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Exploration of Predictive Biomarkers of Early Infliximab Response in Acute Severe Colitis: A Prospective Pilot Study

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Abstract

Background: The outcomes of acute severe ulcerative colitis [ASUC] appear to be dependent on early intervention with the first and/or further infliximab [IFX] doses, although parameters to guide decision-making remain uncertain.
Aim: To assess whether serum/faecal IFX levels and inflammatory biomarkers early after IFX dose can predict ASUC outcomes.
Methods: This prospective pilot study consecutively recruited inpatients with steroid-refractory ASUC, who then received 1–3 IFX rescue doses [5 mg/kg per dose] at the discretion of the treating clinician. Serum IFX, C-reactive protein [CRP], albumin and faecal calprotectin [FC] concentrations were measured daily as an inpatient, and then 7, 14, 28 and 42 days post-first IFX. Faecal IFX was measured 1 day post-IFX. The primary end point was clinical remission (partial Mayo [PM] = 0) and CRP ≤3 mg/l at 6 weeks. Secondary end points were 12-week clinical remission or colectomy during follow-up.
Results: Of 24 ASUC patients with a median follow-up of 28 months [range 13–44], 10 [42%] achieved remission at 6 weeks, 12 [50%] achieved 12-week remission, six [25%] had colectomy. In total, 97% received either two or three IFX doses. Post-first dose, receiver–operator curve-derived cutoffs of the area-under-curve [AUC, Days 4–7] concentrations for serum IFX, FC and PM scores each predicted the primary end point with 100% sensitivity, and predicted future colectomy with 89–94% sensitivity. In multivariate analyses, faecal IFX >1 µg/g (odds ratio [OR] 0.04 [0.2, 0.9]), PM $AUC_{d1\text{-}3}$ < 20 (OR 20.2 [1.01, 404], each $P < 0.05$), FC $AUC_{d1\text{-}3}$ < 10 000 µg/ml [OR 13.6 [0.6, 294], trend only, $p = 0.09$) were each associated with clinical and CRP remission [6 weeks].
Conclusions: In ASUC, post-first dose IFX, early assessment of serum/faecal IFX, calprotectin and PM scores can accurately predict future remission and colectomy, and thus potentially aid in decision-making, i.e. accelerated IFX dosing or surgical planning if/when needed.

Key Words: Ulcerative colitis; infliximab; colectomy

1. Introduction

Infliximab [IFX] rescue therapy for patients with acute severe ulcerative colitis [ASUC] refractory to intravenous steroids is now standard-of-care in centres worldwide, with demonstrated efficacy in inducing remission,[1] reducing rates of colectomy,[2] and preventing future healthcare utilization and morbidity.[3,4] Yet it remains uncertain as to the optimal dosage and timing of IFX doses in this setting, and whether accelerated regimens or higher IFX doses actually improve outcomes. Notably, the dosage of 5 mg/kg and timing at Weeks 0, 2 and 6 of IFX have never been directly evaluated by a randomized, controlled trial for ASUC specifically, but rather this regimen was extrapolated from the IFX registration trials in Crohn's disease and chronic, moderate-to-severe ulcerative colitis.[5–7]

Moreover, if indeed recently promulgated accelerated IFX regimens are more effective,[8] it remains unclear as to the most appropriate indices upon which to base the decision to give IFX earlier and/or at high doses.[9] Currently, this decision-making appears somewhat ad hoc, based on a composite of clinical symptom assessment and/or serum biomarkers such as C-reactive protein [CRP] and albumin, but this is not entirely objective nor evidence-based.[10,11] Even the decision to proceed to colectomy after IFX appears to be variable and based on many subjective factors.[12–14] This raises the possibility that some patients with ASUC could be treated unnecessarily with extra doses of IFX [with the attendant increased costs and potential adverse effects] whilst others are directed unnecessarily, or at least prematurely, towards emergent colectomy [with its potential complications and morbidity].

Hitherto, multiple studies have elucidated markers at baseline predictive of a high risk of colectomy, thus prompting initiation of IFX rescue therapy within the first few days of admission for ASUC, which is now standard practice.[15–17] However, another critical aspect of the clinical decision-making in ASUC is assessing response following the first dose of IFX and whether a second, accelerated or higher dose[s] of IFX should be applied in patients with an inadequate response to the first dose. Hence, there is an urgent unmet need to characterize objective markers which can, as early as possible, reliably and reproducibly predict future outcomes following treatment with IFX rescue therapy for ASUC.

Therefore, in this prospective pilot study, we aimed to assess relationships between, and the predictive capability of, early measurement of serum IFX levels and inflammatory biomarkers after the first IFX dose with reference to future outcomes, including clinical outcomes, biochemical remission and colectomy, in patients with ASUC.

2. Methods

2.1. Study design and recruitment

This prospective, pilot 'real-world' study consecutively recruited consenting patients at least 18 years of age admitted with ASUC, as per modified Truelove–Witts criteria,[18] between June 2013 and June 2015 inclusive. All patients were deemed by their treating clinicians to have not responded adequately to 400 mg/day intravenous [IV] hydrocortisone at or after the third day of admission as per Oxford criteria.[17] All patients then received either one, two or three doses of IFX [5 mg/kg IV per dose] as rescue therapy with the timing and number of IFX doses at the discretion of the treating clinician rather than following a protocol.

2.2. Clinical assessment

Patient demographics, body mass index [BMI] and previous and contemporaneous inflammatory bowel disease [IBD] clinical and treatment data were collected. Partial Mayo scores were calculated daily until discharge and then at Days 7, 14, 28 and 42 by the study investigators. All patients underwent endoscopic assessment prior their first dose of IFX, and had cytomegalovirus [CMV] and other enteric infections excluded. One study investigator [L.B.] determined the endoscopy Mayo subscore in each case. Clinical remission was defined as a partial Mayo score of either 0 or 1.

2.3. Biomarker assessment

All laboratory tests were measured on a daily basis, at a similar time of the morning wherever possible, from baseline (prior to but on the same day of the first IFX dose [denoted 'Day 0']) until the day of discharge inclusive, then at Days 7, 14, 28 and 42 post-first dose IFX after the patient was discharged.

