

**Faecal Calprotectin and UCEIS Predict Short-term Outcomes in Acute Severe Colitis:
Prospective Cohort Study**

Short Title: Predictors of short-term outcome in Acute Severe Colitis

Saransh Jain, M.D.

Saurabh Kedia, M.D., D.M.

Sawan Bopanna, M.D., D.M.

Vikas Sachdeva, Msc.

Peush Sahni, M.S., Ph.D. #

Nihar Ranjan Dash, M.S. #

Sujoy Pal, M.S. #

Sreenivas Vishnubhatla PhD @

Govind Makharia M.D., D.M.

Simon PL Travis DPhil FRCP \$

Vineet Ahuja, M.D., D.M.

Departments of Gastroenterology, #Gastrointestinal Surgery, @Biostatistics

All India Institute of Medical Sciences, New Delhi, India.

\$ Translational Gastroenterology Unit, Oxford University Hospitals, Oxford UK

19 **Address for correspondence:**

20 Vineet Ahuja, M.D.

21 Professor of Gastroenterology,

22 All India Institute of Medical Sciences,

23 New Delhi, India.

24 Email: vineet.aiims@gmail.com

25 Phone No.: 91-11-26593300

26 Fax: 91-11-2658663

27 **Contact information:**

28 Saransh Jain: sjainmamc@gmail.com, Saurabh Kedia: dr.saurabhkedia@yahoo.com, Sawan

29 Bopanna: sawan_bops2001@yahoo.com, Vikas Sachdeva: vikasachdev@gmail.com, Peush

30 Sahni: peush_sahni@hotmail.com, Nihar Ranjan Dash: nagranjan@gmail.com, Sujoy Pal:

31 sujoypal@hotmail.com, Sreenivas Vishnubhatla: sreevishnubhatla@gmail.com, Govind

32 Makharia : govindmakharia@gmail.com, Simon P.L. Travis: simon.travis@ndm.ox.ac.uk,

33 Vineet Ahuja : vineet.aiims@gmail.com

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36 **Non standard abbreviations:** ASC: acute severe colitis, UC: ulcerative colitis, FC: faecal

37 calprotectin, IBD: inflammatory bowel disease, CRP: C-reactive protein, UCEIS: ulcerative

38 colitis endoscopic index of severity, AIIMS: All India Institute of Medical Sciences, CMV:

39 cytomegalovirus, ASA Aminosalicylic acid, EIMs: Extraintestinal manifestations, PPV:

40 positive predictive value, NPV: negative predictive value.

41

42

Abstract

Objective: Early objective markers for failure of intravenous(iv) corticosteroid for acute severe colitis (ASC) can avoid delay in rescue therapy or colectomy. We investigated faecal calprotectin (FC), C-reactive protein (CRP) and endoscopy using the ulcerative colitis endoscopic index of severity (UCEIS) as predictors of steroid-failure following intensive therapy of ASC.

Methods: Consecutive patients with ASC satisfying Truelove and Witts' criteria hospitalized at a single centre from May 2015 to November 2016 were included; all received iv corticosteroids. The primary outcome measure was steroid-failure defined as colectomy and/or rescue therapy with ciclosporin or infliximab during admission. FC levels were measured at admission and on day 3 of intensive therapy. UCEIS was scored at admission and CRP on day 3 of intensive therapy.

Results: 21 of 49(43%) patients failed iv corticosteroids and 15(31%) underwent surgery. FC levels were significantly higher in steroid-failures (2522(590-9654) μ g/g) compared to steroid responders (1530(352-10278) μ g/g) at admission ($p=0.04$), as well as on day 3 of iv corticosteroid therapy (2718(222-9175) μ g/g vs 727(218-4062) μ g/g, $p=0.001$). Steroid-failures had a higher median (range) UCEIS score than responders (6(4-8) vs 5(4-7) ($p=0.001$)). CRP level did not differ significantly between steroid failures and responders. A UCEIS >6 at admission and FC $>1000\mu$ g/g on day 3 were independent predictors of steroid-failure and need for rescue therapy/colectomy.

Conclusions: All patients with UCEIS >6 and day 3 FC $>1000\mu$ g/g failed iv corticosteroids. The UCEIS score on admission and day 3 FC are early predictors of failure of iv corticosteroid therapy.

