Journal of Crohn's and Colitis, 2019, 1527–1536
doi:10.1093/ecco-jcc/jjz103
Advance Access publication May 15, 2019
Original Article

Original Article

Comparison of Adalimumab Serum Drug Levels When Delivered by Pen Versus Syringe in Patients With Inflammatory Bowel Disease. An International, Multicentre Cohort Analysis

Robert D. Little,[a] Isabel E. Chu,[a] Esmerij P. van der Zanden,[b] Emma Flanagan,[c] Sally J. Bell,[c] Peter R. Gibson,[a] Miles P. Sparrow,[a] Edward Shelton,[d] Susan J. Connor,[b] Xavier Roblin,[e] Mark G. Ward[a]

[a]Department of Gastroenterology, Alfred Health and Monash University, Melbourne, VIC, Australia [b]Department of Gastroenterology, Liverpool Hospital and University of New South Wales, Sydney, NSW, Australia [c]Department of Gastroenterology, St Vincent's Hospital and University of Melbourne, Melbourne, VIC, Australia [d]Department of Gastroenterology, Monash Health and Monash University, Melbourne, VIC, Australia [e]Gastro-entérologie et Hépatologie, CHU Saint-Étienne, Saint-Étienne, France

Corresponding author: Dr Mark Ward, MBBS, FRACP, MD, Department of Gastroenterology, Alfred Health and Monash University, Melbourne, VIC 3004, Australia. Tel.: [03] 9076 2223; Fax: [03] 9076 2194; Email: m.ward@alfred.org.au

Abstract

Background: Adalimumab is administered via a pre-filled syringe or spring-loaded pen. In a previous study in Crohn's disease, higher drug levels were observed in syringe users. The aim of this study was to evaluate the impact of delivery device on adalimumab drug levels in patients with Crohn's disease.

Methods: Consecutive Crohn's disease patients treated with maintenance adalimumab [40 mg fortnightly] were recruited from five centres. The first recorded drug level with matched clinical and biochemical markers of disease activity was compared between pen and syringe users.

Results: Of 218 patients, 64% used pen, with a median faecal calprotectin 110 μg/g and serum C-reactive protein 4 mg/L. In comparison to pen, syringe users had higher albumin [39 vs 42 g/L; $p = 0.016$], lower Harvey-Bradshaw Index [2 vs 1; $p = 0.017$], and higher rates of concomitant immunomodulation [54% vs 71%; $p = 0.014$]. Drug levels were equivalent between pen and syringe users [median 5.3 vs 5.2 μg/ml; $p = 0.584$], even after controlling for disease activity and immunomodulation. Syringe users at Alfred Health had higher drug levels than pen [6.1 vs 4.5 μg/ml; $p = 0.039$]; a greater proportion achieved therapeutic levels [75% vs 44%; $p = 0.045$]. A higher proportion of pen users from Saint-Étienne had therapeutic levels [79% vs 42%; $p = 0.027$], yet no significant difference in drug levels [7.9 vs 4.5 μg/ml; $p = 0.119$].

Conclusions: Delivery device does not appear to significantly affect adalimumab drug levels. Given differences between study sites, studies evaluating administration education and technique are warranted.

Key Words: Crohn's disease; adalimumab; therapeutic drug monitoring

1. Introduction

Crohn's disease [CD] is a chronic, autoimmune inflammatory disorder predominantly affecting the gastrointestinal tract.[1] Adalimumab [ADA] and infliximab [IFX], monoclonal antibodies directed against tumour necrosis factor [TNF], are effective for both induction and maintenance of remission in CD.[2–5] The choice between ADA and IFX may be influenced by physician and patient preferences such as perceived issues with adherence, ease of use, dosing interval, and impact on lifestyle and work-related productivity.[6] The majority of patients with luminal CD appear to prefer self-administered subcutaneous injections rather than intravenous infusions.[6] ADA, therefore, is a popular choice among adults with CD.