Serum IFX drug levels were measured by an enzyme-linked immunosorbent assay [ELISA; QS-INFLIXI, Matriks Biotek] as per the manufacturer's instructions and faecal calprotectin [FC]] concentrations were also measured by ELISA [Buhlmann] as per the manufacturer's instructions. Faecal IFX was also measured in the same IFX assay using the faecal extracts prepared for the calprotectin assay. Since both IFX and calprotectin assays were performed in batches [serum and faeces were stored at –20°C], the results did not influence clinical decision-making. Other tests performed by the study hospital laboratories as per standard institutional protocols included serum CRP, serum albumin, plasma haemoglobin and platelet count.

2.4. Study end points

The primary end point for this study was met if a serum CRP ≤3 mg/l and clinical remission was achieved at or before Day 42 [i.e. at the end of 6 weeks ± 5 days, allowing for variability of patients attending infusions or study visits]. The secondary end points for the study were: [1] whether clinical remission was achieved at the end of Week 12 [Day 84 ± 5 days] and [2] whether colectomy had been performed by the end of Week 12 [Day 84 ± 5 days].

The predictive capacity of each biomarker as measured above was also compared to two current predictive indices, the Travis criteria[17] and Ho[19] score, where a high score in each case was attributed at Day 3 of admission as previously described [Day 0 pre-IFX here].[20]

2.5. Statistical analysis

All data were analysed using IBM-SPSS version 20 and/or GraphPad PRISM version 6.0 software. Data were deemed to be non-parametric according to the Shapiro–Wilk test [$p < 0.05$] and thus continuous data were compared with medians and Mann–Whitney tests were applied. Categorical data were presented as proportions and compared using Fisher's exact test. A p value of ≤0.05 was deemed to be significant throughout this study.

To assess the predictive ability of early biomarkers to achieve the primary and secondary end points, receiver–operator curves [ROCs] were produced and the optimal cut-off values were elucidated. To verify the ROCs, the area-under-curve [AUC] was calculated via the trapezoid rule and selected given that the concept of drug exposure [i.e. serum concentration over time] appears an important determinant of drug response. Similarly, the inflammatory marker response to IFX and the relevance of inflammatory burden in ASUC were conceptually well represented by AUC over several days. Therefore, AUC calculations were applied across all biomarkers post-first dose IFX instead of other options such as 'spot' measures, absolute differences or the gradient of changes in biomarker concentrations, which were preliminarily tested. The AUC calculations applied for each biomarker were for both $AUC_{d1\text{-}3}$ [referring to AUC concentration over Days 1–3 post-first dose of IFX] and $AUC_{d4\text{-}7}$ [for AUC for Days 4–7 post-first dose of IFX [see Figure 1].

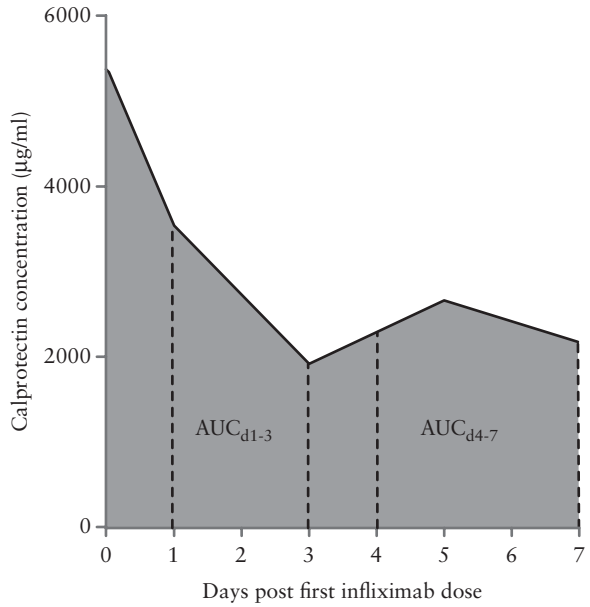

Figure 1. Example graph [using faecal calprotectin, µg/ml] demonstrating the concentration–time curves used to derive AUC_{d1-3} and AUC_{d4-7} calculations in this study.

Cut-off values for each ROC were chosen in a systematic manner so as to achieve in order of the following priority [highest to lowest]: [1] highest sensitivity, [2] same or similar cut-off value for a variable as derived for the primary end point and [3] highest specificity.

Subsequently, multivariate logistic regression analyses were performed to assess factors associated with the primary and secondary end points. This appeared to represent the best fit for these data, especially given the small sample size of the study with variables dichotomized where practicable, according to standard, previously published or ROC-derived cut-off values. Initially, bivariate analyses were performed, represented as odds ratios [ORs] with 95% confidence intervals [95% CIs]. Factors with statistically significant [or trending towards] associations with the individual end points in these analyses were included, as were factors of known or putative importance to the end point[s], such as duration of IV steroids prior to IFX, extensive colitis [E3] phenotype, disease duration since diagnosis and presence of faecal loss of IFX [dichotomized if detected >1 µg/g, no if undetectable, ≤1 µg/g].

Finally, subgroup analyses assessing scatterplots and correlations between serum IFX levels and other biomarkers were performed to further elucidate potentially important relationships between these factors of clinical relevance.

2.6. Ethics approval

The study was approved by the respective Alfred Health and Eastern Health Human Research Ethics Committees at each study site with each subject providing written, informed consent prior to participation in the study.

3. Results

3.1. Overall patient characteristics

Twenty-four consecutive patients who presented with ASUC [16 at The Alfred and eight at Eastern Health] and required IFX rescue therapy consented to be included and completed the study. After admission, all participants had failed IV steroids at Day 3 of admission. At both the initial and the third day of admission, each participant met Truelove–Witts criteria for the most severe category of colitis,[18] with a median of 15 [range 8, 30] bowel movements per day at baseline and eight [5, 16] on the third day of admission plus one or more of visible blood, tachycardia and/or pyrexia in each case. Hence, each was prescribed IFX at a dose of 5 mg/kg intravenously. Prior to IFX, endoscopic severity of colitis in all cases was rated either as moderate or severe [Mayo endoscopy score 2 or 3]. Further characteristics of the cohort are given in Table 1.