Keywords: Acute severe colitis, steroid-failure, prediction

68 Introduction

69 Acute severe colitis (ASC) defined by Truelove and Witts' criteria is a medical emergency
 70 requiring hospitalization and time bound management. ASC complicates the course of
 71 ulcerative colitis (UC) in up to 25% of cases with a third of these episodes being the
 72 presentation of UC^{1,2}. Corticosteroids remain first-line therapy for ASC with a colectomy rate
 73 of 29% that has not changed since the 1970s³. Delay in surgery has been associated with worse
 74 outcomes^{4,5}. There is therefore a need to identify at an early stage those patients at high risk of
 75 iv corticosteroid therapy failure, thereby identifying patients in need of surgery or second line
 76 'rescue' therapy. Several indices have been proposed as predictors of lack of response to
 77 corticosteroids,⁶⁻⁹ with stool frequency and CRP at day 3 (Oxford criteria) being most widely
 78 adopted¹⁰. They are, however, based on stool frequency which is a patient-reported outcome
 79 and a subjective clinical variable dependent on rectal inflammation, which may in turn be
 80 influenced by local therapy. There remains a need for objective markers to complement
 81 existing indices.

82 In this regard, faecal calprotectin (FC) a neutrophil cytosolic protein¹¹ has emerged as a marker
 83 for disease activity in inflammatory bowel disease (IBD). FC has been found to discriminate
 84 between mild, moderate and severe endoscopic appearance of UC¹² and a fall in FC predicts
 85 response to infliximab therapy in UC^{13,14}. A prospective study of 90 patients with ASC showed
 86 that FC was significantly higher in patients undergoing colectomy during the hospital stay, with
 87 a (remarkably precise) level $>1992.5 \mu\text{g/g}$ associated with the need for colectomy over the next
 88 year¹⁵. In this study FC was measured between days 0–4 of admission, so was unable to
 89 determine its value in predicting steroid response at an early stage.

90 Serum CRP level is a marker of systemic inflammation and can be used as marker of
 91 inflammatory burden in ASC, however results have been inconsistent with few studies finding

it to be useful to predict response to iv corticosteroid therapy in ASC previously^{7,8} and few did not^{6,9}.

The Ulcerative Colitis Endoscopic Index of Severity (UCEIS), a validated index that accounts for 86-88% of intra and inter-observer variability in the assessment of endoscopic severity^{16,17}, has been shown to predict early and long-term response to anti-TNF α therapy^{18,19} and predict steroid-failure in ASC. In another retrospective study of 89 patients with ASC, the median UCEIS was significantly higher in steroid non-responders than responders (6 vs 5, p=0.005) and a score of 7 or 8/8 was associated with the need for rescue therapy or colectomy in all patients²⁰. The present study was designed prospectively to assess the role of FC on admission and day 3 of intensive therapy, CRP level on day 3 as well as the UCEIS on admission in predicting response to iv corticosteroid therapy in patients with ASC.

Methods

Patients

All patients with ASC defined by Truelove and Witts' criteria (below) who were hospitalized at All India Institute of Medical Sciences (AIIMS), New Delhi from June 2015 to November 2016 were screened for inclusion. Secondary referrals, children <18 years and patients who had an emergency colectomy without receiving steroids were excluded (Ethical approval AIIMS IRB: IESC/T-277).

Definitions

Ulcerative colitis: Diagnosis based on clinical, radiologic and histological criteria.²¹ Patients with index presentation of ASC that later turned out to be infection or Crohn's colitis were excluded.

Acute severe colitis: Based on Truelove and Witts' criteria (6 or more stools with blood and one or more of following: haemoglobin <105 g/L, erythrocyte sedimentation rate >30 mm/hr, fever >37.8°C, or tachycardia >90/minute^{10,22}).

Steroid-failure: Use of rescue medical therapy (iv ciclosporin 2mg/kg for up to 4 days, oral tacrolimus 0.1 to 0.2 mg/kg (adjusted to trough levels of 5–10 ng/mL) or infliximab 5mg/kg, although cost constraints often mitigated against the latter) during hospitalization (i.e. within 5-7 days of iv corticosteroid).

Disease extent: Maximum macroscopic extent at colonoscopy preceding ASC according to Montreal classification²³. For patients presenting with ASC at diagnosis, extent was determined from the first colonoscopy after discharge or the surgical specimen if they underwent colectomy.

126 Prior steroid use: Any use of systemic corticosteroids prior to the index episode of ASC.

127 Steroid use in first year of diagnosis: Corticosteroids in first year after diagnosis, including the
128 ASC event if within the first year of diagnosis.

