they had been on a thiopurine with therapeutic drug levels for four months or more prior to presentation or were taking methotrexate for the same duration. For the purpose of maintaining consistency across the cohort, those patients on any biologic therapy at the time of admission were excluded.

Laboratory tests

ESR tests were performed on the Ves-matic Junior 20 (Diesse Diagnostica Senese, Monteriggioni, Italy). CRP was assessed on the Roche-Hitachi 747 Analyzer (Roche Diagnostics, Mannheim, Germany, 1996-2008) and the Beckman-Coulter DxC 800 Analyzer, (Beckman-Coulter, Fullerton, USA, 2008-2017) platforms.

Statistics

R version 3.4.0 "*You Stupid Darkness*" (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

2.5 Results

349 presentations with moderate-severe UC resulting in hospital admission were assessed. 158 had an established diagnosis of UC of ≥90 days as well as paired ESR and CRP collected within 24 hours of admission (Table 2.1). Eighty-seven (54%) of patients were male. Forty-three individuals (27%) were on immunomodulators for ≥4 months prior to presentation. Of these 22 were taking 6-mercaptopurine, 19 were taking azathioprine, one patient was taking 6-thioguanine and one oral methotrexate.

There were no statistically significant differences in the proportions of patients separated by immunomodulator status between the groups allocated by the number of TWC satisfied at admission (Figure 2.1). This included the rate of prednisone use at admission. The median dose of prednisone on presentation was 40mg and 32.5mg per day in immunomodulator naïve and immunomodulator positive groups, respectively.

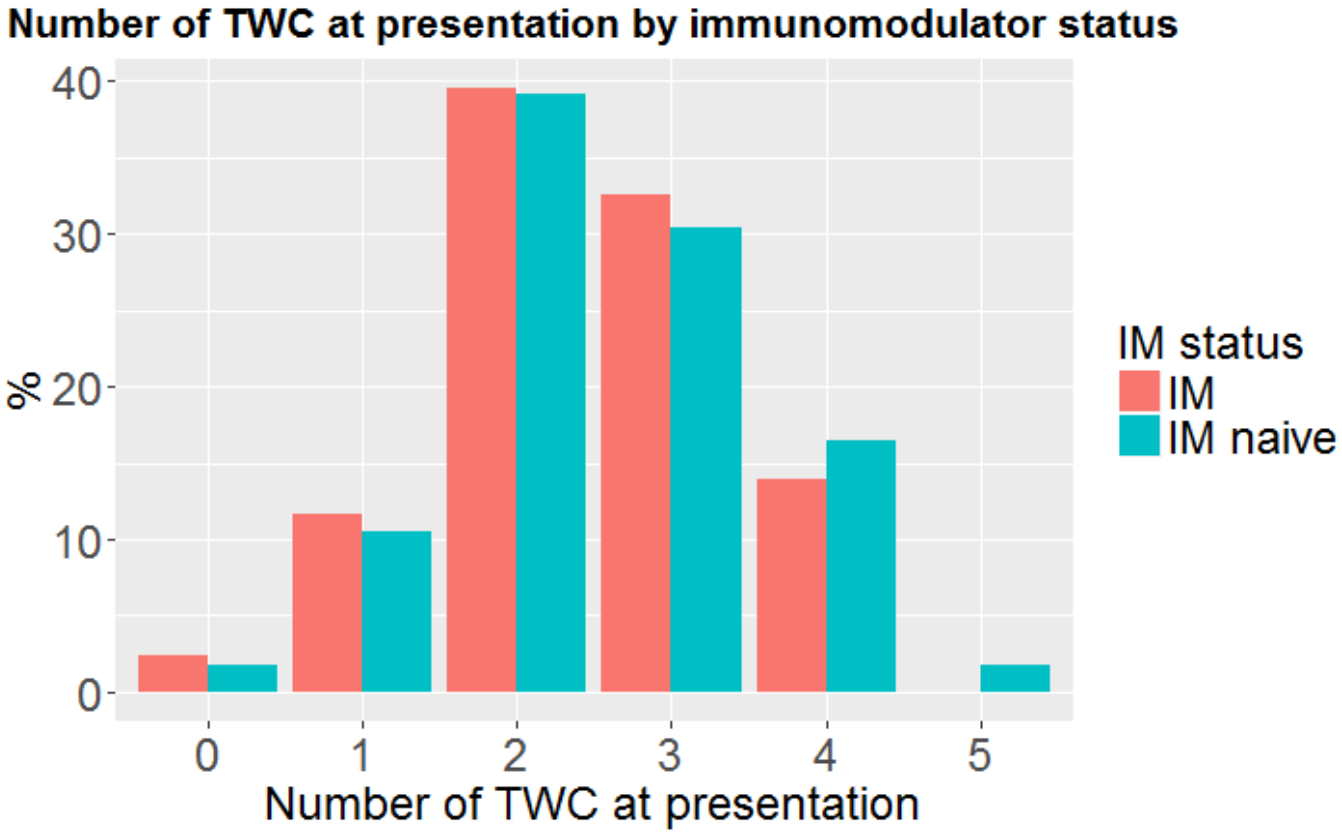

Figure 2.1 Patients separated by immunomodulator status and number of TWC satisfied at admission.

TWC: Truelove and Witts criteria; IM: immunomodulator

Table 2.1 Patient details separated by immunomodulator status

		Total	IM naïve	IM	p-value
n (%)		158	115 (73)	43 (27)	
Gender (%)	M	85 (54)	59 (51)	26 (60)	0.4
	F	73 (46)	56 (49)	17 (40)	
Age at time		33 (26-43)	33 (26-42)	34 (27-43)	0.64
Disease duration in years		4 (2-10)	4 (2-10)	4 (2-11)	0.75
Disease extent (%)	E1-E2	60 (38)	47 (41)	13 (30)	0.3
	E3	98 (62)	68 (59)	30 (70)	
TWC activity (%)	Moderate	21 (13)	15 (13)	6 (14)	1
	Severe	137 (87)	100 (87)	37 (86)	
Prednisone on admission (%)		76(48)	51(44)	25(58)	0.17
Prednisone dose (mg)†		40 (25-50)	40 (25-50)	32.5 (20-50)	0.3
ESR		40 (26-56)	41 (27-58)	40 (24-53)	0.62
CRP		47 (15-95)	50 (18-97)	29 (11-89)	0.25
Albumin*		34 (6)	33 (6)	34 (5)	0.72
Haemoglobin*		121 (22)	122 (22)	120 (22)	0.74
MES~	MES 0	2	1	1	0.89
	MES 1	9	8	1	
	MES 2	46	29	17	
	MES 3	55	38	17	

IM: immunomodulator; IQR: interquartile range; ESR: erythrocyte sedimentation rate, CRP: C-reactive protein; MES: Mayo endoscopic subscore; TWC: Truelove and Witts criteria. Unless otherwise specified all values are expressed as medians with interquartile ranges. †n=112 for IM naïve, n=42 for IM. *Mean and standard deviation. ~MES n=112. Disease extent as per the Montreal classification.

Mann-Whitney U-test revealed no statistically significant correlation between immunomodulator use and CRP (p=0.25) or ESR at presentation (p=0.62).

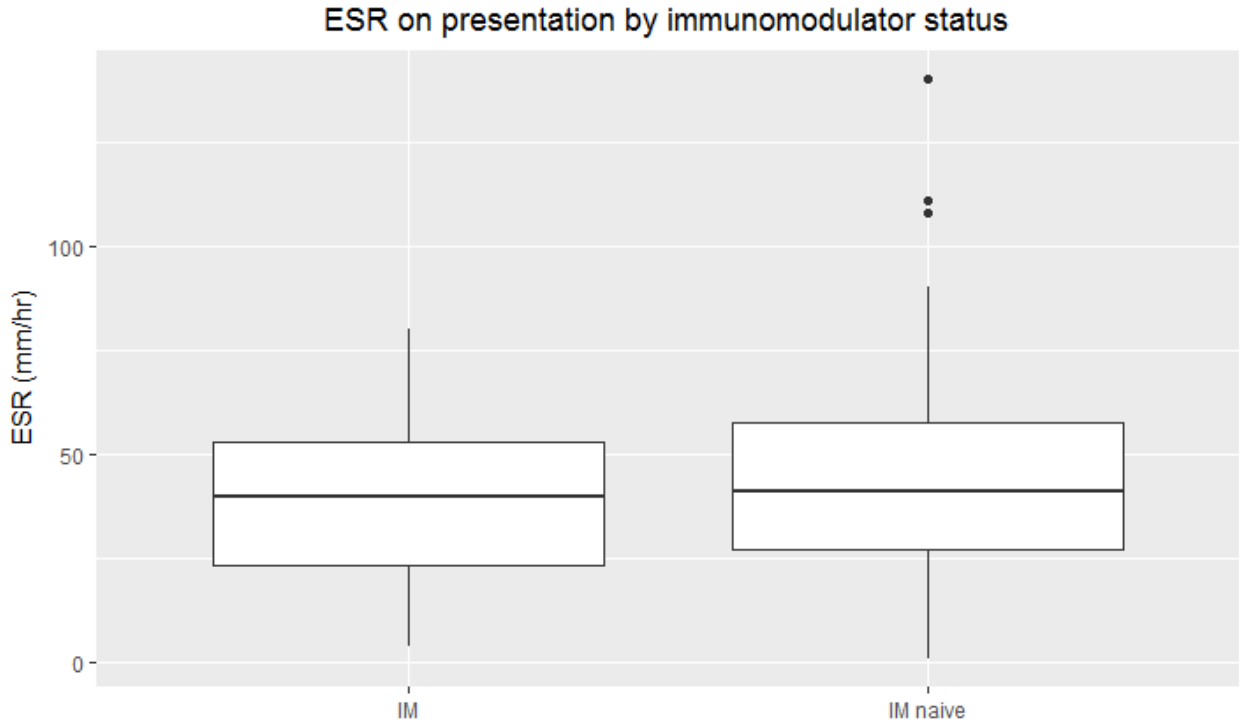

Figure 2.2 ESR on presentation separated by immunomodulator use status

ESR: erythrocyte sedimentation rate; IM: immunomodulator

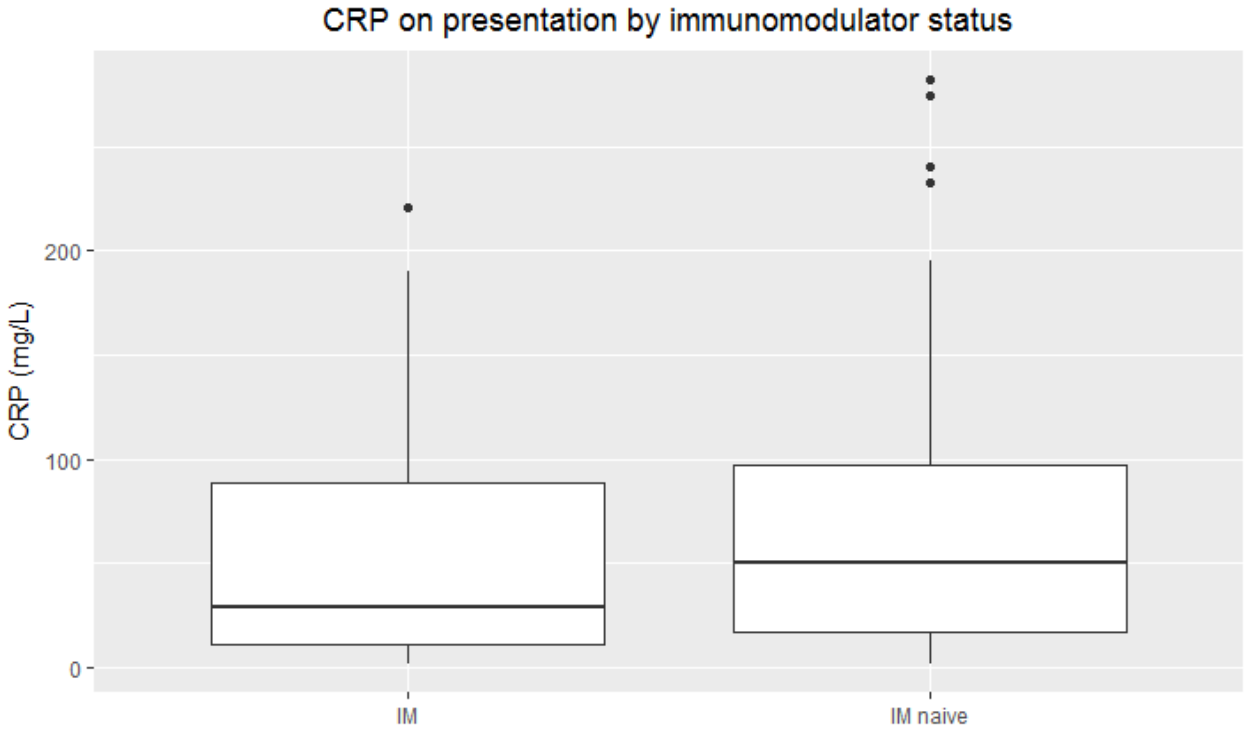

Figure 2.3 CRP on presentation separated by immunomodulator use status

CRP: C-reactive protein; IM: immunomodulator

The effect of oral corticosteroids on markers of systemic inflammation at presentation

A similar analysis of the effect of oral corticosteroids at admission was performed. There were no statistically significant differences found in inflammatory marker levels on the basis of oral corticosteroid intake status on presentation. The uncorrected p-values were p=0.39 and 0.26 for ESR and CRP, respectively.