Ten [42%] patients met the primary end point of clinical remission with a normal CRP at 6 weeks. None of those who achieved this outcome required colectomy by the end of the study follow-up period. Twelve patients [50%] met the secondary end point of clinical remission at 3 months. Six patients [25%] with ASUC in this cohort required colectomy [within 3 years] but no deaths occurred, with a median follow-up of 28 months [range 13, 44].

3.2. Optimal biomarker cut-off values predictive of response to IFX

At the selected cut-off values over the first 3 days after the initial IFX dose, the AUC of partial Mayo and FC levels predicted clinical and CRP remission at 6 weeks, each with a sensitivity of 90% [ROC AUC 0.80 and 0.79; each $p < 0.05$]. Furthermore, the AUC_{d4-7} post-IFX for each of serum IFX level, partial Mayo score and FC level predicted clinical and CRP remission at 6 weeks, each with a sensitivity of 100% [specificity ranging from 36 to 57%, ROC AUC ranging from 0.72 to 0.85; each $p < 0.05$] at the respective optimal ROC cut-off values. In comparison, serum CRP and albumin levels, and CRP/albumin ratio did not exhibit similar predictive accuracy, with non-significant ROCs for both AUC_{d1-3} and AUC_{d4-7} post-IFX. There was minimal or no gain in accuracy by combining biomarkers as composite markers in terms of predictive ability for any of the primary or secondary end points.

Furthermore, at similar cut-off values derived via ROC for the primary end point, serum IFX, partial Mayo score and FC levels [AUC_{d4-7} post-first IFX dose] were each able to predict 'clinical remission at 12 weeks' and 'colectomy' with sensitivities ranging from 89 to 100% [ROC AUCs ranging from 0.86 to 0.96, each $p < 0.05$] [Table 2].

Finally, the sensitivities of AUC measurements [derived post-IFX as above] for serum IFX, partial Mayo score and FC consistently outperformed the sensitivities of Travis and Ho high-risk scores [derived pre-IFX] in relation to all three end points in this study, as shown in Table 2.

3.3. Factors associated with clinical and CRP remission [6 weeks] and clinical remission [12 weeks] post-IFX

To ascertain factors potentially associated with, or predictive of, clinical and CRP remission at 6 weeks post-first dose of IFX, bivariate analyses were initially performed, as shown in Table 3. Dichotomous cut-off values for relevant biomarker variables were assigned based on the cut-off values derived from the ROC analyses above [Table 2].

Regarding the primary end point, those who did not receive a prolonged course of IV steroids prior to receiving IFX, nor had detectable faecal IFX levels [>1 µg/g at Day 1 post-first dose of IFX] and those with relatively lower FC and/or serum IFX levels were more likely to achieve remission at 6 weeks. Moreover, those with more extensive colitis or longer disease duration were more likely to achieve clinical remission at 12 weeks.

Table 1. Characteristics of patient cohort [$n = 24$]

Variable as of presentation with ASUC [Day 1]		
Age, years (median [range])		36 [18, 72]
Sex	Male	12 [50%]
	Female	12 [50%]
Disease duration since diagnosis, months (median [range])		38 [1, 372]
Disease extent [Montreal criteria]	E1	0
	E2	13 [57%]
	E3	10 [43%]
Smoking status	Non-smokers	15 [62%]
	Ex-smokers	9 [38%]
	Current smokers	0
Body mass index, kg/m^2 (median [range])		24 [17, 35]
Number of days received IV steroids prior first IFX (median [range])		5 [3, 8]
Concomitant medications	Treatment naïve [first presentation]	5 [21%]
	Aminosalicylates	20 [83%]
	Immunomodulators	8 [33%]
	Steroids [within prior 3 months]	17 [71%]
Number of IFX infusions given for ASUC salvage therapy [5 mg/kg IV]	1	3 [13%]
	2	17 [71%]
	3	4 [16%]
Duration between 1st and 2nd IFX salvage dose, days (median [range])		5 [1, 23]
Colectomy rate at 30 days		2 [8%]
Colectomy rate during follow-up		6 [25%]
Death during follow-up		0 [0%]
Study follow-up period, months (median [range])		28 [13, 44]
Endoscopic severity [Mayo score]	***1***	0 [0%]
	2	6 [25%]
	3	18 [75%]
Partial Mayo score at baseline (median [range])		8 [6, 11]
Time to CRP normalization (median [range])		17 [1, 170]
Baseline CRP, mg/l (median [range])		26 [4, 171]
Baseline faecal calprotectin, µg/ml ×10^3 (median [range])		5.8 [1.6, 100.1]
Baseline serum albumin, g/l (median [range])		25 [12, 34]
Baseline plasma haemoglobin, g/dl (median [range])		11.2 [8.0, 13.3]
CLINICAL & CRP REMISSION at 6 weeks	Partial Mayo 0 or 1, **plus** CRP ≤ 3 mg/l	10 [42%]
CLINICAL REMISSION at 12 weeks	Partial Mayo 0 or 1	12 [50%]

ASUC, acute severe ulcerative colitis; IV, intravenous; IFX, infliximab; CRP, C-reactive protein.

3.4. Concept of 'IFX refractoriness' in ASUC patients in this cohort

Given that elevated IFX levels [as per IFX $AUC_{d4\text{-}7}$] in this cohort were accurately predictive of negative outcomes, including non-remission at 6 and 12 weeks, and future colectomy with sensitivities of ≥90%, further exploration of the factors associated with higher IFX $AUC_{d4\text{-}7}$ levels was performed. Based on Spearman's correlation analyses, the partial Mayo score and FC levels were each strongly correlated with higher IFX $AUC_{d4\text{-}7}$, as shown in Figure 2. Furthermore, the duration of IV steroids administered pre-IFX [days] and the faecal IFX concentration [measured at Day 1 post-first IFX dose] were each strongly positively correlated with IFX $AUC_{d4\text{-}7}$, whereas the duration between the first and second IFX doses was inversely correlated [each $p < 0.05$].