129 Charlson co-morbidity index²⁴: to record and stratify co-morbidities.

130 UCEIS¹⁷: The sum of three descriptors: vascular pattern (scored 0-2); bleeding (scored 0-3);
131 and erosions and ulcers (scored 0-3), range 0-8, assessed in the most severely affected area at
132 flexible sigmoidoscopy (performed by SJ or SB). *Both the operators had experience of grading
133 endoscopic activity using the UCEIS in >100 patients with ulcerative colitis before the start of
134 the study.*

135 Faecal calprotectin: Faecal samples were collected and stored at -80°C within 1 hour of
136 collection. All samples were analysed by sandwich ELISA utilizing 2 antibodies to different
137 epitopes of human calprotectin in accordance with manufacturer's instructions (DIA source
138 Immuno Assay SA, Belgium). All samples were analysed at end of study. Lab personnel were
139 blinded to the outcomes.

140 **Study design**

141 Prospective observational cohort study that collected data on baseline demographics, prior
142 therapy for UC, plain abdominal radiograph and endoscopic assessment of severity
143 (UCEIS)¹⁷ by unprepared flexible sigmoidoscopy within 24h of admission, clinical
144 observations of pulse rate, temperature, blood pressure and stool frequency, laboratory
145 parameters during the hospital stay. Serum CRP was done on day 3 of iv corticosteroid therapy
146 and faecal samples were taken on admission for culture, *C. difficile toxin assay* by ELISA and
147 calprotectin, which was repeated on the first morning sample from day 3 of iv corticosteroid
148 therapy, where the day of admission was counted as day 1.

149 Outcome was failure to respond to iv corticosteroid therapy (use of rescue therapy or colectomy,
150 above).

151 **Management**

152 All patients received intravenous and rectal hydrocortisone (400mg/d iv, 200mg/d pr), whilst
153 continuing 5ASA therapy, according to guidelines,²⁵ as well as antibiotics (ciprofloxacin and
154 metronidazole) given the prevalence of gastrointestinal infection in India. Blood transfusion
155 was given as required (haemoglobin <80g/L), mucosal biopsies were taken at flexible
156 sigmoidoscopy to exclude CMV infection. Oxford criteria⁷ were used to identify patients at
157 high risk of colectomy and if unresponsive to 5-7 days of iv corticosteroids, rescue therapy or
158 colectomy was advised. The choice, decision and timing was made after joint medical-surgical
159 review and patient counselling. Patients responding to iv corticosteroids were discharged on
160 40mg/day prednisolone with a taper period of 3 to 4 months, along with azathioprine.

161 **Statistical analysis**

162 Continuous variables were expressed as the mean \pm SD and non-Gaussian distribution as median
163 and range. Categorical variables were summarized as frequencies with percentages.
164 Quantitative variables at admission were compared using Student's t test or Mann-Whitney U
165 test and qualitative variables by Chi-square test. Comparison of the means of continuous
166 variables for two groups was based on analysis of variance or the nonparametric Kruskal-
167 Wallis test, where indicated. Receiver operator characteristic curves were used to identify cut-
168 off with optimal sensitivity and specificity. Clinically relevant variables with p values <0.1
169 were considered for multivariable analysis, which was performed for steroid-failure as outcome
170 variable. A subgroup analysis excluding patients with CMV IHC positivity was done to identify
171 independent predictors of steroid failure in this subgroup. P values <0.05 were considered

172 statistically significant. Analyses were performed using Stata software 11.2 (College Station,
173 Texas, USA)
174

Results

Patients

53 patients with 57 episodes of ASC were hospitalized during the study period; 8/53 were excluded (Figure 1), so 45 patients with 49 episodes of ASC were included in the study. Response to iv corticosteroids occurred in 28/49 episodes and failure in 21/49. Of these 8/21 received rescue therapy (3 ciclosporin, 2 tacrolimus, 2 infliximab and 1 patient received iv immunoglobulin^{26,27}) and 15/21 had surgery, including 2 after rescue therapy.

Baseline demographic and clinical characteristics

Mean age on admission was 36±12 years, 74% male and 8% presented with ASC at diagnosis (see Table 1 for demographics). 71% had 2 or more Truelove and Witts' criteria on admission and 33% had Charlson co-morbidity index ≥ 1 .