The effect of disease extent on markers of systemic inflammation at presentation

A similar analysis of disease extent was performed. There were no statistically significant differences found in inflammatory marker levels on the basis of disease extent. The uncorrected p-values were p=0.15 and 0.72 for ESR and CRP, respectively. The two proctitis cases (E1) were included with the left-sided (E2) cases for this analysis.

2.5 Discussion

The main finding of this prospective observational study is that systemic immunosuppression with non-biologic agents did not significantly affect the levels of systemic markers of inflammation at the time of presentation in established cases presenting with acute moderate-severe UC.

Strengths of this study include prospective data collection, sample size, consistencies between patient groups split by immunomodulator status, collection of oral corticosteroid data, consistencies of clinical and laboratory assessment, the inclusion of patients with moderate colitis and the exclusion of patients on biologic agents.

Restricting study eligibility to established cases of UC was performed for two key reasons. Firstly, it was to allow for an appropriate comparison between like groups.

The likelihood of incident UC cases also being on systemic immunosuppression for another indication is low. This will result in the preferential allocation of incident cases to the immunomodulator naïve group causing bias. Additionally, a patient having a diagnosis for more than 90 days will have a lower likelihood of being assessed during their index flare.

Whilst the exclusion of patients on biologic therapy may initially seem to limit the external validity of the findings, it was an important feature of the study protocol. It removed the potent influence of these agents on inflammatory markers at presentation.

Of interest, a preliminary analysis with a larger sample (n=270) including index presentations and patients transferred from other hospitals (using presentation data from the transferring facility) *did* find a statistically significant lower CRP but not ESR in immunomodulator users (see Appendix A). This observation was not found to have statistical significance following the application of robust regression with Huber weighting.

Shortcomings of this study include the change in CRP analyser during the study period. An assessment of machine batch effect was not evaluated.

Other indices that might affect ESR in particular, other inflammatory disorders, mean corpuscular volume and haematocrit were not incorporated in the analysis.[39, 43] The observation that there were

no statistically significant differences between immunomodulator status cohorts in gender, haemoglobin and albumin concentrations is a reassuring finding.

On the level of the individual patient, the serial CRP concentration is one of the few informative and objective measures of disease acuity and treatment response that will alter management during the hospital admission.[26, 44] This study provides the previously lacking reassurance that baseline immunosuppression status need *not* be taken into account when interpreting the ESR or CRP levels on presentation in established UC patients not on biologic therapy.

As many of the IBD severity and prognostic indices in contemporary use incorporate either the ESR or CRP as objective markers of disease acuity, this study has high translatability to patients with a secure diagnosis of UC not in their initial flare.[4, 25, 26]

Chapter 3

The derivation of a risk algorithm predicting failure of intravenous corticosteroid therapy in acute ulcerative colitis from clinical criteria available on presentation

3.1 Literature review

In the setting of acute UC intravenous corticosteroid therapy has a response rate of between 50-70%.[45] The response rate is subject to many variables including the definition of acute UC, local protocols relating to timing of implementation of second-line therapies, and the definition of an adequate clinical response to therapy. Variations in these parameters have hindered meta-analyses and direct inter-study comparisons between these predominantly observational studies.

Since the 1970s there have been many UC activity indices formulated and extensively reviewed elsewhere.[5, 6, 45] Few have been validated in prospectively collected patient cohorts and at least four offer a risk score on the likelihood of colectomy-by-discharge. These are the Oxford[26], Swedish[44], Seo[46] and Ho[25] indices. These indices use clinical parameters present or collated up to day three of intravenous corticosteroid therapy (Table 3.1).

Table 3.1 Prognostic indices for acute UC[47]

Table 1. Prognostic scores.[13–19]

Index	Variables	Diagnostic accuracy
First 24 h		
Truelove Witts	Hematochezia, pulse rate, temperature, Hgb, ESR	50% risk of colectomy when three or more additional criteria
St. Marks	Stool frequency >12	55% risk of colectomy
72–96 h		
Seo	Stool frequency, hematochezia, nocturnal bowel movements, abdominal pain, and activity level	Negative predictive value 97%; positive predictive value 52%
Ho	Stool frequency, colonic dilation, hypoalbuminemia	85% sensitivity in original study; validation: 66% require second-line therapy and 33% require colectomy in high-risk group
Oxford	Stool frequency >8/day; or 3–8/day and CRP >45	Positive predictive value 85%
Swedish	CRP and stool frequency	Positive predictive value ~70%
Italian	<40% reduction in stool frequency at day 5	

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

There is a dearth of prognostic scores that have been trained to use variables available at the time of presentation to predict clinical outcomes during the admission. As will be discussed in more detail below, this is, in part, due to the focus on the outcome of colectomy-by-discharge.

Exceptions include a retrospective study in abstract form from Edinburgh.[48] It derived a risk score for colectomy-by-discharge based on data from admission and day three of corticosteroid therapy. It was found that colectomy-by-discharge was predicted by extensive disease, albumin <30g/L and CRP ≥20 mg/L on admission, and albumin and stool frequency at day three. This study also addressed corticosteroid therapy response and found that 71% of those who satisfied two or less of the criteria at admission responded adequately to corticosteroids and avoided treatment escalation.

A prospective study of 89 acute severe UC presentations to Oxford showed that rescue therapy was required in 11/14 (79%) cases with an ulcerative colitis endoscopic index of severity (UCEIS) of >6/8.[49]

Jain *et al* reported a small prospective study of 49 acute severe UC patients.[50] Patients with a UCEIS score of >6/8 at admission combined with a day three faecal calprotectin of >1000μg/g uniformly failed intravenous corticosteroid therapy. It was of interest that CRP at admission or on day three and admission faecal calprotectin were *not* identified as independent risk factors for subsequent corticosteroid therapy failure.

Two older studies, also from India were designed to prospectively assess for early parameters predicting intravenous corticosteroid treatment failure.[51, 52] One assessed 55 consecutive acute UC presentations from 50 patients.[52] Only ten of the 55 did not respond to corticosteroid therapy. After multivariate analysis haemoglobin <90g/L, CRP >18.6mg/L, prothrombin time >1.4 and fibrinogen <22mg/L independently predicted corticosteroid therapy failure with AUCs between 77 and 80%. A logistic regression approach combining these parameters was not undertaken in this study.

Another study of 30 patients from the mid-1990s treated cases with intravenous corticosteroids for a mean of 9.2 days (range 2-20) with a response rate of 60%.[51] It found that the admission parameters of stool frequency ≥9 per day, pulse rate ≥120/minute, temperature ≥38°C, albumin ≤ 20g/l, mucosal tags on plain x-ray abdomen and extensive colitis predicted a poor response to corticosteroid therapy.

Much more recently, a study in abstract form from Melbourne has identified an admission faecal calprotectin of >1645μg/g and CRP to albumin ratio (CAR) of >1.34 as being highly predictive of corticosteroid therapy failure with a 96% PPV.[53]

The changing face of acute ulcerative colitis treatment outcomes

The success of corticosteroid therapy was classically defined as avoidance of colectomy-by-discharge or colectomy within a defined timeframe of hospital admission. The advent of rescue medical therapy in the 1990s interrupted the progression between corticosteroid failure and immediate referral for colectomy. Despite this protocol change, a 2007 meta-analysis indicated colectomy-by-discharge rates had remained constant at around 27% between the 1970s and the early 2000s.[45] The analysis of the 2008 and 2010 UK IBD audit data revealed colectomy rates of 19% and 17% respectively. However, due to a lack of ESR data, the inclusion criteria for severe colitis included a CRP of >10mg/L. Additionally, no haemoglobin data were available for the 2010 cohort.[11]

Despite these omissions, there appears to be a trend towards lower colectomy by discharge rates in the UK coinciding with the widespread uptake of rescue medical therapy and the addition of infliximab to the armamentarium for this indication in the late 1990s. This decrease may in part be due to the observed effect of a delay to the timing of an 'inevitable' colectomy following the institution of rescue therapy.[3, 26, 54] Medium to long term data reveal an ongoing risk of colectomy in the recently discharged severe UC patient group. This risk being most marked up to two years from the severe UC admission.[2, 55]

With a reduction in colectomy-by-discharge rates, the maturing of rescue medical therapy and the increasing availability and use of maintenance biologic therapy, the progression from corticosteroid therapy failure to early colectomy has been irrevocably disrupted. Concomitantly, advances in severe UC treatment success rates also influence the relationship between clinical indices observed early during the admission and the resulting colectomy-by-discharge outcomes.

In contrast, a clinical outcome that has remained relatively constant during this time is that of failure of corticosteroid therapy. Overall this rate is approximately 40% when inclusion criteria are restricted to patients with severe UC activity as defined by the TWC.[45] This is in part due to the consistency of the recommended corticosteroid regimen established in the mid-1970s. In most first world settings it comprises a total daily intravenous dose of 400mg hydrocortisone or 60mg methylprednisolone or equivalent dose of corticosteroid for a minimum of five days.[56]

Assessing response to corticosteroid therapy

Appropriately, the definition of corticosteroid therapy failure is usually a clinically driven decision. This decision is likely to have poor inter-observer agreement, although this has never been tested.

The Day 3 (Oxford) criteria is the tool that has had a profound impact on clinical decision making for this presentation.[26] It is an assessment of corticosteroid response applied on the third calendar day of intravenous corticosteroid therapy. If at this stage the bowel frequency is >8 or 3-8 per day with a CRP of >45mg/L then the risk of subsequent colectomy during the admission was found to be 85%. This high positive predictive value lays solid grounds for treatment escalation.

The Oxford criteria were derived from 51 presentations of TWC severe UC with 14 proceeding to rescue therapy with cyclosporine. A total of 15 (29%) patients underwent colectomy during the admission. In keeping with the contemporary practice and the disease severity of individual cases, eight of the colectomy cases were not administered rescue therapy. Another observation of this study was the high colectomy-by-discharge rate in cyclosporine treated individuals with only 7/14 and 4/14 avoiding colectomy during the admission and at study completion with a median follow-up of 12 months, respectively.

Additionally, intravenous corticosteroids were continued for up to eight days in total prior to the commencement of rescue therapy. This is one of a number of era-related protocol components that may have contributed to the higher colectomy-by-discharge rate than we see in contemporaneous series. Long-term follow up data did show a trend towards lower colectomy rates within four years of presentation for those administered cyclosporine therapy within five days or less of intravenous corticosteroids versus those commenced after more than five days of intravenous corticosteroids.[55] This observation did not reach statistical significance by Log-rank test.

Despite the changes wrought by improvements in contemporary severe UC practice, the Oxford criteria retains its utility as a hurdle for continuing with intravenous corticosteroids versus treatment escalation. Whilst this practice may seem somewhat dated, continuing to follow this paradigm enhances the consistency between practitioners and hence between published series that strictly apply this clinical decision making tool.

A recent comparison of the accuracy of established risk scores (MES, Oxford, Edinburgh, Swedish) did analyse clinical outcomes including intravenous corticosteroid failure, rescue therapy administration and colectomy-by-discharge.[57] For all clinical endpoints the Swedish score had the highest AUC. In this Portuguese study of 112 TWC qualifying severe UC patients, corticosteroid treatment failure was defined by a clinical as opposed to a criteria-led decision for escalation to rescue medical therapy or surgery.