Additionally, the three-dimensional scatterplots further illustrate the clustering of cases with a triumvirate of higher serum IFX and FC levels, and partial Mayo scores in association with adverse prognostic cofactors including those either subsequently receiving a colectomy and/or evidence for faecal loss of IFX [Figure 3A, B]. Conversely, Figure 3C shows the clustering of those patients who achieved clinical and CRP remission at 6 weeks, with typically lower levels of serum IFX, FC and partial Mayo scores, again emphasizing the inter-relationship between these variables.

4. Discussion

Whereas the factors associated with poorer outcomes in ASUC at baseline [either pre-steroids or pre-IFX] have been well characterized,[15,17] those factors immediately after the first dose of IFX that are potentially predictive of IFX-induced remission at 6 weeks have not been elucidated. The current prospective study of patients with strictly defined ASUC receiving IFX rescue therapy has addressed this issue in a real-world setting. Several key observations have been made that may inform clinical decision-making at this critical juncture.

First, early serial FC measurement is a more discriminative predictor of post-IFX outcomes than serum CRP or serum albumin, the foci of standard care. Previously, Kohn *et al.*[10] have shown that an elevated CRP at Days 3 and 7 post-IFX and Gibson *et al.*[11] found that a pre-IFX albumin level <22 g/l [with a trend to CRP/albumin ratio > 1.7] were associated with colectomy 2 and 3 months later, respectively. However, consistent with this study, Ho *et al.* demonstrated that baseline FC levels were higher in those who subsequently required colectomy and/or in IFX non-responders.[21]

Secondly, the serial measurement of symptoms as per partial Mayo scores was of similar accuracy to FC in predicting clinical and CRP remission at 6 weeks, and the secondary end points. It is known that

Table 2. ROC-derived optimal cutoffs assessing the ability of early biomarker levels [AUC] post-infliximab to accurately predict [A] clinical and CRP remission at 6 weeks, and [B] colectomy within 1 year of follow-up

	Optimal AUC cutoff[1]	Sensitivity [%]	Specificity [%]	AUC [95% CI]
[A] PREDICTING CRP & CLINICAL REMISSION AT 6 WEEKS				
REFERENCE INDICES:[17,19]	*for Day 0 prior to initial IFX dose*			
Travis index – high risk	–	46	64	–
Ho score – high risk	–	57	65	–
	for AUC Days 1–3 post-initial IFX dose			
Serum infliximab, mg/ml	<120	90	36	0.67 [0.45, 0.90]
Partial Mayo score	<20	90	64	0.80 [0.61, 0.99]
Faecal calprotectin, µg/ml	<10,000	90	64	0.79 [0.59, 0.98]
Serum CRP, mg/l	<100	90	36	0.61 [0.38, 0.85]
Serum albumin, g/l	>79	79	36	0.54 [0.30, 0.77]
Serum CRP/albumin ratio	<2.0	70	50	0.59 [0.36, 0.83]
COMPOSITE VARIABLES: [2]				
IFX + Calpro	<220	90	71	0.82 [0.61, 1.00]
Partial Mayo + Calpro	<260	90	71	0.81 [0.62, 0.99]
Partial Mayo + Calpro + IFX	<350	90	79	0.84 [0.66,1.00]
	for AUC Days 4–7 post-initial IFX dose			
Serum infliximab, mg/ml	<216	100	36	0.72 [0.51, 0.93]
Partial Mayo score	<20	100	50	0.75 [0.55, 0.96]
Faecal calprotectin, µg/ml	<8000	100	57	0.85 [0.68, 1.00]
Serum CRP, mg/l	<50	80	43	0.70 [0.48, 0.92]
Serum albumin, g/l	>67	70	29	0.55 [0.22, 0.85]
Serum CRP/albumin ratio	<2.0	75	38	0.59 [0.33, 0.84]
COMPOSITE VARIABLES: [2]				
IFX + Calpro	<250	90	63	0.84 [0.68, 1.00]
Partial Mayo + Calpro	<205	100	71	0.86 [0.71, 1.00]
Partial Mayo + Calpro + IFX	<400	100	57	0.83 [0.66, 1.00]
[B] PREDICTING CLINICAL REMISSION AT 12 WEEKS [3]				
REFERENCE INDICES:	*for Day 0 prior initial IFX dose*			
Travis index – high risk	–	62	55	–
Ho score – high risk	–	57	47	–
	for AUC Days 1–3 post-initial IFX dose			
Partial Mayo + Calpro + IFX	<350	70	72	0.70 [0.48, 0.92]
	for AUC Days 4–7-post initial IFX dose			
Serum infliximab	<216	92	64	0.75 [0.52, 0.97]
Partial Mayo score	<20	100	64	0.75 [0.52, 0.98]
IFX + Calpro	<320	92	64	0.76 [0.56, 0.97]
Partial Mayo + Calpro	<260	92	37	0.73 [0.51, 0.94]
Partial Mayo + Calpro + IFX	<400	92	64	0.76 [0.55, 0.98]
[C] PREDICTING AVOIDANCE of COLECTOMY within 12 months [3]				
REFERENCE INDICES:	*for Day 0 prior initial IFX dose*			
Travis index – high risk	–	82	31	–
Ho score – high risk	–	82	43	–
	for AUC Days 1–3 post-initial IFX dose			
Partial Mayo score	<20	61	100	0.86 [0.71, 1.00]
Partial Mayo + Calpro + IFX	<350	61	82	0.68 [0.48, 0.89]
	for AUC Days 4–7 post-initial IFX dose			
Serum infliximab	<216	94	66	0.89 [0.75, 1.00]
Partial Mayo score	<20	94	100	0.96 [0.89, 1.00]
Faecal calprotectin	<11,000	89	50	0.82 [0.63, 1.00]
IFX + Calpro	<320	83	83	0.91 [0.77, 1.00]
Partial Mayo + Calpro	<260	83	50	0.91 [0.79, 1.00]
Partial Mayo + Calpro + IFX	<400	89	73	0.94 [0.84, 1.00]

ROC, receiver–operator curve; AUC, area-under-curve; IFX, infliximab; Calpro, calprotectin; CRP, C-reactive protein.

[1]Based on ROC to achieve in order of priority: [1] highest sensitivity, [2] same or similar cutoff for variable as derived for primary end point, [3] highest specificity.