Clinical and laboratory parameters during hospitalisation:

Median stool frequency on the day of presentation was 12(6-20) which decreased to 7 (2-18) on day 3 of iv corticosteroids (Table 2). Median haemoglobin (range) was 98 (47-155) g/L and ESR on admission was 45 (10-82) mm/hr and 25% received blood transfusion(s) (Table 2). Median FC on admission was 1776 (352-10278) $\mu\text{g/g}$ and 880 (218-9175) $\mu\text{g/g}$ on day 3 ($p=0.03$). Median UCEIS was 5 (4-8). Median CRP level on day 3 of iv corticosteroid was 23.8 (1.4-209) mg/L. One patient was positive for *C difficile* toxin in stool and CMV immunohistochemistry (IHC) was positive in 10/49 (20%) episodes. Patients received iv corticosteroids for a median 5(3-10) days with duration of hospital stay being 11(5–36) days. Four patients developed toxic megacolon, all of whom underwent colectomy. One patient died on post-operative day 3 from ventricular arrhythmia due to hypokalemia.

Comparison of clinical and laboratory parameters between corticosteroid-responders and-failures

Patients who failed iv corticosteroids (i.e. needing rescue therapy *and/or* colectomy) had a higher number of additional Truelove and Witts' criteria on admission (64% (3-4) vs 34% (1-2), $p=0.04$) and a higher frequency of steroid use in the first year of diagnosis, although this did not quite reach significance (14/28(76%) vs 16/21(50%) $p=0.06$). They also had a higher stool frequency on day 3: 8(3-18) vs 6(2-10) ($p=0.02$). There was no difference in age of presentation, sex, extent of disease, duration of UC prior to ASC, prior use of azathioprine, steroids, or tobacco, co-morbidities or presence of EIMs.

Faecal calprotectin

FC was significantly higher in steroid failures than responders, both on admission (2522 (590-9654) $\mu\text{g/g}$ vs 1530 (352-10278) $\mu\text{g/g}$ ($p=0.04$)), and at day 3 ((2718 (222-9175) vs 726.5 (218-4062) $\mu\text{g/g}$, $p=0.001$)). There was no difference between admission and day 3 value of FC in steroid-failures ((2522(590-9654) vs 2718 (222-9175) $\mu\text{g/g}$ ($p=0.9$)), where as among the responders the day 3 FC decreased significantly from baseline (1530(352-10278) to 726(218-4062) $\mu\text{g/g}$ ($p=0.001$))(Table 2).

There was a 21% fall in FC value (from admission to day 3) among steroid-responders as compared to 16% rise in steroid failures ($p=0.05$). A failure of FC to fall on day 3 was associated with steroid failure in 69% (11/16) of patients as compared to 30% (10/33) when it fell on day 3 ($p = 0.01$).

Endoscopic activity

Steroid-failures had a higher median UCEIS at baseline (6(4-8) vs 5(4-7), $p=0.001$) compared to responders (Table 2).

220 *C-reactive protein*

221 Median CRP levels on day 3 of iv corticosteroid therapy did not differ significantly between
 222 steroid- failures and –responders (36 (2.7-209) mg/L vs 19.6 (1.4-178) mg/L (p=0.4)) (Table
 223 2).

224 *Other parameters*

225 Steroid-failures and responders did not differ in terms of stool frequency or ESR on admission,
 226 or other parameters (Table 1 and 2), including CMV-positivity in mucosal biopsies. Duration
 227 of iv corticosteroids did not differ between steroid-failures and -responders, although steroid-
 228 failures had a longer duration of hospital stay 20(10-36) vs 8(5-21) days (p=0.001).13/23 (57%)
 229 patients satisfying Oxford criteria (stool frequency on day 3 > 8/day or stool frequency between
 230 3 to 8/day with CRP >45 mg/l) failed iv corticosteroid compared to 8/26 (31%) patients who
 231 did not satisfy the criteria (Table 4).

232 **Multivariable analysis**

233 On univariate analysissteroid use in the first year, more than 2 additional Truelove and Witts’
 234 criteria on admission, day 3 stool frequency >8, day 3 FC>1000µg/g and UCEIS>6 met the
 235 criteria for multivariable analysis between steroid-failures and –responders. On multivariable
 236 analysis only UCEIS score>6 (Odds ratio 8.6 (95% confidence interval 1.3-56, p= 0.02)) and
 237 FC >1000µg/g on day 3 of iv corticosteroids (OR 7.9(95%CI 2.0-31.8, p= 0.004)) remained
 238 significantly associated with steroid-failure (Table 3).

239 **Faecal calprotectin and UCEIS as predictors of steroid-response:**

240 Cut-offs for FC thresholds to predict steroid-failure were derived from receiver-operating
 241 characteristic (ROC) curves for FC on admission and day 3.