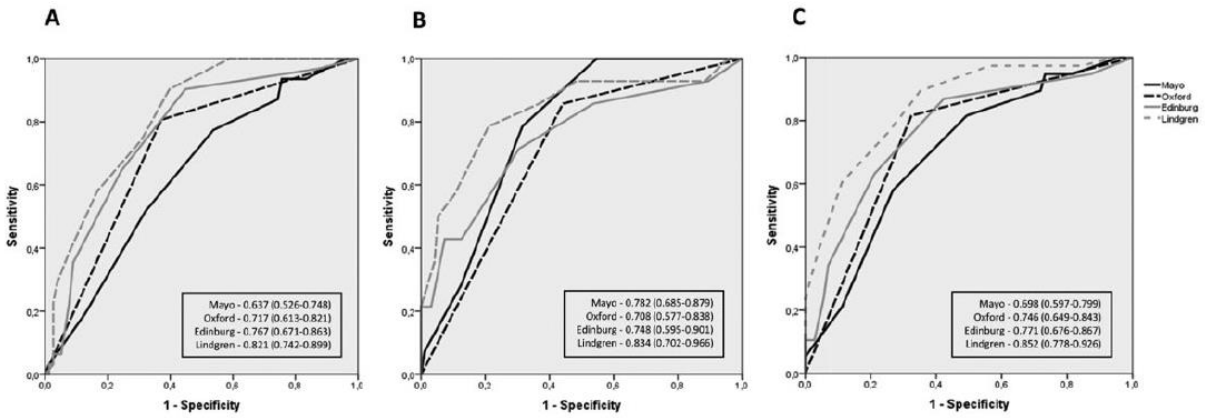

FIGURE 2. Receiver operating characteristic curve showing the different acute severe ulcerative colitis risk scores used to predict need of rescue medical therapy (A), surgery (B), and steroid refractoriness (C). Values are expressed as OR (95% CI).

Figure 3.1 ROC curves for four risk prediction scores for the clinical outcomes of rescue therapy, colectomy-by-discharge and corticosteroid treatment failure.[57]

Whilst this study presented a useful comparison between a large retrospective series of severe UC patients, the fact that these risk scores predominantly utilise day three indices limits the validity of these results to the question of predictive indices assessable on presentation.

A paradigm shift: The utility of a continuous risk score for corticosteroid therapy failure as an index of disease activity

The Truelove and Witts paradigm of classifying disease severity into ordinal assignations of mild, moderate and severe disease activity has influenced subsequent iterations of UC activity indices. Whilst this scale continues to be a useful guide for patient disposition in acute UC presentations, a significant proportion of cases requiring admission on clinical grounds for intravenous corticosteroids do not meet the severe UC criteria.

Continuous risk scores such as the Swedish and Seo indices do address this issue. However, the Seo index also allocates patients into one of three ordinal groups based on activity. Despite having a reported 97% negative predictive value for colectomy at 72 hours, this index has not had widespread uptake and in one study its agreement with an expert clinician's judgement was only 47%.[58] The Swedish fulminant colitis index, like the Day 3 (Oxford) criteria is focussed on identifying those with a high risk of colectomy as opposed to being a global activity index.

There are other precedents for continuous risk scores linked with clinical outcomes elsewhere in the fields of gastroenterology and hepatology. One of the most successful is the Model for End-stage Liver Disease (MELD) score. It is widely used to determine appropriateness and priority for orthotopic liver transplant.[59]

The MELD score was a slight alteration to a risk score already derived from 231 cirrhotic patients for predicting patient survival post elective trans-jugular intrahepatic portosystemic shunt (TIPS) insertion.[60] It incorporated the international normalised ratio (INR), total serum bilirubin and creatinine to determine the three month mortality risk. The formula was then slightly altered and validated on a large and heterogeneous series of cirrhotic patients. The derived score is discretised into MELD score bands (≤9, 10-19, 20-29, 30-39, ≥40) with associated increasing rates of three month mortality.

The benefits of a validated continuous risk score include the ability to prognosticate an individual's risk of a defined outcome based on clinical variables that are proven discriminators without having to assign patients into risk categories. This allows the clinician to personalise subsequent management decisions.

In the context of severe UC the foreknowledge of a symptomatic patient's chance of responding to intravenous corticosteroids is useful. This knowledge can be used as background for decision making regarding the requirement for, timing and type of treatment escalation. It could give the clinician an evidence base for a more definitive, top-down treatment approach avoiding sequential therapy and potential side effects of immunosuppression with multiple concurrent agents.[61, 62] There is also the potential for improved early colectomy avoidance and post-colectomy outcomes with the institution of rescue therapy prior to the current day 3-5 paradigm.[55] Finally, the identification of outpatients at high risk of complications will help guide both clinical decision making and patient acceptance of escalations of care such as hospital admission.

In summary, colectomy-by-discharge rates for severe UC have changed with the adoption of advances in rescue therapy protocols. Conversely, rates of corticosteroid response have remained relatively constant. The latter is a useful clinical outcome forming the basis of a risk score that will be able to assess disease activity at the moderate-severe end of the activity spectrum and potentially influence the acute management of these cases.

3.2 Aims

- To use clinical data from acute UC presentations obtainable within 24 hours of admission to identify clinical parameters that independently predict the failure of corticosteroid therapy.

- Employ these parameters to derive a continuous risk score for failure of intravenous corticosteroid therapy for acute UC.

3.3 Hypothesis

Risk factors for the failure of corticosteroid therapy present at admission are likely to include: elevated CRP, ESR, bowel frequency; decreased albumin concentration; current corticosteroid and systemic immunosuppressant usage status and may *not* include all of the TWC parameters.

3.4 Methods

Patients

As part of the inflammatory bowel disease (IBD) program commencing in 1996, all subjects admitted to the Royal Brisbane and Women's Hospital with any complications of IBD, including an acute flare of their disease, are referred to the IBD team. Data on these admissions have been prospectively collected on a dedicated software platform, IBD Prime. Patients included in this study were 349 consecutive admissions between 1996 and 2017 with acute UC requiring hospital admission. Repeat presentations by the same individual within a 12 month period of the initial presentation were excluded. Written informed consent was obtained from all patients included in this study and the study protocol approved by the Royal Brisbane and Women's Hospital medical ethics committee.

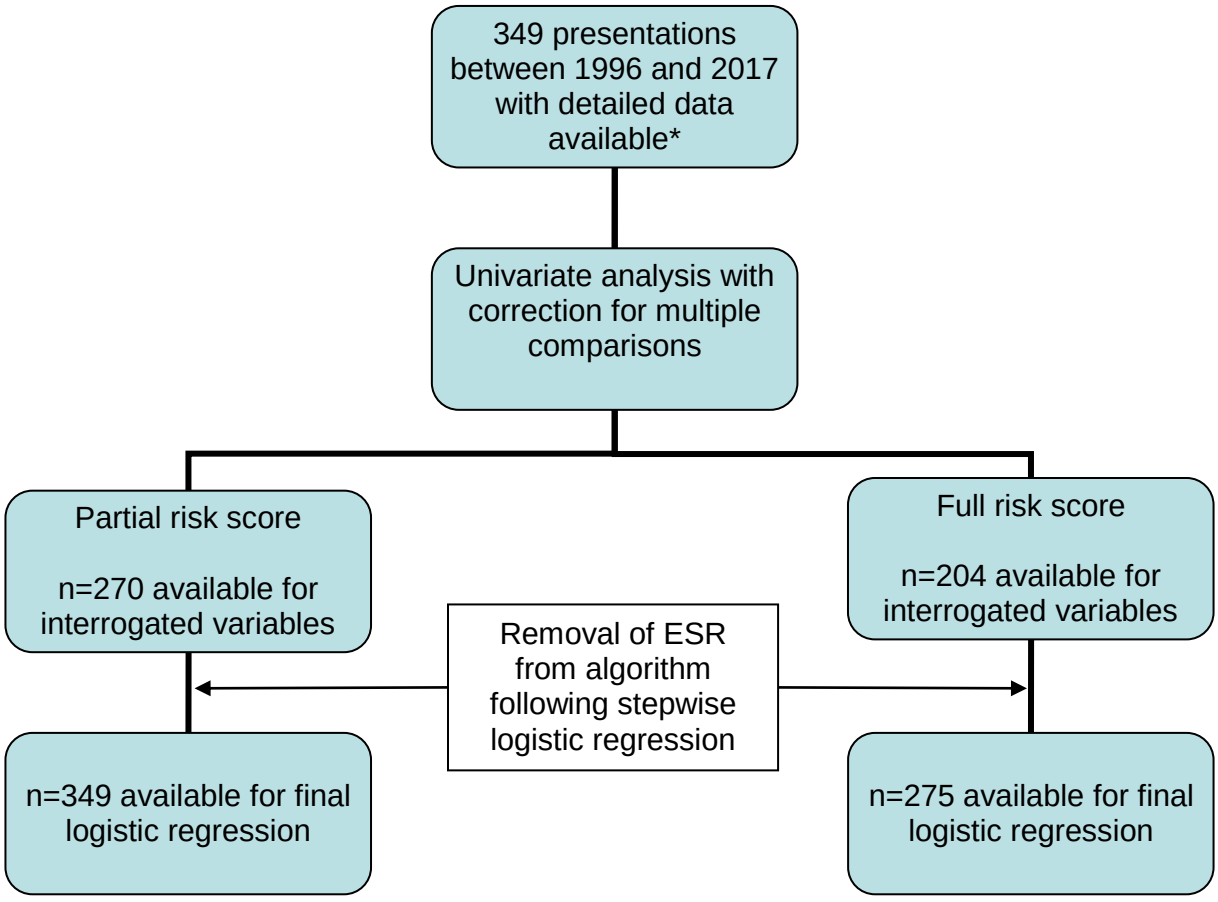

Figure 3.2 Progression of statistical analysis for clinical and endoscopic risk scores. *Data set was incomplete for ESR (n=270), MES (n=275) and faecal calprotectin (n=166).

Patients on concomitant immunomodulator, oral corticosteroid and/or 5-aminosalicylates at the time of admission were included in the study. Patients were considered to be taking oral

corticosteroids if they presented with a >24 hour history of oral corticosteroid administration prescribed for UC. Patients were considered to be taking an immunomodulator if they had been on a thiopurine with therapeutic drug levels for four months or more prior to presentation or were on methotrexate for the same duration. For the purpose of maintaining consistency across the cohort, those patients on biologic therapy at the time of admission were excluded. Maintenance infliximab for refractory or corticosteroid dependent moderate-severe UC was first reimbursed by the Pharmaceutical Benefits Scheme (Australian Commonwealth Government payer) in 2015.

The diagnosis of UC was confirmed by histology together with endoscopic, radiological and clinical correlation. Patients were treated with a standardised treatment protocol by one of two subspecialist IBD physicians.[3] The Day 3 and Day 7 (Oxford) criteria were used to determine corticosteroid treatment failure.[26] When indicated a choice of surgery or rescue therapy initially with intravenous cyclosporine A (1999-) and later with infliximab (2001-) was offered. The choice of rescue agent and/or surgery when appropriate was made in close collaboration with the patient.

Laboratory tests

ESR tests were performed on the Ves-matic Junior 20 (Diesse Diagnostica Senese, Monteriggioni, Italy). CRP was assessed on the Roche-Hitachi 747 Analyzer (Roche Diagnostics, Mannheim, Germany, 1996-2008) and the Beckman-Coulter DxC 800 Analyzer, (Beckman-Coulter, Fullerton, USA, 2008-2017) platforms.

Statistics

R version 3.4.0 "*You Stupid Darkness*" (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.
Statistics

3.5 Results

Data from 349 presentations of acute UC admitted to hospital for intravenous corticosteroid therapy generated by 327 individuals were interrogated. The dataset was incomplete for some variables (Table 3.3). A total of 17 variables were assessed with univariate analysis to determine statistically significant differences between responders and non-responders to corticosteroid therapy.