[2]Sum of separate variables with corrections to standardize variables with serum infliximab levels as reference.

[3]Only statistically significant [or trending to significant, i.e. $p < 0.10$] variables shown for the secondary end points.

symptoms such as rectal bleeding and bowel frequency are strongly correlated with endoscopic activity and, to some extent, outcomes in active UC,[15,22,23] but perhaps the most important message to be gleaned here might be that stricter, daily recording and calculation of the partial Mayo score may help to objectify measurement of symptomatic response to treatment and better inform treatment decision-making.

Table 3. Factors significantly [or trending towards, i.e. $p \leq 0.1$] associated with [A] clinical and CRP remission at 6 weeks post-infliximab, [B] clinical remission at 12 weeks post-infliximab and [C] colectomy for patients with ASUC in this cohort

	Bivariate analysis		Multivariate analysis	
Variable	Odds ratio [95% CI]	*p* value	Odds ratio [95% CI]	*p* value
[A] Clinical and CRP remission at 6 weeks				
Age ≥ 40 years	0.3 [0.04, 1.6]	0.1		
Male sex	1.0 [0.2, 5.1]	1.0		
High BMI ≥ 30 kg/m^2	0.3 [0.06, 1.8]	0.2		
Current or ex-smoker	0.6 [0.1, 3.2]	0.5		
Duration since diagnosis ≥ 3 years	2.0 [0.4, 10.4]	0.4		
Extensive colitis [E3, index colonoscopy]	1.3 [0.3, 6.8]	0.7		
Severe [Mayo 3] colitis pre-IFX	0.6 [0.1, 4.1]	0.6		
Concurrent immunomodulator ≥ 3 months pre-admission	0.8 [0.1, 4.4]	0.7		
Long IV steroid course pre-IFX [≥5 days]	0.4 [0.01, 0.7]	0.02		
Faecal IFX positive [>1 mg/ml]	0.2 [0.03, 0.8]	0.04	0.04 [0.02, 0.9]	0.04
Low baseline serum albumin [≤22 g/l]	0.8 [0.2, 4.4]	0.8		
High baseline serum CRP [≥50 mg/l]	1.5 [0.2, 12.9]	0.7		
High baseline serum CRP [≥30 mg/l]	0.7 [0.1, 3.4]	0.6		
High baseline CRP/alb ratio [≥1.6]	1.8 [0.3, 9.7]	0.5		
Faecal calprotectin AUC3 <10 000 µg/ml	16.2 [1.6, 167]	<0.01	13.6 [0.6, 294.6]	0.09
Serum IFX level AUC3 <120 mg/ml	5.0 [0.5, 51.7]	0.1		
Partial Mayo score AUC3 <20	14.7 [2.0, 109]	<0.01	20.2 [1.01, 404.4]	0.049
Composite PM + Calpro + IFX AUC3 <350	33.0 [2.9, 374]	<0.01		
Faecal calprotectin AUC4–7 <8000 µg/ml	133.3 [18.1, 983]	<0.01		
Serum IFX level AUC4–7 <216 mg/ml	55.6 [7.5, 410.5]	<0.01		
Partial Mayo score AUC4–7 <20	100.0 [13.6, 737]	<0.01		
Composite PM + Calpro + IFX AUCD4–7 <400	133.3 [18.1, 983]	<0.01		
[B] Clinical remission at 12 weeks				
Age ≥ 40 years	0.5 [0.1, 2.8]	0.5		
Male sex	2.8 [0.5, 14.7]	0.2		
High BMI ≥ 30 kg/m^2	0.5 [0.1, 2.7]	0.4		
Current or ex-smoker	2.3 [0.4, 12.7]	0.3		
Duration since diagnosis ≥3 years	6.0 [1.01, 35.4]	0.04		
Extensive colitis [E3, index colonoscopy]	4.3 [0.8, 24.3]	0.1		
Severe colitis [Mayo 3] pre-IFX	1.3 [0.2, 8.0]	0.8		
Concurrent immunomodulator ≥3 months pre-admission	1.7 [0.3, 9.4]	0.6		
Long IV steroid course pre-IFX [≥5 days]	0.2 [0.03, 1.5]	0.1		
Faecal IFX positive [>1 mg/ml]	0.2 [0.01, 1.7]	0.1		
Low baseline serum albumin [≤22 g/l]	0.7 [0.3, 1.7]	0.5		
High baseline serum CRP [≥50 mg/l]	0.8 [0.1, 7.0]	0.9		
High baseline serum CRP [≥30 mg/L]	1.0 [0.2, 5.2]	1.0		
High baseline CRP/alb ratio [≥1.6]	0.9 [0.2, 4.8]	0.9		
Faecal calprotectin AUC3 <10 000 µg/ml	5.8 [1.0, 34.6]	0.05		
Serum IFX level AUC3 <120 mg/ml	1.3 [0.2, 8.0]	0.8		
Partial Mayo score AUC3 <20	4.3 [0.8, 24.2]	0.09		
Composite PM + Calpro + IFX AUC 3 <350	6.0 [1.02, 35.4]	0.04		
Faecal calprotectin AUC4–7 <8000 µg/ml	2.8 [0.5, 16.0]	0.2		
Serum IFX level AUC4–7 <216 mg/ml	108.3 [14.6, 803]	<0.01		
Partial Mayo score AUC4–7 <20	227.5 [31, 1690]	<0.01	34.2 [2.8, 416.4]	0.02
Composite PM + Calpro + IFX AUCD4–7<400	21.0 [1.9, 227.2]	<0.01		
Clinical & CRP remission achieved at 6 weeks	36.7 [4.5, 297.9]	<0.01		
[C] Colectomy [until end of followup]				
Age ≥ 40 years	5.2 [0.7, 37.9]	0.1		
Male sex	0.4 [0.1, 2.7]	0.3		
High BMI ≥ 30 kg/m^2	0.5 [0.1, 3.4]	0.5		
Current or ex-smoker	0.3 [0.02, 2.6]	0.2		
Duration since diagnosis ≥3 years	0.4 [0.1, 2.7]	0.3		
Extensive colitis [E3, index colonoscopy]	0.5 [0.1, 3.5]	0.5		
Severe colitis [Mayo 3] pre-IFX	1.9 [0.2, 20.8]	0.6		
Concurrent immunomodulator ≥3 months pre-admission	0.3 [0.03, 3.3]	0.3		
Long IV steroid course pre-IFX [≥5 days]	3.5 [0.5, 24.6]	0.2		
Faecal IFX positive [>1 mg/ml]	10.5 [1.03, 107.2]	0.04	176 [2.1, 14,452]	0.01
Low baseline serum albumin [≤22 g/l]	0.5 [0.1, 3.3]	0.5		
High baseline serum CRP [≥50 mg/l]	4.0 [0.4, 37.8]	0.2		