FC > 1800 µg/g on admission could discriminate between steroid-failures and -responders with a sensitivity of 66%, specificity 64% and AUC 67% (95% CI 51.8-82.2). FC > 1000 µg/g on day 3 of iv corticosteroids could discriminate steroid-failure with a sensitivity of 71%, specificity 75% and AUC 77% (95% CI 62.8-91.3, Figure 3).

UCEIS > 6 had a specificity 93% and PPV of 80% in predicting steroid-failure however had a low sensitivity (38%) and NPV (67%) (Figure 5 and Table 4).

All patients with day 3 FC > 1000 µg/g and UCEIS > 6 failed iv corticosteroids. If either one was present, 17/26 (65%) failed iv corticosteroids whereas if both were absent, only 4/23 (17%) failed iv corticosteroids ($p=0.001$) with OR 8.9 (2.3-34) (Table 4).

Those with UCEIS ≤ 6 tended to have lower median calprotectin on day 3 than those with UCEIS > 6 (785 (218-7427) µg/g vs 1993 (393-9175) µg/g, $p=0.07$) (figure 4).

We performed a subgroup analysis of episodes without CMV infection ($n=39$) which showed similar results with day 3 FC > 1000 µg/g and UCEIS > 6 being only independent predictor of steroid failure (supplementary appendix).

Prediction score:

A simple predictive score was derived based upon UCEIS and FC. This score was defined as:

Risk score: FC > 1000 µg/g + UCEIS > 6, where FC > 1000 µg/g = 1 and UCEIS > 6 = 1; and FC < 1000 µg/g = 0 and UCEIS < 6 = 0

This score ranged from 0 – 2, with the score of 0, 1 and 2 having PPV of 17%, 65% and 100% respectively for steroid failure in ASC. Also 83% of those who had a score 0 responded to iv corticosteroids (Table 5).

Discussion

There is a large therapeutic gap for patients with ASC when treated conventionally with intravenous corticosteroids. This gap is bridged either by medical rescue therapy (infliximab/ciclosporin/tacrolimus), or by colectomy. Timely intervention in patients with ASC not responding to iv corticosteroids is of paramount importance, because delays in initiating rescue therapy or surgery are associated with a higher rate of complications, including death^{4,5}. The third day of intensive corticosteroid therapy has become the most important junction in the treatment of patients with ASC, since clinical (stool frequency) and laboratory parameters (CRP) on day 3 can predict the likelihood of needing colectomy²⁸. However stool frequency is a semi-objective measure, may be affected by topical therapy and may not reflect the true inflammatory burden of the disease. There is, therefore, a need for objective parameter(s) to identify as early as possible in the disease course those patients who will need medical rescue therapy or surgery. An earlier prospective study from India evaluated 55 episodes of ASC and found haemoglobin <90 g/L, CRP >18.6 mg/L and prolongation of PT >2 seconds at admission to independently predict steroid-failure²⁹. Another study on 30 patients with ASC found that an admission stool frequency ≥ 9 , pulse rate ≥ 120 /minute, temperature $\geq 38^{\circ}\text{C}$, albumin ≤ 20 g/L together with pancolitis independently predicted steroid-failure³⁰. The present study, for the first time, has prospectively evaluated two objective markers: UCEIS and faecal calprotectin in predicting steroid response in patients with ASC.

The rate of steroid-failure rate (21/49, 43%) and colectomy (15/45, 33%) in our study at AIIMS, New Delhi are similar to reports from Western centres^{3,31,32}. We also found no difference in median age, disease duration, extent, course, prior immunomodulator use, haemoglobin, or albumin between steroid-failures and –responders. As an insight into the similarities between UC in India and elsewhere^{20,28}, we also found a significant association between steroid-failure

and steroid use in first year of disease, ≥ 2 Truelove and Witts' criteria on admission and higher median stool frequency on day 3. These all reflect a higher inflammatory burden of disease.

However, unlike other studies, median CRP levels were no different between steroid-failures and –responders. Consequently the Oxford third day index was also not significantly different between the two groups. The CRP is not uniformly elevated during active UC³³ and although some indices for predicting colectomy include CRP^{7,8,29}, others do not^{6,9}. This may reflect the differences between predicting steroid-failure and predicting colectomy.

In contrast, median FC levels (both at admission and day 3) and endoscopic severity were higher in steroid-failures than steroid-responders. Indeed, the only independent predictors of steroid-failure after adjusting for other variables were FC and UCEIS on day3, and the combination of the two identified all steroid-failures in this prospective cohort.