Raw data

Of the 349 presentations, 188 (54%) were male. The median age of presentation was 33 (IQR 24-46). Extensive disease predominated with 234 (67%) having Montreal classification E3 disease. Median disease duration was two years (IQR 0.25-7.21) with 90 (26%) of presentations being the first presentation of IBD. In all, 141 (40%) met the predefined criteria for complete clinical response to intravenous corticosteroid therapy while 208 (60%) did not respond.

At the time of presentation 151 (43%) of patients were taking oral corticosteroids whilst only 80 (23%) were on systemic immunosuppression for more than four months. The median dose of oral prednisone on presentation was 40mg (n=102, IQR 25-50mg) in the corticosteroid therapy failure group and 40mg (n=43, IQR 30-50mg) in the responders (p=0.29).

Univariate analysis

Seven variables were statistically disparate between corticosteroid therapy responders and non-responders after familywise error correction for multiple comparisons using the Bonferroni method. These were disease extent (extensive versus not extensive; p=0.0046), disease duration (p=0.017), oral prednisone therapy on admission (p=0.0048), ESR (p=0.036), CRP (p=0.011), haemoglobin (p=0.026), albumin ($p=6.85e^{-6}$), Mayo endoscopic subscore (MES; $p=1.21e^{-8}$).

Table 3.2 Patient characteristics by corticosteroid therapy response status

		Total (IQR, %)	Responder (IQR, %)	Failure (IQR, %)	p-value	Corrected p-value*
N		349	141 (40)	208 (60)		
Male gender		188 (54)	68 (48)	120 (58)	0.1	1
Age		33 (24-46)	32 (24-47)	34 (25-44)	0.68	1
Extent: E1-E2		114 (33)	62 (44)	52 (25)	0.00027	0.0046
E3		234 (67)	78 (56)	156 (75)		
Disease duration (years)		2 (0.25-7.21)	2.1 (1-9)	1.7 (0.1-5.4)	0.001	0.017
First UC episode		90 (26)	25 (18)	65 (31)	0.007	0.12
Oral prednisone		151 (43)	44 (31)	107 (51)	0.00028	0.0048
Immunomodulator		80 (23)	32 (23)	48 (23)	1	1
Bowel frequency		10 (8-15)	10 (7-13)	10 (8-15)	0.028	0.48
ESR (n=270)		42 (27-59)	37 (24-48)	47 (30-63)	0.0021	0.036
CRP		55 (21-112)	39 (11-100)	62 (28-122)	0.00063	0.011
Temperature		37 (36.7-37.4)	37 (36.6-37.4)	37 (36.7-37.5)	0.24	1
Pulse Rate		88 (76-98)	88 (80-99)	88 (75-96)	0.21	1
Haemoglobin		122 (106-138)	127 (109-140)	118 (103-134)	0.0015	0.026
Albumin		32 (28-36)	34 (30-37)	30 (26-35)	4.03e-7	6.85e-6
Number of TWC	0	7 (2)	6 (4)	1 (1)	0.029	0.49
	1	35 (10)	21 (15)	14 (7)		
	2	163 (47)	58 (41)	105 (50)		
	3	94 (27)	42 (30)	52 (25)		
	4	44 (13)	12 (9)	32 (15)		
	5	5 (1)	1 (1)	4 (2)		
Faecal calprotectin (n=166)		2100 (1025-3795)	1600 (860-3000)	2500 (1300-4400)	0.0085	0.14
MES (n=275)						
MES 0	0	2 (1)	2 (2)	0 (0)	7.15e-10	1.21e-8
MES 1	1	15 (5)	12 (11)	3 (2)		
MES 2	2	122 (44)	64 (60)	58 (34)		
MES 3	3	136 (49)	30 (32)	108 (64)		

UC: ulcerative colitis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TWC: Truelove and Witts criteria; MES: Mayo endoscopic subscore. Immunomodulator use included: azathioprine, 6-mercaptopurine, 6-thioguanine and methotrexate therapy for ≥4 months. *P-values corrected for familywise error using the Bonferroni method.

Stepwise logistic regression models

Partial (clinical) risk score for corticosteroid therapy failure

Clinical variables not reliant on an endoscopic evaluation that were statistically correlated with corticosteroid non response and additional variables included in the TWC were assessed in a multivariate general linear model. To identify the minimum data required to predict corticosteroid non response, stepwise regression was then applied to this model. The variables entered into the equation were: disease duration, oral prednisone therapy on admission, ESR, CRP, haemoglobin, albumin, bowel frequency, body temperature, pulse rate. A complete data set of n=270 was available for this analysis.

Following stepwise logistic regression the variables in the model were reduced to disease duration, bowel frequency, albumin, oral prednisone on admission, and CRP.

The removal of ESR from the variables in this model allowed the training dataset to be expanded to n=349. The logistic regression model was rerun with the five listed variables in the larger data set producing the following result.

Partial (clinical) risk score for corticosteroid therapy failure =

0.0971890 - 0.2446537Oral prednisone on admission - 0.0116896Bowel frequency
- 0.0004597CRP(mg/L) + 0.00.0171600Albumin(g/L) + 0.0079754Disease duration (years)

Validation and testing

Leave-one-out cross validation was performed.

The partial risk score for corticosteroid therapy failure algorithm was applied to the data set of 349 individuals with available data generating 349 score results (Table 3.3). Each result was assessed as a potential cut off or threshold for defining patients at high risk for corticosteroid therapy failure. As expected, increasing the threshold score used to define corticosteroid failure (thus including more individuals with a lower risk score result) reduced the positive predictive value of the algorithm (Figure 3.3).

A threshold score of 0.305 produced an 80% PPV for corticosteroid failure. This threshold decreased to 0.234, 0.179 and 0.151 for 85%, 89% and 92% PPVs for corticosteroid failure, respectively.

Table 3.3. Partial risk of corticosteroid therapy failure logistic model cut offs at various PPV intervals

Threshold	Sensitivity	Specificity	Accuracy	PPV	NPV	% of cases
0.151	0.163462	0.978723	0.492837	0.918919	0.442308	11
0.179	0.225962	0.957447	0.52149	0.886792	0.456081	15
0.234	0.293269	0.921986	0.547278	0.847222	0.469314	21
0.305	0.403846	0.851064	0.584527	0.8	0.491803	30

PPV: positive predictive value; NPV: negative predictive value; % cases: the percent of the total number of cases below the corresponding threshold

The 80%, 85%, 89% and 92% PPV thresholds encompassed 30%, 21%, 15% and 11% of the 349 presentations, respectively.

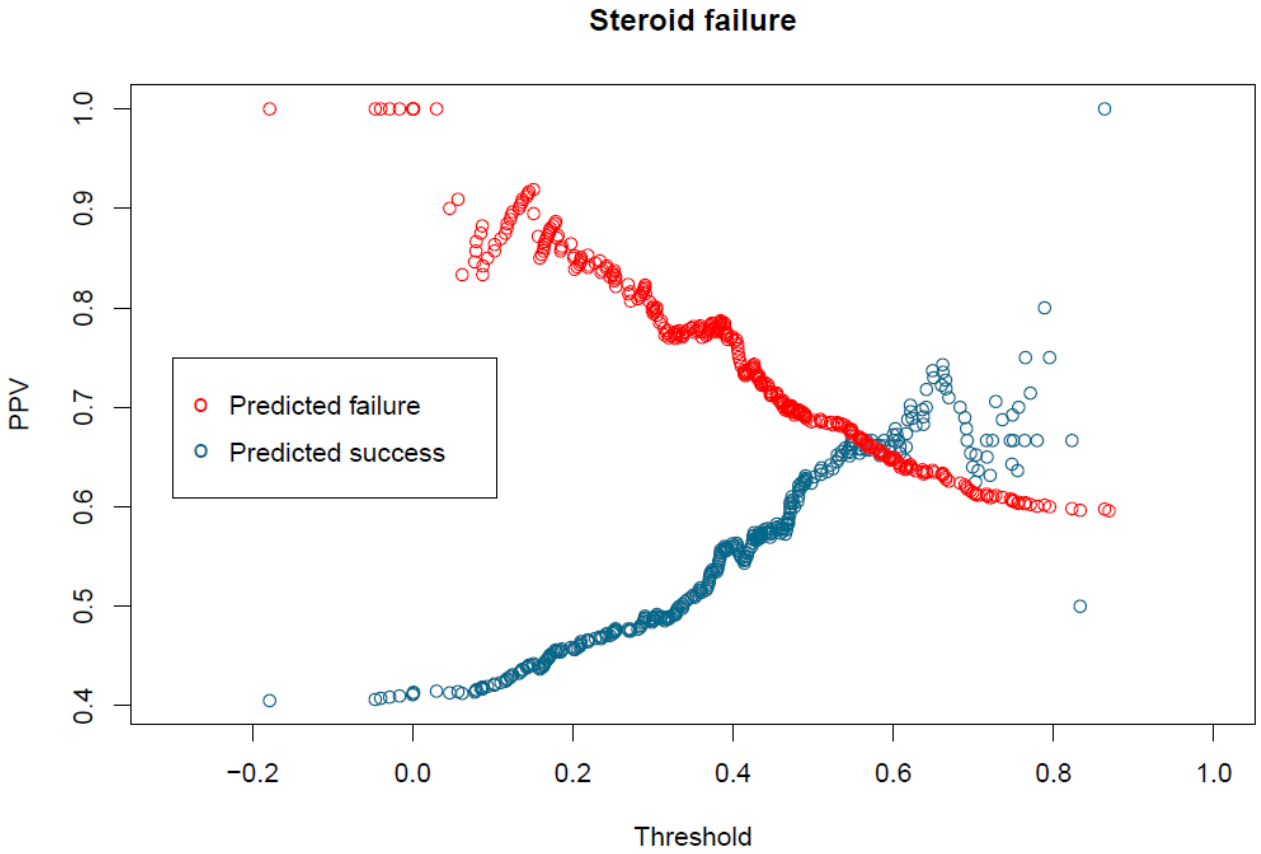

Figure 3.3 PPV for corticosteroid therapy failure at various thresholds of partial risk of corticosteroid failure scores (n=349). PPV: positive predictive value.

Full risk score for corticosteroid therapy failure

Statistically significant clinical variables in addition to the remaining variables contributing to the TWC were assessed in a stepwise logistic regression model on the basis of response or non-response to corticosteroid therapy. The variables entered into the equation were: disease duration, oral prednisone therapy on admission, ESR, CRP, haemoglobin, albumin, bowel frequency, body temperature, pulse rate, Mayo endoscopic subscore and disease extent. A complete data set with n=204 was available for the stepwise analysis.

Following application of the stepwise logistic regression model the variables remaining in the model were reduced to: bowel frequency, CRP, albumin, oral prednisone on admission and MES.
The restricted logistic regression model was then trained on an expanded data set of 275 presentations with data available for the five restricted variables producing the following result.

Full risk score for corticosteroid therapy failure:

0.770800 – 0.2214970Mayo endoscopic subscore – 0.2039264Oral prednisone on admission –0.0090635Bowel frequency – 0.0003810CRP(mg/L) + 0.0120914Albumin(g/L)

Validation and testing

Leave-one-out cross validation was used.

The full risk score for corticosteroid therapy failure score was applied to the data set of 275 individuals with available data (Table 3.4). As with the partial risk score, increasing the threshold used to predict corticosteroid failure reduced the positive predictive value of the algorithm (Figure 3.4).

A threshold of 0.307 produced an 85% PPV for corticosteroid failure. This threshold decreased to 0.161 and 0.0824 for 89% and 95% PPVs, respectively.

Table 3.4 Risk of corticosteroid therapy failure logistic regression model cut offs at discrete PPV intervals

Threshold	Sensitivity	Specificity	Accuracy	PPV	NPV	% cases
0.0824	0.125749	0.990741	0.465455	0.954545	0.422925	8
0.161	0.281437	0.944444	0.541818	0.886792	0.459459	19
0.307	0.526946	0.851852	0.654545	0.846154	0.538012	38

PPV: positive predictive value; NPV: negative predictive value; % cases: the percent of the total number of cases below the corresponding threshold

The 85%, 89% and 95% PPV thresholds encompassed 38%, 19% and 8% of the 275 presentations, respectively.