Table 3. *Continued*

Variable	Bivariate analysis		Multivariate analysis	
	Odds ratio [95% CI]	*p* value	Odds ratio [95% CI]	*p* value
High baseline serum CRP [≥30 mg/l]	3.1 [0.5, 22.0]	0.2		
High baseline CRP/alb ratio [≥1.6]	0.5 [0.1, 3.3]	0.5		
Faecal calprotectin AUC3 <10 000 µg/ml	0.3 [0.04, 1.8]	0.2		
Serum IFX level AUC3 <120 mg/ml	1.9 [0.2, 20.8]	0.6		
Partial Mayo score AUC3 <20	0.01 [0.001, 0.08]	<0.01		
Composite PM + Calpro + IFX AUC3 <350	0.1 [0.01, 1.3]	0.06		
Faecal calpro AUC4–7 <11 000 µg/ml	0.2 [0.03, 1.5]	0.1	0.2 [0.07, 0.8]	0.02
Serum IFX level AUC4–7 <216 mg/ml	0.03 [0.02, 0.4]	<0.01		
Partial Mayo score AUC4–7 <20	0.001 [0.0001, 0.01]	<0.01		
Composite PM + Calpro + IFX AUCD4–7 <400	0.04 [0.003, 0.5]	<0.01		

ASUC, acute severe ulcerative colitis; CRP, C-reactive protein; IV, intravenous; IFX, infliximab; AUC, area-under-curve; BMI, body mass index.

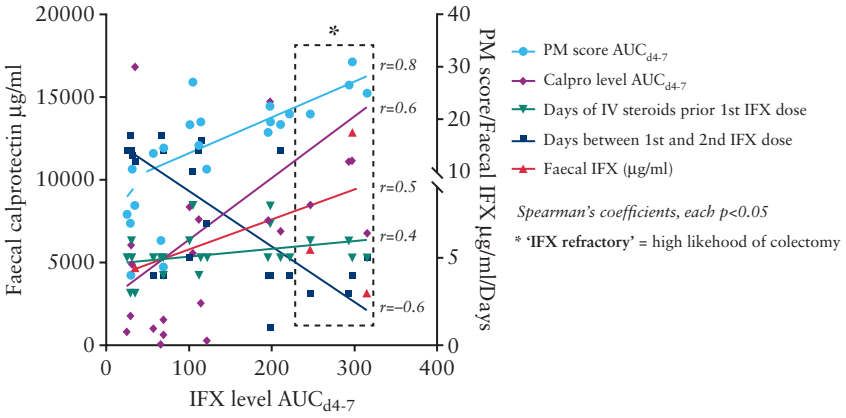

Figure 2. Scatter plot with lines of best fit depicting correlations between variables and 'IFX refractoriness' in ASUC. Patients with data points within box [annotated with '*'] are at higher risk of colectomy [sensitivity 94% when IFX AUCD4–7 >216, see Table 2].

Thirdly, this study suggests that measuring the area under the time–concentration curves of biomarkers over the first 1–7 days, rather than one-off measure[s], might be more robust and responsive to change. While predictive indices pre-salvage therapy such as the Travis or Ho scores[20] remain useful guides in determining the need for salvage therapy, it is the management after the initial IFX dose that is a more sophisticated, nuanced challenge for clinicians. AUC measurement enables the integration of both disease burden at baseline and early response to IFX, thus informing decision-making on further IFX dosing and/or prediction of future colectomy risk with high sensitivities, superior to Travis and Ho scores pre-IFX for instance, as we have shown. Although AUC assessment has been applied previously, for instance by Brandse *et al*. and Papamichael *et al*.,[24–26] this was not directed at early predictive assessment but rather looked at serum or faecal IFX AUC concentrations after 2, 6 or 14 weeks. Yet from a clinical perspective, such time points are unhelpful given that they preclude earlier intervention such as accelerated IFX dosing. In addition, the studies above included patients with moderate-to-severe colitis, but only a small proportion required hospitalization; hence, by definition, few had ASUC.[24] While calculation of AUC might complicate bedside arithmetic, this type of modelling could easily be derived from an online calculator or via a smartphone application. Another advantage is that AUC calculations do not require strict, daily testing to achieve reasonable accuracy, and thus alternative daily biomarker measurements may be sufficient, reducing cost and inconvenience.

Fourthly, we found that AUC concentrations of serum IFX levels were lower, not higher, over Days 4–7 post-first IFX dose, both in early remitters and in those avoiding future colectomy. This is in contrast again to the findings of Brandse *et al*., but the current cohort was greater in number and more strictly characterized with all meeting standard criteria for ASUC.[24] This finding appears counterintuitive and an underlying mechanistic explanation is certainly beyond the scope of this observational study. Yet we speculate that lower serum IFX levels could relate to either more efficient binding and/or utilization of IFX at inflamed target sites resulting in greater likelihood of remission compared to non-remitters in whom, for reasons unknown, optimal efficacy is not achieved. This finding requires replication and evaluation in future studies, given it could imply two opposing scenarios: first, that the higher serum drug levels in early IFX non-responders might reflect a state of 'IFX refractoriness' [as also depicted in the clustering of cases, along with other negative prognostic cofactors, in Figures 2 and 3] where more drug is required to overcome this and/or early colectomy is perhaps advisable. Conversely, those with lower AUC post-first IFX may not require further doses; for instance, three patients in this cohort only received one dose of IFX rescue therapy yet all three achieved clinical remission at 3 months and had avoided colectomy through to the end of follow-up.