A novel finding in our study was that when FC failed to fall between admission and day 3, then this was associated with steroid-failure in 69% patients. We found that FC both at admission and day 3 to be predictive of steroid-failure and FC level at day 3 to be an independent predictor of steroid-failure, 68% patients with day 3 FC > 1000 $\mu\text{g/g}$ had steroid failure as compared to only 22% with FC < 1000 $\mu\text{g/g}$ (OR (95% CI) 8 (2-32)). FC broadly correlates with the severity of colitis¹² and serial levels have been shown to predict response to infliximab therapy^{13,14}. An earlier study showed FC to be higher in steroid-refractory patients with ASC¹⁵, but it lacked FC measurements on a specific day, which limited applicability of the results in clinical practice. FC predicted steroid-failure better than CRP, which may mean that FC is a more direct marker of intestinal inflammation.

The median UCEIS was significantly higher (6/8 vs 5/8) in steroid-failures compared to responders. This difference appears small, but confirms a retrospective study of 86 patients²⁰. The UCEIS was an independent predictor of steroid-failure (OR=9, 95%CI=1.3-56) and 8/10

with a UCEIS of 7 or 8/8 were steroid-failures. It may be argued that the UCEIS is not objective, since the endoscopist may well be the clinician looking after the patient: however, every patient admitted with ASC has (or should have) a flexible sigmoidoscopy within 24h of admission, not only to confirm the severity of colitis, but also to exclude complications such as CMV. As far as objectivity is concerned, the UCEIS accounts for 86-88% of inter-observer variation in multiple populations across the world¹⁷. A simple prediction score based on day 3 FC >1000 µg/g and UCEIS >6 can be used to predict steroid failure, with 0 meaning both negative, 1 meaning either positive and 2 meaning both positive to give a PPV of 17%, 65% and 100% prediction of steroid failure.

The primary outcome of the present study was steroid failure, which was defined as the need for surgery/rescue therapy. We realize that the choice of rescue therapy was not uniform, but this reflects the real world. The choice of rescue therapy was based on multiple factors including cost, availability, potential for complications, physician and patient choice. Nevertheless, the choice of rescue therapy could not affect the primary outcome.

There are, of course, other limitations to this study. The sample size, although prospective, is small. It is similar to that which lead to the Oxford criteria⁷ which changed practice. It is from a single centre from North India, but the demographics and outcomes are very similar to those from elsewhere. Access to biological or medical rescue therapy may differ in India to elsewhere, tending to a bias towards colectomy, but this is the real world for most and our numbers suggest otherwise. It is, however, conceivable that higher dose infliximab may have reduced the rate of colectomy but not every study concurs³⁴. Although the UCEIS scoring system is a quantitative and objective parameter, an external *post hoc* (based on videoclip) assessment of the UCEIS by blinded investigators would have improved the study. We simply

334 need better treatment for ASC and early predictors of the failure of conventional steroid
335 therapy.

336 A combination of a faecal calprotectin $>1000 \mu\text{g/g}$ on day 3 and UCEIS ≥ 6 on admission with
337 ASC are associated with all patients who will fail iv corticosteroids and need medical rescue
338 therapy or colectomy. The sooner objective measures of early response to treatment for ASC
339 are adopted, the fewer delays there should be in decision-making between physicians and
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348 **Saransh Jain:** Study design, acquisition of data, analysis and interpretation of data, drafting
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350 **Saurabh Kedia:** Analysis and interpretation of data, drafting of the manuscript, critical
351 revision and final approval of the manuscript.

352 **Sreenivas Vishnubhatla:** Statistical analysis, critical revision and final approval of the
353 manuscript.

354 **Sawan Bopanna, Vikas Sachdeva, Peush Sahni, Nihar Ranjan Dash, Sujoy Pal, Govind**
355 **Makharia:** Acquisition of data, drafting of the manuscript, critical revision and final approval
356 of the manuscript.

357 **Simon P.L. Travis:** analysis and interpretation of data; drafting of the manuscript, critical
358 revision and final approval of the manuscript.

359 **Vineet Ahuja:** study concept and design, analysis and interpretation of data; study supervision,
360 drafting of the manuscript, critical revision and final approval of the manuscript.