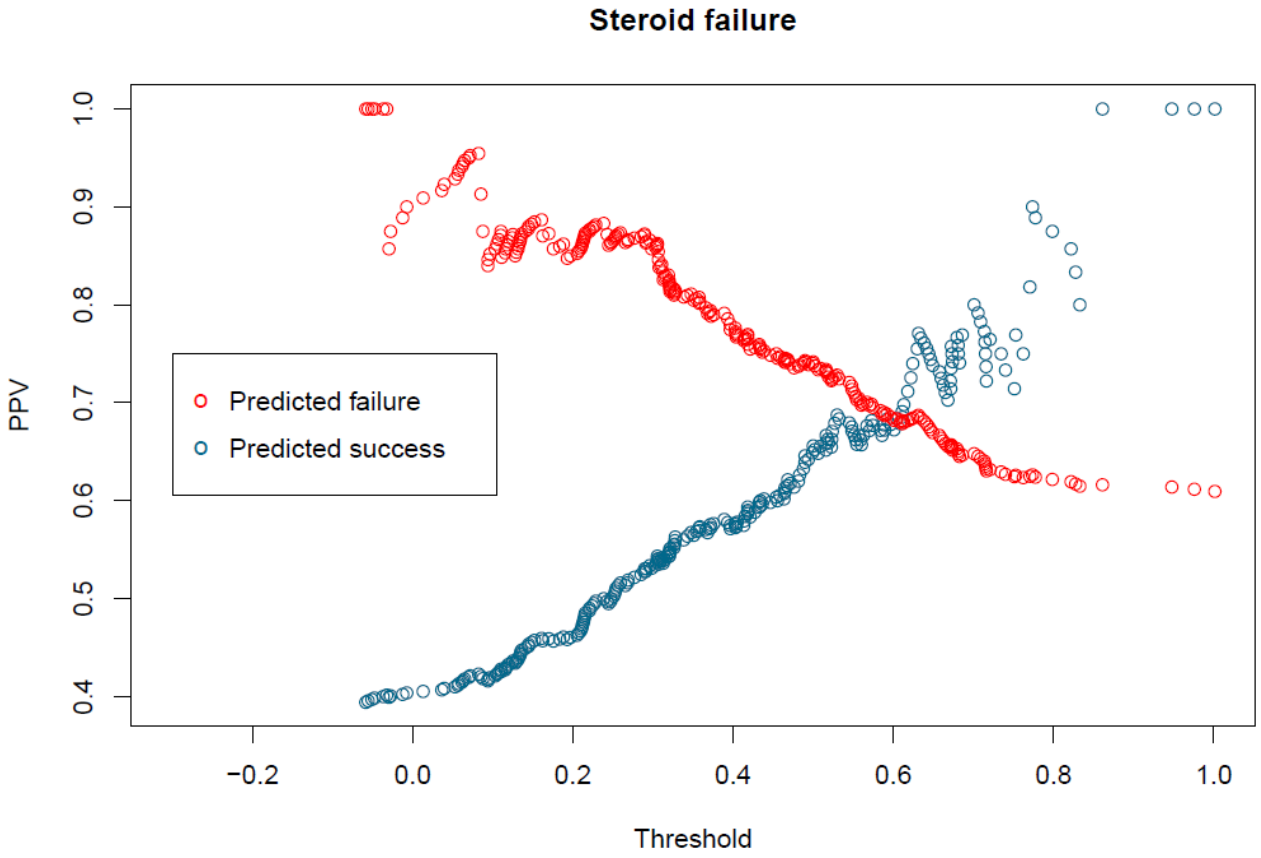

Figure 3.4 PPV for corticosteroid failure at various thresholds of full risk of corticosteroid failure scores (n=275). PPV: positive predictive value.

Alternative analytic approaches

Bayesian networks and deep learning analyses were also trialled to derive a risk score. Neither of these approaches produced greater discriminatory powers than the stepwise logistic regression models.

Colectomy-by-discharge risk analysis

A logistic regression model was also applied to the colectomy by 90 days clinical outcome. A meaningful risk score for colectomy by 90 days was not able to be derived from this admission data.

3.6 Discussion

A continuous risk score for failure of corticosteroid therapy based on clinical, endoscopic and laboratory parameters available at the time of presentation was derived from 349 presentations of acute UC.

This algorithm was derived from a large real-world cohort of patients treated at a single centre. As seen in other contemporaneous series, there was a predominance of male cases (54%). The fact that the study was performed at a large referral centre led to a skew towards corticosteroid treatment failure (60%). Those patients who presented directly to the referral centre had a corticosteroid failure rate of 50%. This figure is at the lower end the previously reported response range of 50-70%.[45]

This risk score is informative with regards to treatment failure and can provide an estimation of risk that is personalised to the patient and the severity of their immediate presentation. It is also versatile as it can be applied with or without the information obtained from an endoscopic assessment that, depending on the time and place of the presentation, may not always be available within 24 hours.

With the additional data from the endoscopic assessment a larger proportion (38% versus 21%) of patients were able to be identified as being high (>85% PPV) risk for corticosteroid failure. The observed relationship between endoscopic severity and the failure of medical therapy has been well described elsewhere.[6, 57, 63]

Whilst the UCEIS score has been found to have a better receiver operating characteristic (ROC) score for predicting the need for colectomy-by-discharge, the MES remains in widespread usage.[64] There is a balance to be struck between accessibility and the performance characteristics of the endoscopic scoring system. It was for these reasons and for consistency across the cohort that the MES was employed.

Whilst this continuous risk score incorporates five clinical, laboratory and endoscopic parameters at a time, all are available from either the patient history or promptly available laboratory tests or endoscopy. As the algorithm is not a simple 'rule' based score, in the clinical setting it will be accessed via an online or application-based clinical calculator (Figure 3.5). Whilst this may seem a barrier to its application at the bedside, in the new era of personalised medicine clinicians are more accepting of computer-based algorithms accessed through clinical calculators.[65] Another example in

gastroenterology of a score requiring calculation is the widely used MELD score. Like the MELD score, the risk of steroid failure score can be applied as an assessment of disease severity in acute UC at a given point in time that is linked to an important and informative clinical outcome.

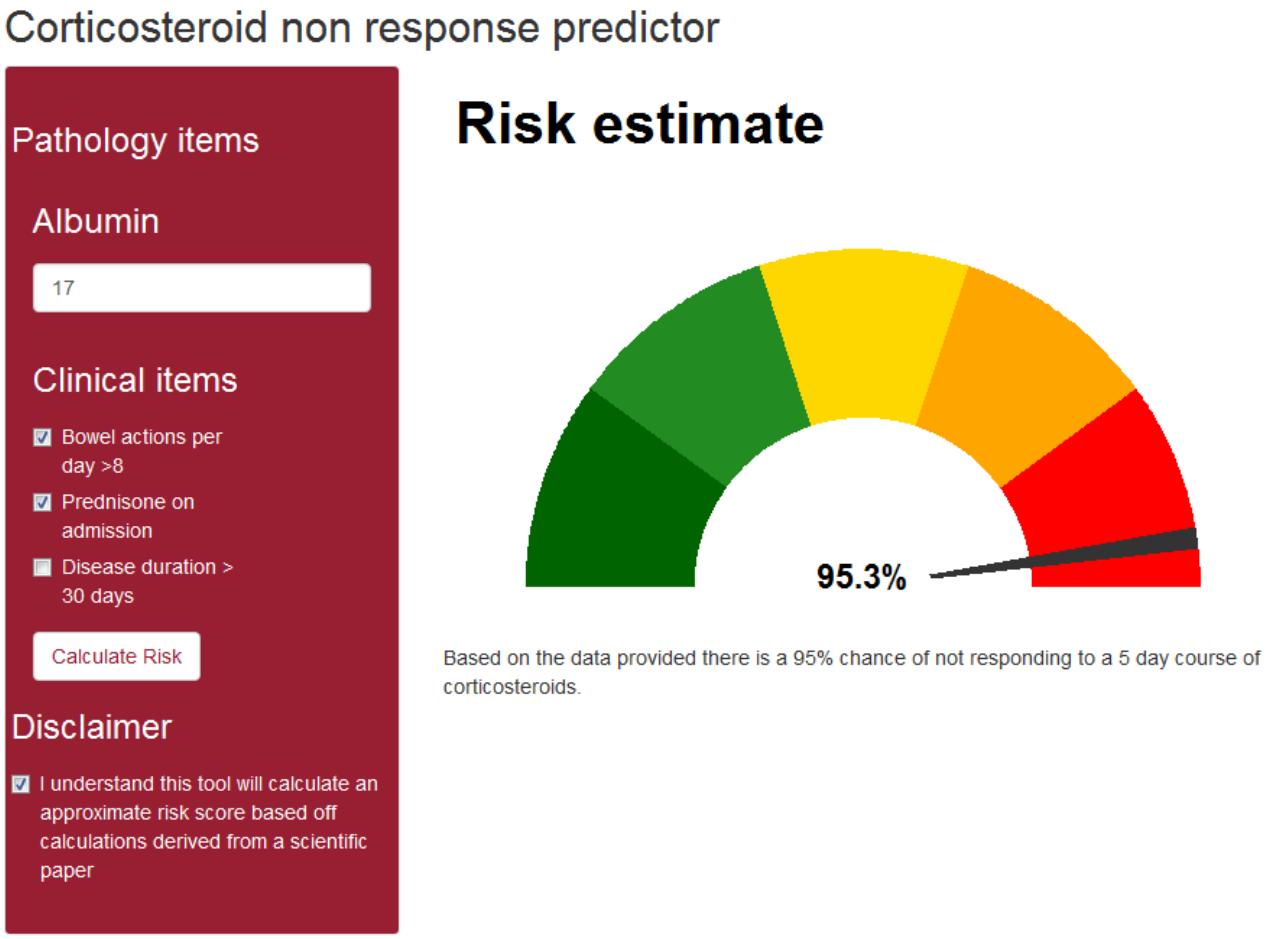

Figure 3.5 Example of an online clinical risk calculator result for an adapted partial (clinical) risk score showing a case at high risk of corticosteroid treatment failure

Strengths of this study include prospective data collection, the size of the series and the number of parameters assessed. Compared to previously reported scores, only the abstract from Edinburgh that analysed 444 admissions generated by 323 patients used a larger total number of cases in its derivation cohort. Of these, detailed data was available for 97 admissions.

The performance characteristics of this score compare favourably to those previously described to identify the likelihood of corticosteroid failure based on admission indices. The study of 49 patients from India identified just two variables that provided a PPV of 100% for corticosteroid failure.[50] However, the faecal calprotectin analysis was taken on a day three specimen so this algorithm is not purely based on presentation indices.

Whilst studies such as the recent abstract from Melbourne utilising admission faecal calprotectin are of interest, the practicality of using the faecal calprotectin to influence clinical management prior to day three of intravenous corticosteroid therapy requires either a reproducible and accurate bedside testing platform or a very prompt turnaround from a conventional laboratory based platform.

Additionally, when considering the reproducibility of results from quantitative faecal assays, faecal collection and sample processing methods must be closely controlled to ensure uniformity. Even when these precautions are taken and the same testing platform is used, faecal calprotectin results can vary widely during the same day in the same patient with severe UC.[66] As seen in previous chapters and the appendices of this thesis, in the experience of our centre this test performs poorly at separating moderate from severe levels of disease activity.

Whilst other studies have sought to replicate easily applicable rule-based criteria such as that used in the Oxford index, this may be a simplistic approach. By applying the risk score a more personalised assessment of the risk of treatment failure is undertaken. The slight inconvenience of having to input the relevant data into a clinical calculator is outweighed by the informed and nuanced approach to individual patient care that is afforded by the tool.

Shortcomings of the study include, single centre design. However, this does confer a degree of consistency in patient management and data collection. The series reflects a real world cohort of acute UC patients that has external validity to other analogous healthcare settings. Testing the newly derived score in a validation cohort will inform its performance characteristics in an independent series.

The long duration of data collection spans a few eras of acute UC management. The series is uniquely placed to answer questions relating to corticosteroid therapy for biologic naïve patients. It spans the era of access to salvage medical therapy for all presentations. It also presents the findings from a series of patients free from maintenance biologics. In a similar way that immunomodulator therapy has been observed to alter severe UC outcomes, maintenance biologic therapy is likely to alter the natural history of the presentation, management and the likelihood of response to the offered treatment.

Not all clinical indices evaluated in the univariate analysis were available for each patient. There were incomplete of data sets only for ESR (270/349), MES (275/349) and faecal calprotectin

(166/349) variables. This incompleteness of data could potentially lead to decreased representativeness of these variables in the univariate analysis.