Finally, in this ASUC cohort the presence of faecal loss of IFX [Day 1 post-first IFX dose, ≥1.0 µg/g] was consistently and strongly associated both with a reduced likelihood of achieving remission at 6 weeks and with a higher risk of future colectomy. Notably, as in

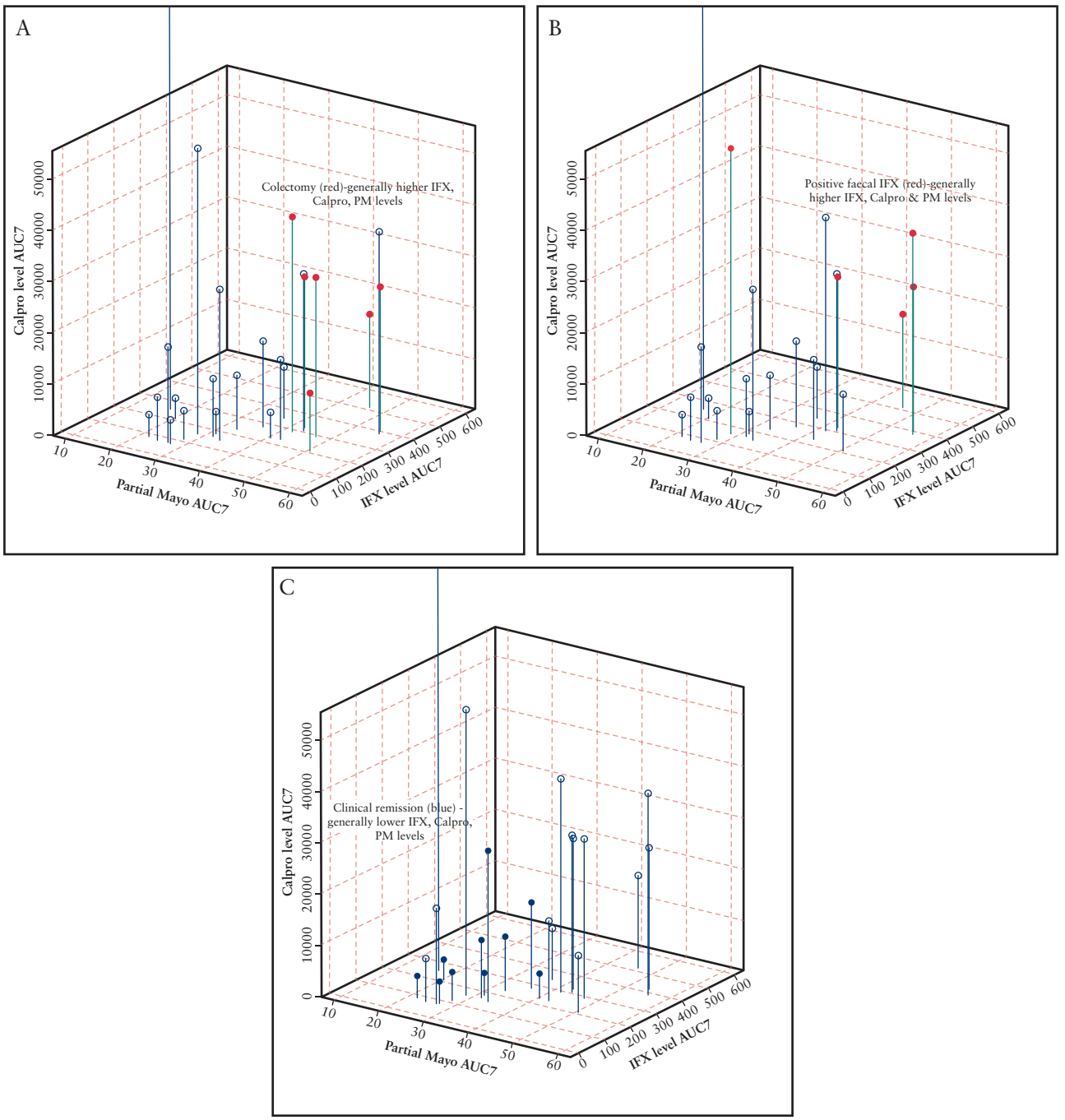

Figure 3. Three-dimensional scatter plots illustrating the relationships between serum infliximab, faecal calprotectin levels and partial Mayo scores with adverse prognostic cofactors such as [A] future colectomy, [B] presence of faecal loss of IFX [in red, each clustering where IFX, FC and PM AUC7 are higher] and, positively, [C] clinical and CRP remission [in blue, clustering where IFX, FC and PM AUC7 are generally lower].

Brandse *et al.*,[25] there was no correlation between serum IFX levels and faecal IFX levels. Thus, faecal loss of IFX does not appear to be a strong cofactor in the apparent IFX refractoriness demonstrated in a subset of this study. Nevertheless, it appears an independent predictor of poor prognosis post-IFX, even when accounting for other potential confounders such as FC concentration, given the findings of the multivariate analysis [Table 2].

This study has significant limitations. Its small sample size and observational design limit the ability to attribute causality or to further delineate mechanisms. In this real-world scenario, IFX delivery was not protocol-driven, extra doses were given according to the discretion of the clinician and endoscopic remission [post-IFX] was not assessed in this study. However, the study has multiple strengths such as its prospective design, the unselected nature of inclusion of patients fulfilling strict criteria of ASUC and the complete follow-up for at least 12 months to assess colectomy rates. Therefore, the findings are potentially more applicable to patients with ASUC elsewhere compared with previous studies in this genre. Also, all had endoscopic assessment pre-IFX confirming moderate-to-severe activity and excluding confounders such as CMV colitis. Furthermore,

the serial, high-volume testing of relevant biomarkers ensured a comprehensive assessment of potential predictors of remission and/or colectomy post-IFX.