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482 **Figure legends:**

483 Figure 1: CONSORT diagram: Study population

484 Figure 2: Box-plots showing faecal calprotectin level in steroid responders and failure at
 485 admission and on day 3. Faecal calprotectin fell in responders (1530(352- 10278) vs 726(218-
 486 4062) $\mu\text{g/g}$) ($p=0.003$) and did not in non-responders (2522(590- 9654) vs 2718(222-
 487 9175) $\mu\text{g/g}$) ($p= 0.9$)

488 Figure 3: ROC curves of Faecal calprotectin day 3 for prediction of steroid-failure(AUROC
 489 77(62.8-91.3) %)

490 Figure 4: Box-plot representing day 3 Faecal calprotectin in patients with UCEIS ≤ 6 and >6
 491 ($p=0.07$)

492 Figure 5: Bar chart representing percentage of patients with steroid-failure with increasing
 493 UCEIS

494 Figure 6: Flexible sigmoidoscopy image of patients showing a) UCEIS= 4 ($V2/B1/U1 = 4/8$)
 495 and b) UCEIS =7 ($V2/B3/U2 = 7/8$)

496

497 Table 1: Comparison of baseline demographic and clinical characteristics in steroid-responders
 498 and failures among patients with acute severe colitis

	Total n=49	Steroid-responder n=28	Steroid-failure n= 21	p value
Age (year)	36.1 ± 11.9	37.6 ± 12.9	34.2 ± 10.4	0.3
Duration of UC prior to ASC (months)	36 (1-180)	36 (1-180)	36 (1-168)	0.4
Male (%)	36 (74)	20 (71)	16 (76)	0.7
Index presentation of UC as ASC (%)	4 (8.2)	3 (10.7)	1 (4.8)	0.6
Extent				0.8
E2 (left sided colitis)	15 (32)	9(33)	6(30)	
E3 (extensive colitis)	32 (68)	18 (67)	14(70)	
Prior azathioprine	22 (45)	12(43)	10 (48)	0.7
Previous ASC	12 (25)	8(28)	4(19)	0.5
Prior steroid use	39 (80)	21 (75)	18 (86)	0.5
Steroid use in 1 st year of diagnosis of UC	30 (61)	14(50)	16 (76)	0.06
Tobacco user	10 (20)	8 (29)	2 (10)	0.15
Presence of EIMs	15 (31)	7(25)	8(38)	0.3
Number of Truelove criteria on admission in addition to bloody stool frequency ≥6				0.04

1	14 (29)	7 (25)	7 (33)	
2	21 (42)	16 (57)	5 (24)	
3 or more	14 (29)	5 (18)	9 (43)	
Charlson comorbidity index ≥ 1	16 (33)	11(39)	5(24)	0.3
Pulse rate on admission (/min)	100 (69-142)	100 (80-142)	100 (69-128)	0.7
Stool frequency on admission (/day)	12 (6-20)	10 (6 -18)	12 (6-20)	0.08
Pulse rate beginning day 3 (/min)	84 (60-110)	84 (60-110)	86 (64-102)	0.8
Stool frequency during day 3(/day)	7 (2-18)	6 (2-10)	8 (3-18)	0.02

499 Value(s) provided as mean \pm standard deviation, median (range) or n (%) as appropriate. Extent
500 not available in 2 patients. UC: ulcerative colitis, ASC: acute severe colitis, EIMs:
501 extraintestinal manifestations

502

503 Table 2: Comparison of laboratory parameters and management in steroid-responders and
 504 steroid-failures among patients with acute severe colitis

	Total n= 49	Steroid responder n=28	Steroid-failure n= 21	p value
Haemoglobin on admission (g/L)	98 (47-155)	97 (47-155)	98 (5.3-15)	0.7
ESR on admission(mm/hr)	45 (10-82)	42 (10-57)	47 (22-82)	0.3
Blood transfusion n (%)	12(25)	5(18)	7 (33)	0.2
White cell count (x10 ⁹ /L)	8.6 (3.8-30.7)	9.5 (3.8-30.7)	7.9 (4.3-14.3)	0.2
Platelet day on admission(x10 ⁹ /L)	359 (50.9- 764)	355.5 (50.9- 764)	370 (132- 578)	0.9
Albumin on admission (g/L)	30 (10 -46)	30 (20- 46)	27 (10-43)	0.14
Median faecal calprotectin on admission (µg/g, range)	1776 (352-10278)	1530 (352-10278)	2522 (590- 9654)	0.04
UCEIS on admission	5(4-8)	5 (4-7)	6 (4-8)	0.01
UCEIS >6 n (%)	10 (20)	2 (7)	8 (38.1)	0.01