Both partial and full corticosteroid failure risk tools were trained on a dataset of 349 presentations of moderate-severe acute UC. The rate of intravenous corticosteroid failure was 60% in this cohort. The training of this algorithm was optimised for predicting treatment failure, and as a result the predictive characteristics for treatment success were affected (Figures 3.3 and 3.4).

In more detail, the sensitivity and NPV calculations are affected by the fact that at the higher PPV and specificity thresholds there is a higher rate of false negative results. That is, there is a large number of patients whose risk scores lie above the threshold who go on to experience intravenous corticosteroid therapy failure.

In a real-world situation, identifying those at the highest level of risk for intravenous corticosteroid treatment failure is the most clinically useful group to identify for possible early treatment escalation. As the default clinical pathway is to continue with at least three days of intravenous corticosteroid therapy, the application of this algorithm is unlikely to affect the treatment outcomes for those patients with falsely negative risk scores who present with indices reflecting lower disease burden and treatment refractoriness.

Both the sensitivity and NPV can be altered by increasing the threshold value. However, this will result in the identification of a group with a more heterogenous risk profile for intravenous corticosteroid therapy failure. If these risk scores are to be implemented and subsequently evaluated for their ability to improve patient outcomes then clinicians would prefer the identification of a group at very high risk for corticosteroid therapy failure prior to changing currently accepted clinical practice.

With regards to changing current clinical practice, this new risk score is able to inform the clinician as to the risk of corticosteroid therapy failure. The earlier institution of treatment escalation in the form of intensified medical therapy or surgery has been observed to confer improved medium and long term outcomes in severe UC patients.[55]

In summary, a risk score based on a logistic model including the admission indices of oral corticosteroid failure, bowel frequency, CRP, albumin and either disease duration or MES was

trained on a set of 349 presentations of acute UC. This as yet unvalidated score has the potential to inform clinicians as to the timing of treatment escalation in acute UC.

3.7 Future directions

The next step for the developed risk scores are making them widely available for clinical use, initially on an internet-based platform.

A randomised clinical trial utilising the risk scores as a basis for the implementation of early treatment escalation is currently in the early stages of development.

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5.0 Appendices

Appendix A

A preliminary analysis of an n=270 cohort of patients for Chapter 1. This was a larger cohort of 270 presentations including 37 patients admitted with TWC moderate colitis and also those transferred from other facilities. This draft chapter includes the statistical approach undertaken in an attempt to obtain an expression that would accurately predict an ESR result based on a CRP and clinical variables at the time of presentation.

As the root-mean-square deviation (RMSD) for the predicted ESR was 35.6mm/h it was determined to be of limited utility. Subsequently, the statistical approach detailed in the body of the thesis was pursued.

Methods

Study population

During the study period 233 presentations with acute severe UC and 37 with moderately severe UC had paired ESR and CRP levels recorded within 24 hours of first presentation to hospital (Table 4). These presentations were generated by 252 individuals. There were no deaths of hospitalised patients as a consequence of UC, surgery or any subsequent complications.

Statistics

Spearman's correlation was used to assess associations between raw ESR and CRP data. Robust regression with Huber weighting was used to assess the relationship between CRP and the ESR corrected for albumin, haemoglobin, gender and age. The residuals for the ESR values of the severe cases corrected for albumin and haemoglobin were correlated with the actual CRP values by Spearman's correlation.

The results of the robust regression were also used to determine a formula predicting an ESR corrected for albumin and haemoglobin. Leave-one-out cross validation was used to determine the robustness of this formula. Spearman's correlation was used to correlate the relationship between the predicted and actual ESR value for the severe cases only.

Root mean squared difference (RMSD) was used to determine the accuracy of the ESR prediction formula.

Results

There was a male predominance of 54% with a median age of 32 years at admission. Nineteen percent of cases were index (first) presentations of UC. The median time elapsed between UC diagnosis and admission was two years (IQR 1-8 years). Two thirds (67%) of cases had disease proximal to the splenic flexure. Less than one quarter (23%) of cases were receiving systemic immunosuppression at the time of presentation.

Table 4. Moderately-acute severe UC patient characteristics

	Presentations (n=270)
Gender (male)	146 (54%)
Median age at admission (range)	32 (15-86)
Disease duration (median years)	2 (0-44)
First UC presentation	50 (19%)
Relapse	220 (81%)
Presentation location	
Tertiary centre	190 (70%)
Other hospital	80 (30%)
Severity of episode by TWC	
Moderate	37 (14%)
Severe	233 (86%)
Disease extent (Montreal classification)	
Proctitis (E1)	2 (1%)
Left-sided (E2)	87 (32%)
Extensive (E3)	181 (67%)
Immunosuppression on admission	
None	207 (77%)
Yes	63 (23%)

UC: ulcerative colitis; TWC: Truelove and Witts criteria

Raw ESR and CRP data

Both ESR and CRP on presentation were not normally distributed. The median (IQR) ESR was 42mm/h (27-59mm/h). The median (IQR) CRP was 54mg/L (21-103mg/L).

ESR-CRP correlation, robust regression, predicted ESR formula

Initial analyses revealed a weak correlation between the raw ESR and CRP (rho=0.38 by Spearman's correlation). The addition of 35 moderately severe cases modestly increased the strength of the correlation (rho=0.51 by Spearman's correlation, robust regression t-statistic for ESR=8.49). The moderately severe cases were subsequently removed from further analysis in the ESR prediction calculations due to their tendency to reduce the correlative and predictive power of the series in these analyses.

Correction by robust regression of the severely affected patient CRP against the ESR corrected for paired albumin and haemoglobin concentrations but not with the addition of either age or gender yielded significant t-statistics of 5.98, -4.73 and 5.00 for ESR, albumin and haemoglobin, respectively. The intercept/constant was 6.96.

The Spearman's correlation of the Huber weighted residuals from the CRP ~ ESR+Alb+Hb robust regression were correlated against CRP giving a rho=0.80.

Formula cross-validation and performance appraisal

Leave-one-out cross-validation produced an inter-quartile range for the intercept of 6.08-7.71.

The intercept and beta coefficients for the significant variables were used to construct a formula initially solved for CRP. The formula was then solved for ESR to derive the equation:

predicted ESR= (CRP + 3.37xalbumin - 1.04xhaemoglobin – 7)/1.08.

The predicted ESR was plotted and correlated against the actual ESR giving a Spearman's correlation of rho=0.67, R^2=0.45. Therefore, following this model 45% of the variance of the CRP can be attributed to the paired combination of ESR corrected for albumin and haemoglobin results.

The RMSD for the predicted ESR was 35.6mm/h.

Determining and applying the CRP cut off for the n=270 cohort

Compared to the traditional ESR cut off of >30mm/h, the lowest CRP value that produces a PPV of 81% was ≥20mg/L. This threshold captures 88% of cases with an ESR >30mm/h. The area under the curve is 0.77. A cut off of 142mg/L was required to generate a PPV of 91% (Table 5); however this captured only 22% of cases. The PPV is consistently above 80% for CRP cut off values from

20-200mg/L (Figure 2). The level of accuracy was consistently between 77-78% for CRP cut off values from 10-20mg/L (Figure 3).

Table 5. CRP cut off positive predictive values for a paired ESR of >30mm/h

CRP cut off (≥mg/L)	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**Accuracy (%)**
10	78	76	95	36	78
20	81	65	88	51	77
30	83	55	78	63	74
142	91	34	22	95	43

CRP: C-reactive protein; PPV: positive predictive value for CRP cut off having a matched ESR >30mm/h; NPV: negative predictive value for CRP cut off having a matched ESR >30mm/h

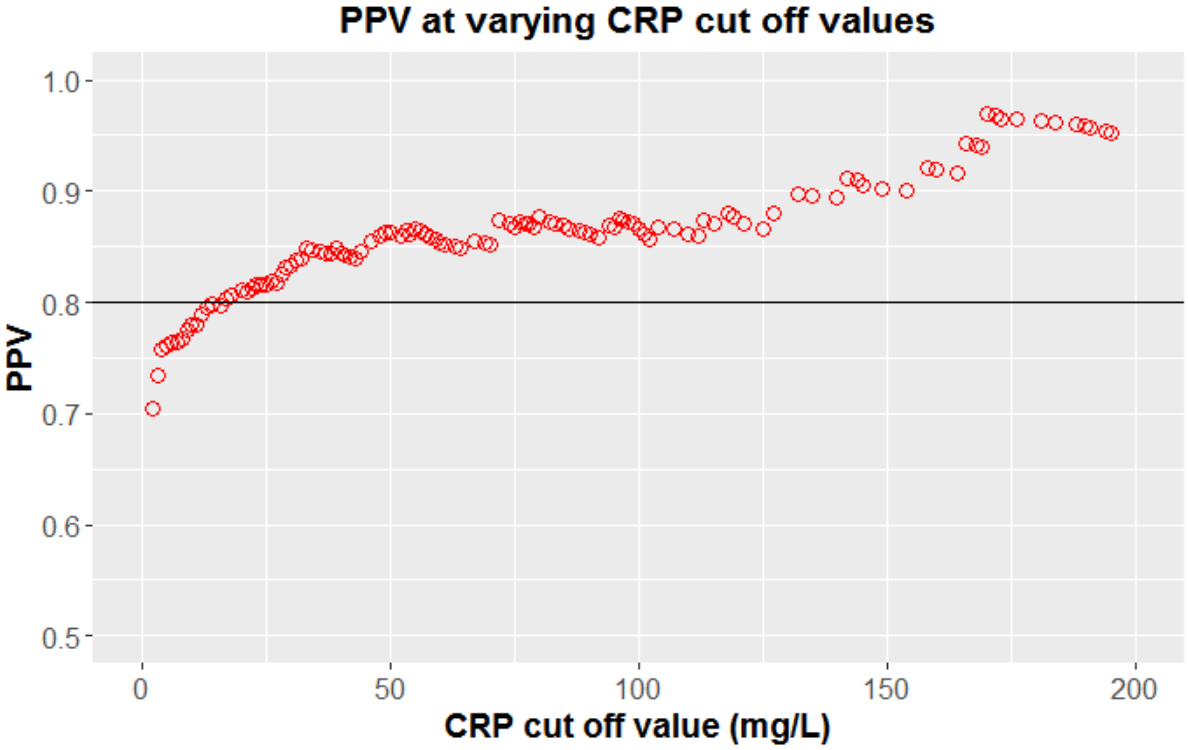

Figure 2. Positive predictive value for a matched ESR >30mm/h for a paired CRP cutoff

The application of the new CRP≥20mg/L cut off to the data altered the number of TWC fulfilled for 61/270 (23%) of patients (Figure 3). Conversely, 209 or 77% of presentations remained unchanged in their number of TWC satisfied on admission.