In conclusion, this prospective pilot 'real world' study has suggested that early and serial measurements of FC, serum and faecal IFX and partial Mayo scores are more useful in predicting outcomes than the traditional inflammatory markers, CRP and/or albumin, and other published risk indices. It has also provided strong evidence that an AUC-based representation of each biomarker provides greater predictive value than one-off measures for short- and long-term outcomes after the first dose of IFX rescue for ASUC following steroid failure. Faecal loss of drug on Day 1 post-IFX was an independent predictor of poor prognosis, aligning with the notion that intestinal inflammatory burden is the key, objective determinant that should guide treatment and inform prognosis. We also showed, somewhat counterintuitively, that AUC of serum IFX levels post-first dose tended to be lower in patients achieving early remission at 6 weeks compared to non-remitters. We propose that further prospective, larger-scale evaluation of these findings post-IFX in ASUC will aid clinicians in delivering optimal care in a more objective, cost-effective and prognosis-based manner, and will also aid provide further understanding of the unique interplay of inflammatory burden, anti-tumour necrosis factor pharmacokinetics and response in ASUC.

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Conflict of Interest

The authors have no conflicts of interests to declare.

References

1. Laharie D, Bourreille A, Branche J, *et al*.; Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. *Lancet* 2012;**380**:1909–15.
2. Järnerot G, Hertervig E, Friis-Liby I, *et al*. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. *Gastroenterology* 2005;**128**:1805–11.
3. Löwenberg M, Duijvis NW, Ponsioen C, van den Brink GR, Fockens P, D'Haens GR. Length of hospital stay and associated hospital costs with infliximab versus cyclosporine in severe ulcerative colitis. *Eur J Gastroenterol Hepatol* 2014;**26**:1240–6.
4. Williams JG, Alam MF, Alrubaiy L, *et al*. Comparison Of iNfliximab and ciclosporin in STeroid Resistant Ulcerative Colitis: pragmatic randomised Trial and economic evaluation (CONSTRUCT). *Health Technol Assess* 2016;**20**:1–320.
5. Rutgeerts P, Sandborn WJ, Feagan BG, *et al*. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005;**353**:2462–76.
6. Rutgeerts P, Feagan BG, Lichtenstein GR, *et al*. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. *Gastroenterology* 2004;**126**:402–13.
7. Monterubbianesi R, Aratari A, Armuzzi A, *et al*.; Italian Group for the study of Inflammatory Bowel Disease [IG-IBD]. Infliximab three-dose induction regimen in severe corticosteroid-refractory ulcerative colitis: early and late outcome and predictors of colectomy. *J Crohns Colitis* 2014;**8**:852–8.
8. Hindryckx P, Novak G, Vande Casteele N, *et al*. Review article: dose optimisation of infliximab for acute severe ulcerative colitis. *Aliment Pharmacol Ther* 2017;**45**:617–30.
9. Seah D, De Cruz P. Review article: the practical management of acute severe ulcerative colitis. *Aliment Pharmacol Ther* 2016;**43**:482–513.
10. Kohn A, Daperno M, Armuzzi A, *et al*. Infliximab in severe ulcerative colitis: short-term results of different infusion regimens and long-term follow-up. *Aliment Pharmacol Ther* 2007;**26**:747–56.
11. Gibson DJ, Heetun ZS, Redmond CE, *et al*. An accelerated infliximab induction regimen reduces the need for early colectomy in patients with acute severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2015;**13**:330–335.e1.
12. Byrne CM, Tan KK, Young JM, Selby W, Solomon MJ. Patient and clinician preferences for surgical and medical treatment options in ulcerative colitis. *Colorectal Dis* 2014;**16**:285–92.
13. Rini C, Jandorf L, Goldsmith RE, Manne SL, Harpaz N, Itzkowitz SH. Interpersonal influences on patients' surgical decision making: the role of close others. *J Behav Med* 2011;**34**:396–407.
14. Chhabra KR, Sacks GD, Dimick JB. Surgical decision making: challenging dogma and incorporating patient preferences. *JAMA* 2017;**317**:357–8.
15. Lindgren SC, Flood LM, Kilander AF, Löfberg R, Persson TB, Sjödahl RI. Early predictors of glucocorticosteroid treatment failure in severe and moderately severe attacks of ulcerative colitis. *Eur J Gastroenterol Hepatol* 1998;**10**:831–5.
16. Travis S, Satsangi J, Lémann M. Predicting the need for colectomy in severe ulcerative colitis: a critical appraisal of clinical parameters and currently available biomarkers. *Gut* 2011;**60**:3–9.
17. Travis SP, Farrant JM, Ricketts C, *et al*. Predicting outcome in severe ulcerative colitis. *Gut* 1996;**38**:905–10.
18. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955;**2**:1041–8.
19. Ho GT, Mowat C, Goddard CJ, *et al*. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther* 2004;**19**:1079–87.
20. Lynch RW, Churchhouse AM, Protheroe A, Arnott ID; UK IBD Audit Steering Group. Predicting outcome in acute severe ulcerative colitis: comparison of the Travis and Ho scores using UK IBD audit data. *Aliment Pharmacol Ther* 2016;**43**:1132–41.
21. Ho GT, Lee HM, Brydon G, *et al*. Fecal calprotectin predicts the clinical course of acute severe ulcerative colitis. *Am J Gastroenterol* 2009;**104**:673–8.
22. Arias MT, Vande Casteele N, Vermeire S, *et al*. A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. *Clin Gastroenterol Hepatol* 2015;**13**:531–8.
23. Colombel JF, Keir ME, Scherl A, *et al*. Discrepancies between patient-reported outcomes, and endoscopic and histological appearance in UC. *Gut* 2016;doi: 10.1136/gutjnl-2016-312307.
24. Brandse JF, Mathôt RA, van der Kleij D, *et al*. Pharmacokinetic features and presence of antidrug antibodies associate with response to infliximab induction therapy in patients with moderate to severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2016;**14**:251–8.e1–2.
25. Brandse JF, van den Brink GR, Wildenberg ME, *et al*. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. *Gastroenterology* 2015;**149**:350–5.e2.
26. Papamichael K, Van Stappen T, Vande Casteele N, *et al*. Infliximab concentration thresholds during induction therapy are associated with short-term mucosal healing in patients with ulcerative colitis. *Clin Gastroenterol Hepatol* 2016;**14**:543–9.