Haemoglobin day 3 (g/L)	95 (61- 144)	96 (61- 144)	94 (64- 132)	0.7
Platelet day 3(x10 ⁹ /L)	317(121-622)	315 (147- 622)	325 (121- 503)	0.4
CRP day 3 (mg/L)	23.8 (1.4-209)	19.6 (1.4-178)	36 (2.7-209)	0.4
Albumin day 3 (g/L)	30 (11-46)	31 (21-46)	27 (11-40)	0.09
Median faecal calprotectin day 3 (µg/g, range)	880 (218- 9175)	727 (218-4062)	2718 (222-9175)	0.001
Meeting Oxford third day criteria* n (%)	23 (47)	10 (36)	13 (62)	0.06
Stool positive for <i>C difficile</i> toxin n (%)	1 (2)	0	1 (5)	
Toxic megacolon during admission (n %)	4(8)	0	4(19)	
Median duration of iv steroid (days, range)	5 (3-10)	5(5-8)	5(3-10)	0.12
Median duration of admission (day, range)	11 (5-36)	8(5-21)	20 (10-36)	0.001
Mortality n (%)	1(2)	0	1(5)	

505 Value(s) provided as mean \pm standard deviation, median (range) or n (%) as appropriate. Extent
506 was not available in 2 patients. *Oxford third day criteria: stool frequency $>8/d$ or CRP >45
507 mg/L *and* 3-8 stools on third day of iv corticosteroid therapy. UC: ulcerative colitis, ASC: acute
508 severe colitis, EIMs: extraintestinal manifestations, RDW: red cell distribution width, ESR
509 erythrocyte sedimentation rate, TLC: total leukocyte count, CRP: C-reactive protein, UCEIS:
510 ulcerative colitis endoscopic severity index

511

512 **Table 3: Multivariate analysis for predictors of steroid response:**

Steroid-failure				
	Univariate		Multivariable	
	OR(95% CI)	p value	OR(95%CI)	p value
Steroid use in 1 st year	3.2(0.9-11.1)	0.06		ns
Number of Truelove criteria on admission>2	3.4(0.9-12.6)	0.06		ns
Oxford third day criteria#	2.9(0.9-9.4)	0.06		ns
Day 3 faecal calprotectin >1000 µg/g	7.9(1.9-31.7)	0.004	7.9(2-31.8)	0.004
UCEIS on admission >6	8.6(1.3-56)	0.02	8.6(1.3-56)	0.02

513 #Oxford third day criteria: stool frequency >8/d or CRP >45 mg/L and 3-8 stools on third day

514 of iv corticosteroid therapy

515 UCEIS: ulcerative colitis endoscopic severity index OR (95% CI) Odds ratio (95%

516 Confidence interval)

517

518

Table 4: Comparison of Oxford third day index, faecal calprotectin and UCEIS for prediction of steroid-failure:

	Sensitivity	Specificity	PPV	NPV	OR (95% CI)
Oxford third day index #	62%	64%	56%	69%	2.9(0.9-9.4)
Day 3 faecal calprotectin > 1000 µg/g	71%	75%	68%	78%	7.9(2-31.8)
UCEIS > 6	38%	93%	80%	67%	8.6(1.3-56)
Either UCEIS > 6 or FC > 1000 µg/g	81%	68%	65%	83%	8.9 (2.3-34)
Both UCEIS > 6 and FC > 1000 µg/g	29%	100%	100%	65%	

Oxford third day criteria: stool frequency >8/d or CRP >45 mg/L and 3-8 stools on third day of iv corticosteroid therapy

FC: faecal calprotectin, UCEIS: ulcerative colitis endoscopic severity index, PPV: positive predictive value, NPV: negative predictive value, OR (95% CI): odds ratio (95% confidence interval)

527 Table 5: Prediction score for steroid failure on basis of day 3 faecal calprotectin and UCEIS:

528

Score		Sensitivity	specificity	PPV	NPV
0	UCEIS ≤ 6 and Day 3 FC < 1000 μ g/g	19%	32%	17%	34%
1	UCEIS >6 or Day 3 FC $\geq 1000\mu$ g/g	81%	68%	65%	83%
2	UCEIS >6 and Day 3 FC $\geq 1000\mu$ g/g	29%	100%	100%	65%

529

530 FC: faecal calprotectin, UCEIS: ulcerative colitis endoscopic severity index, PPV: positive

531 predictive value, NPV: negative predictive value