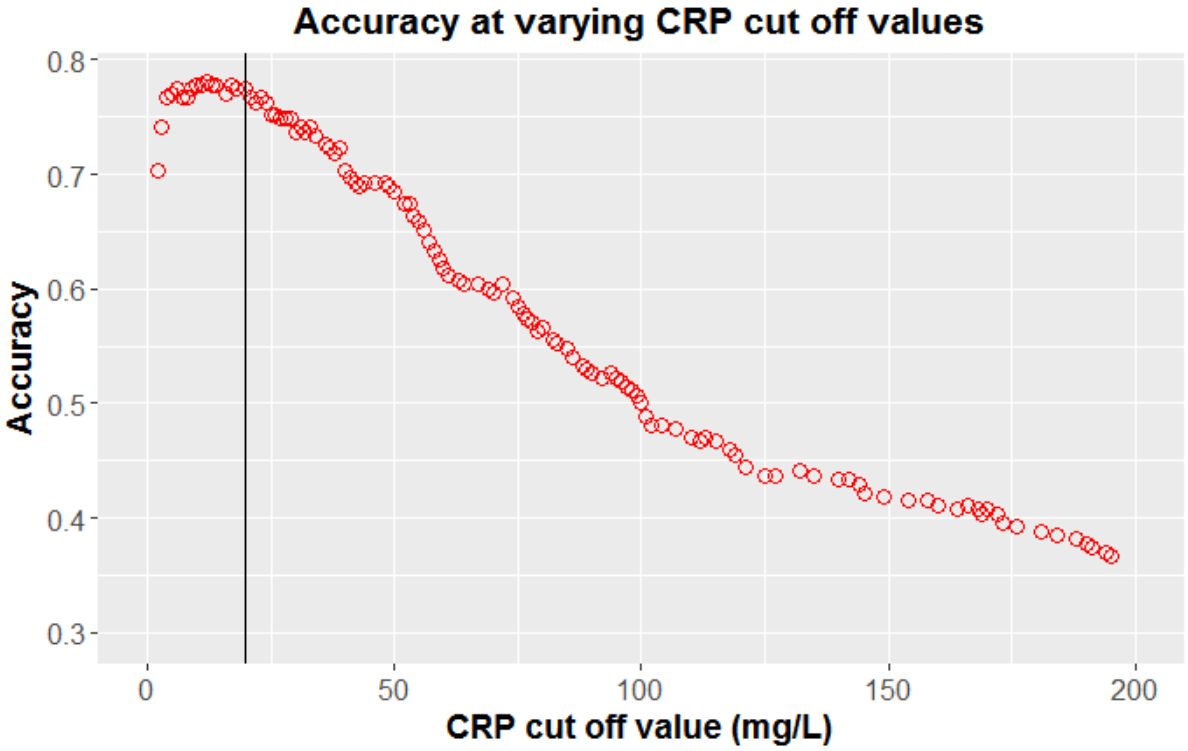

Figure 3. CRP accuracy at varying CRP cut off values.

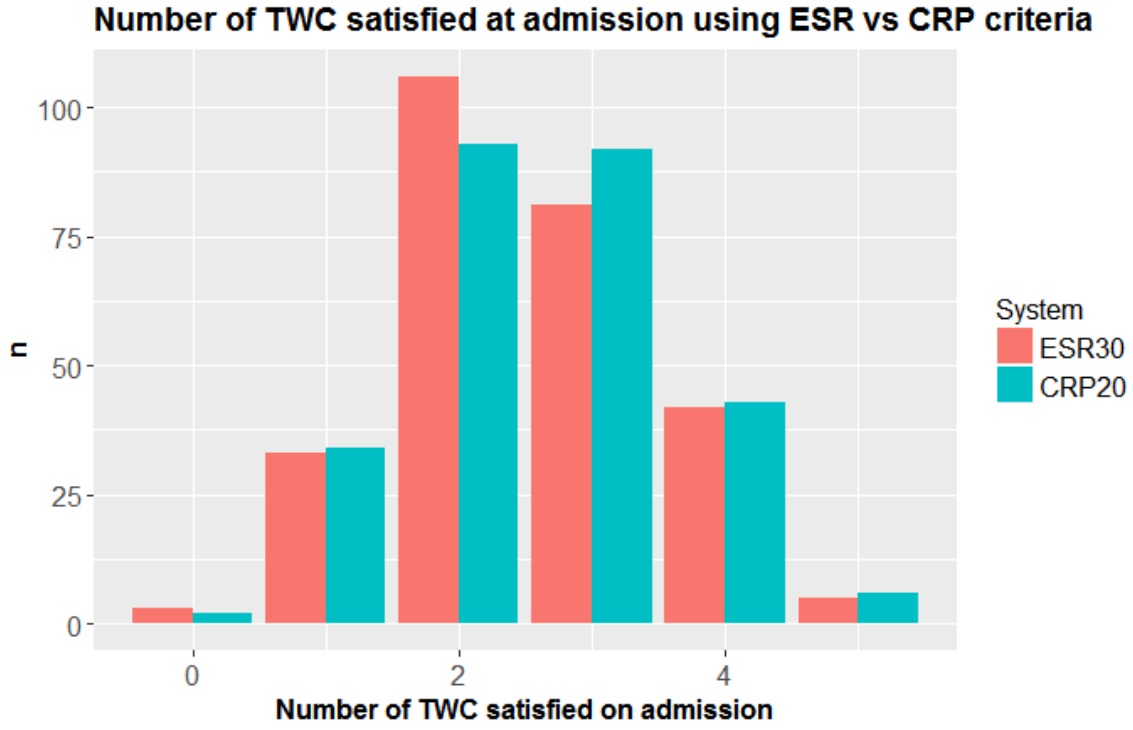

Figure 4. Number of Truelove and Witts criteria present at admission by ESR and CRP criteria.

There was a net increase in the number of TWC in 17 (6%) patients when the CRP≥20mg/L criteria was applied. Thirty-nine patients underwent an increase in their total number of severity criteria including eight having an upgraded diagnosis to severe from moderate colitis. Four of these

upgraded patients experienced corticosteroid therapy failure. In this group there were no colectomies performed prior to discharge.

Overall, 22 patients underwent a decrease in their severity criteria with nine patients being downgraded from severe to moderate disease status. Five of this group experienced corticosteroid therapy failure. Two of the nine patients progressed to inpatient colectomy following an incomplete response to rescue therapy.

Clinical outcomes

Following the application of the CRP≥20mg/L cut off there were no statistically significant changes to the number of patients with corticosteroid therapy failure in either the moderate versus the severe group or in any single TWC allocation group (Figure 5).

There were no statistically significant differences by Chi-squared or Fisher's exact tests in colectomy-by-discharge rate by number of TWC satisfied on admission between the ESR>30 and CRP≥20 criteria (Figure 6).

The number of colectomies prior to discharge in the moderate group did increase from two to four cases but this observation did not reach statistical significance. All four moderate cases that proceeded to colectomy-by-discharge satisfied at least one or more TWC.

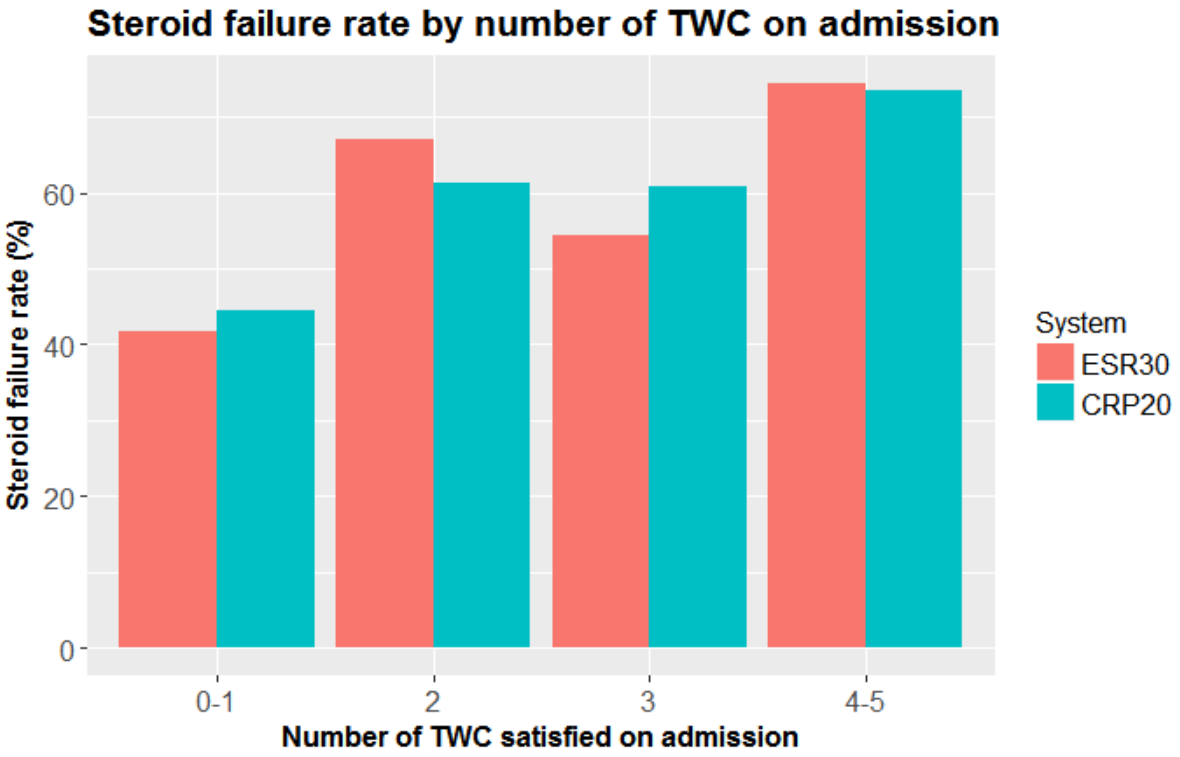

Figure 5. Corticosteroid failure rate by number of TWC and inflammatory marker classification system

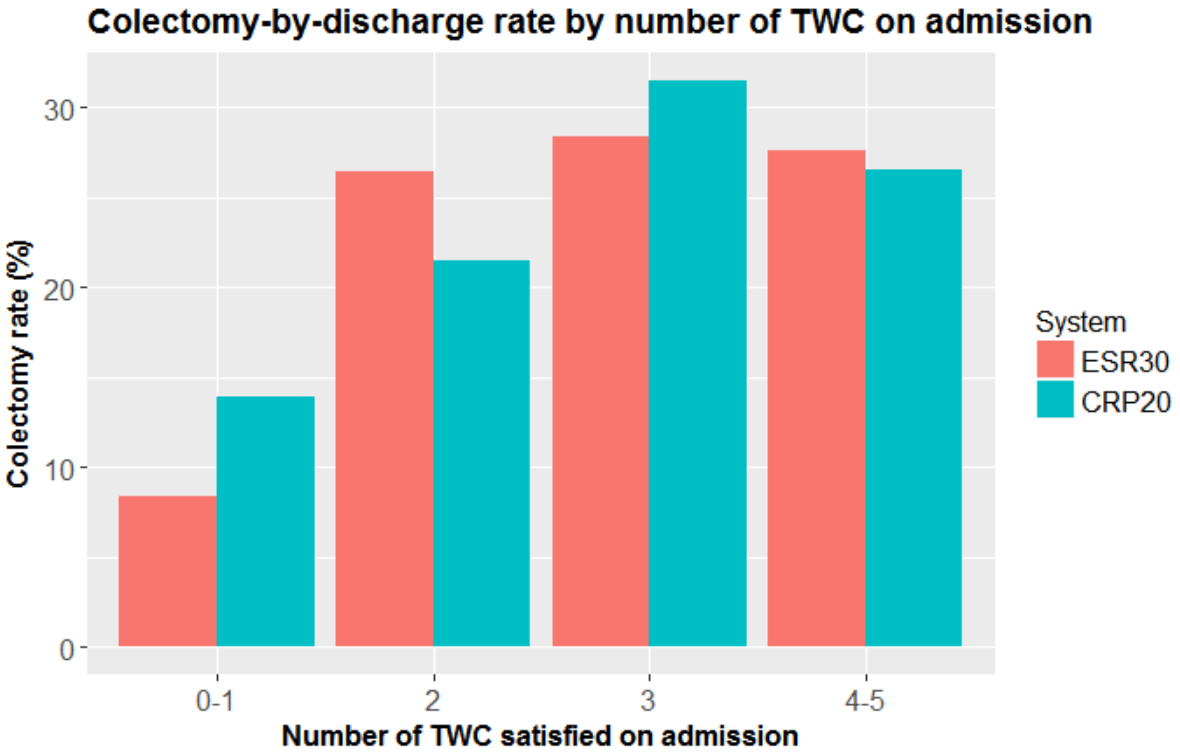

Figure 6. Colectomy-by-discharge rate by number of TWC fulfilled at admission and inflammatory marker allocation system

Other markers of disease severity on admission to hospital

Faecal calprotectin

Faecal calprotectin results were available for 117 patients (Figure 5). The median calprotectin in the moderate colitis group was 2000µg/g (n=27, IQR 1300-3667µg/g) and 2000µg/g (n=23, IQR 1033-3633µg/g) for ESR>30 and CRP≥20 criteria, respectively. For the severe colitis group the median calprotectin was 2100µg/g (n=90, IQR 937-4608µg/g) and 2100µg/g (n=94, IQR 1200-4608µg/g) for ESR>30 and CRP≥20 criteria respectively. There were no statistically significant differences between ESR>30 and CRP≥20 allocated moderate or severe groups.

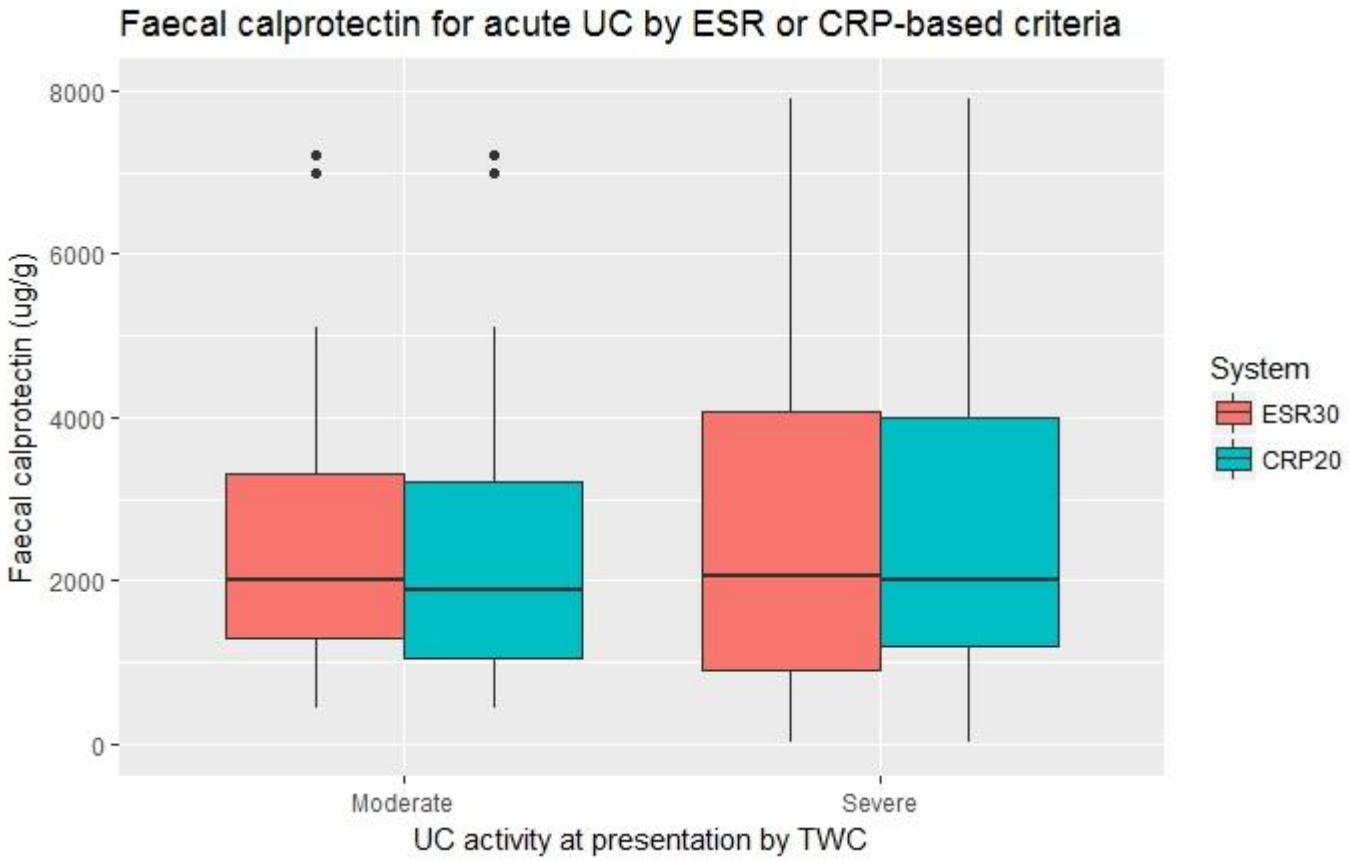

Figure 7. Faecal calprotectin at presentation by TWC activity on presentation and inflammatory marker allocation system. (12 data points not visualised due to y-axis limitation of 8000µg/g).

Mayo endoscopic subscore

Mayo endoscopic subscore results were available for 203 patients (Figure 8). There were no statistically significant differences found in MES results between ESR>30 and CRP≥20 allocated subgroups. There were also no significant differences found between groups allocated by the number of TWC satisfied on admission (TWC 0-1 and 4-5 groups combined).

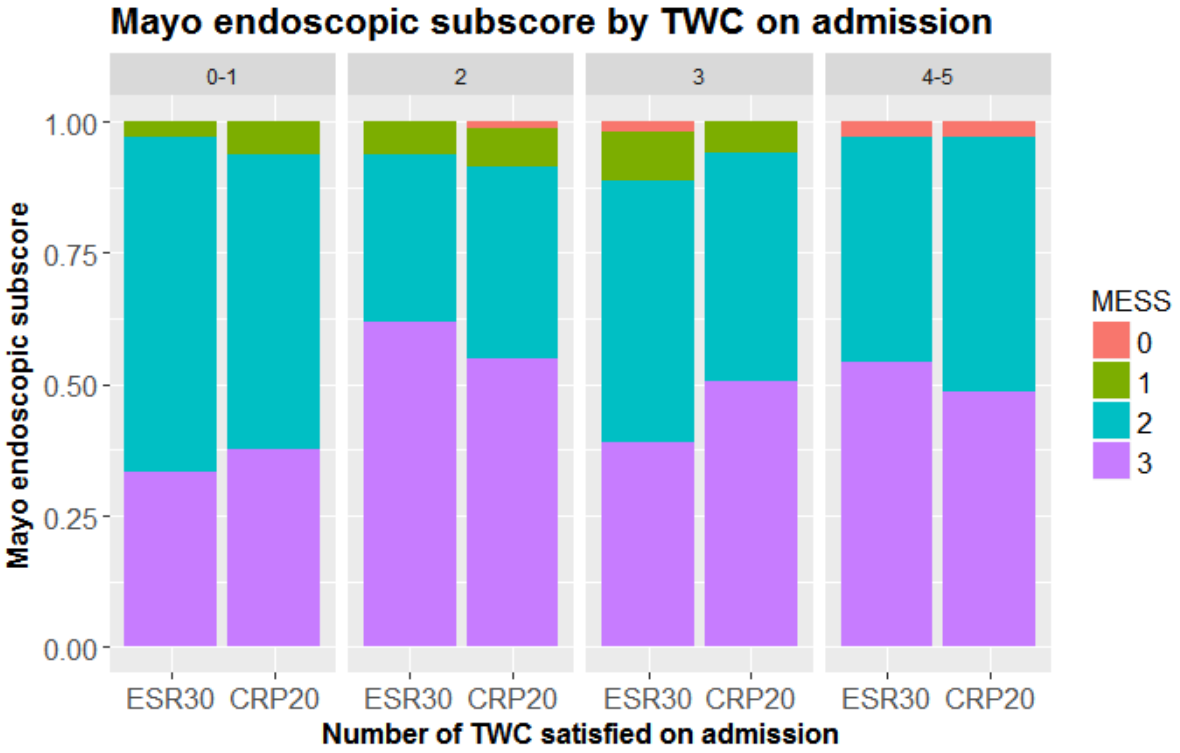

Figure 8. Mayo endoscopic subscore at presentation by TWC fulfilled and inflammatory marker allocation criteria

Trial of cut off with the inclusion of immunosuppression data

The results of the analyses performed in Chapter 2 reveal a trend towards a reduction in the presenting CRP level observed in patients on systemic immunosuppression. The cut off model was rerun with an adjustment of -14mg/L to the CRP concentration in those patients on systemic immunosuppression.

The CRP cut off conferring an 81% PPV increased to of ≥23mg/L. There were minimal changes to other resultant parameters including the overall accuracy of the cut off threshold.

The effect of disease extent on inflammatory markers in acute severe ulcerative colitis

Mann-Whitney U-test analysis revealed no statistically significant correlation between disease distribution when the series was divided into extensive and left-sided disease (including proctitis) and either ESR or CRP level at presentation, p=0.29, p=0.59, respectively. Similarly, robust regression did not identify a statistically significant relationship between disease distribution and inflammatory marker levels.

Appendix B

Abstract submitted to Digestive Disease Week 2018 and accepted for poster presentation

MARKERS OF SYSTEMIC INFLAMMATION IN MODERATE-SEVERE ULCERATIVE COLITIS: WHAT LEVEL OF C-REACTIVE PROTEIN CONSTITUTES ACUTE SEVERE ULCERATIVE COLITIS?

Background: The erythrocyte sedimentation rate (ESR) as a component of the Truelove and Witts Criteria (TWC) is the traditional inflammatory marker used for the assessment of ulcerative colitis (UC) activity. An ESR >30mm/hr is one of the four additional TWC that when present in addition to the mandatory stool frequency criteria, assigns the patient to the severe activity category. In some settings satisfying the TWC for severe disease is a requirement for the reimbursement of infliximab for use as rescue medical therapy following failure of intravenous corticosteroid therapy.

In current clinical practice the C-reactive protein (CRP) is the most frequently assessed blood derived marker of systemic inflammation.

Aim: To determine the equivalent CRP cut off for an ESR of >30mm/hr in patients with moderate-severely active UC admitted for intravenous corticosteroid therapy.

Methods: A retrospective analysis of prospectively collected data from 233 patients with TWC qualifying acute severe and 37 with moderately active UC admitted at a single tertiary referral centre was performed. Each patient had paired ESR and CRP assays performed in addition to the collection of other laboratory, clinical and endoscopic parameters. Statistical analyses were performed to determine a corresponding CRP cut off for acute severe UC on the basis of the positive predictive value and accuracy for having a paired ESR >30mm/hr. The entire series was allocated into groups by disease activity using the new CRP cut off in place of the ESR criteria. The characteristics of the allocation groups were compared using clinical outcome measures and an assessment of faecal calprotectin and Mayo endoscopic subscore (MES) at presentation.

Results: A CRP cut off of ≥20mg/L generated an 81% positive predictive value with a sensitivity of 88% and an accuracy of 77% for having a paired ESR >30mm/hr. Using the CRP cut off in place of the ESR criteria eight patients were upgraded from moderate to severe UC. Four of these patients experienced steroid therapy failure. Nine patients were down staged from severe to moderately severe UC. Five of these patients experienced steroid therapy failure with two undergoing surgery during the admission. Disease activity allocation was unchanged in 94% of cases. There were no statistically significant differences between disease activity groups allocated by the traditional ESR versus the new CRP-based criteria in the presenting faecal calprotectin, MES or the clinical outcomes of intravenous corticosteroid failure or colectomy prior to discharge.

Conclusion: The proposed CRP≥20mg/L cut off is an inclusive and sensitive cut off that when incorporated into the TWC, replacing the traditional ESR >30mm/hr criteria, had similar performance characteristics to the ESR criteria when used for the assessment of UC disease activity.

Appendix C

Human Research Ethics Committee approval letter

Queensland
Government

Metro North
Hospital and Health Service

Royal Brisbane & Women's Hospital
Human Research Ethics Committee

Enquiries to:	Ulani Hearn Assistant Coordinator
Telephone:	07 3646 6132
Facsimile:	07 3646 5849
File Ref:	HREC/14/QRBW/323
Email:	RBWH-Ethics@health.qld.gov.au

A/Professor Graham Radford-Smith
Department of Gastroenterology & Hepatology
Level 9, Ned Hanlon Building
Royal Brisbane & Women's Hospital
Herston Qld 4029

Dear Dr Radford-Smith,

Re: Ref N^{o}: HREC/14/QRBW/323: The Natural History of Inflammatory Bowel Disease: A Longitudinal Study of Disease Aetiology, Pathogenesis and Outcomes

I refer to correspondence dated 29 September 2017 in regard to this study. On 10 September 2017, I noted the Annual Report dated 04 October 2017 and approved of an extension for the study for an additional 3-year period, i.e. until 10 October 2020.

This will be noted by the Committee at its 13 November 2017 meeting.

Please provide a copy of this approval to each of the Principal Investigators who should provide a copy to their own Research Governance office.

It should be noted that all requirements of the original approval still apply. Please continue to provide at least annual progress reports until the study has been completed.

Please accept our best wishes for the remainder of the study and should you have any queries, please do not hesitate to contact the Research Ethics Coordinator on 07 3646 5490.

Yours sincerely,

Dr Allison Sutherland
A/Chairperson Human Research Ethics Committee
Royal Brisbane & Women's Hospital (EC 00172)
24.10.2017