Despite the efficacy of ADA, approximately 20–40% of patients with luminal CD fail to respond to induction, and a further 15% to 54% of patients subsequently lose response by 12 months, depending on the definition.[2,7,8] Understanding the mechanisms that drive primary and secondary loss of response to ADA, and subsequent identification of strategies to optimise therapy, are important. Measuring drug levels and anti-drug antibodies with therapeutic drug monitoring [TDM] may be useful in this setting and is now often integrated into routine clinical practice. A clear exposure-response relationship has been demonstrated between IFX drug levels and disease outcomes, but the data are less consistent for ADA. The majority of the literature demonstrates an ADA exposure-response relationship,[2,9–11] yet some studies report a less robust association.[12,13] The reason for this discordance is unclear. In comparison to an intravenous infusion, the subcutaneous route may introduce variation in ADA absorption due to patient-specific factors, including injection technique, immune recognition at the skin, or the type of delivery device. ADA is administered via a pre-filled syringe or a spring-loaded automatic pen. Industry-funded preliminary data of healthy volunteers appear to show no overall difference in the bioavailability of both ADA and a biosimilar between delivery devices.[14–16] In contrast, in an intensive pharmacokinetic study of ADA in CD, we found that the mode of delivery contributed to serum drug level variance. Syringe compared with pen use was predictive of higher trough drug level on both multiple regression and univariate linear regression, although numbers were very small.[17] However, whether there is bioequivalence in ADA drug levels between pen and syringe devices has not yet been explored in a dedicated study in patients with IBD.

The aim of this study was to explore the relationship between choice of delivery device and ADA drug level in patients with CD, using a dedicated multicentre cohort analysis.

2. Materials and Methods

2.1. Patients

Adult patients with luminal CD established on maintenance ADA [defined as >14 weeks of treatment at a dose of 40 mg fortnightly] from the outpatient clinics of Alfred Health, Monash Health, St Vincent's Hospital and Liverpool Hospital in Australia, and Centre Hospitalier Universitaire [CHU] Saint-Étienne in France, were included. The diagnosis of CD was based on standard endoscopic, histopathological, and radiological criteria.[18] Consecutive patients with available serum drug levels, administering ADA using the same delivery device [pen or syringe] for at least 3 months, were included. Patients with isolated perianal disease, or those receiving past or current dose-intensified ADA, were excluded. Four of five sites educate patients in subcutaneous abdominal injections only. Liverpool Hospital educates a minority of patients who do not tolerate abdominal injections to administer ADA into the thigh. All Australian sites as well as Saint-Étienne before 2017 used a citrate-containing formulation of ADA.

2.2. Design

Data were collected retrospectively from the electronic medical record at participating centres. Outcomes were assessed in two scenarios. Results of TDM [drug levels and, where detected, anti-drug antibody titres] according to delivery device were recorded and compared with disease activity. Disease activity was assessed clinically using the Harvey-Bradshaw Index [HBI],[19] biochemically using C-reactive protein [CRP] and, as a surrogate of mucosal inflammation, faecal calprotectin [FCP], all recorded within 3 months of TDM. ADA drug levels >4.9 μg/ml were considered therapeutic, as previously reported.[10,20] Active disease was defined as HBI >4, CRP >5 mg/L or FCP >150 μg/g.[21,22] The indication for TDM was recorded as one of four possibilities: suspected loss of response, routine [proactive], suspected adverse anti-TNF effects, or other [not specified]. Delivery device status was retrieved from prescriptions and cross-checked against medical records. Second, we analysed a subgroup of patients drawn from all four Australian centres with sub-therapeutic drug levels, who were subsequently escalated to weekly ADA 40 mg due to secondary loss of response. These patients were followed for 12 months to compare the rate of treatment failure between pen and syringe delivery device, defined as switching within class [to an alternative anti-TNF agent], switching out of class, or requiring oral corticosteroids, IBD-related surgery, or IBD-related hospitalisation. The study was approved by the institutional ethics review committees of participating centres or was deemed to meet the Australian National Health and Medical Research Council criteria for exemption.

2.3. Laboratory methods

All sites measured ADA drug levels at trough, using a drug-sensitive commercial sandwich enzyme-linked immunosorbent assay [ELISA] as per manufacturer's instructions. ELISA drug kits varied as follows, according to site: Shikari® Q-ADA [Matriks Biotek, Turkey]—Alfred Health, Monash Health, St Vincent's Hospital, and 54% of samples from Liverpool Hospital; LISA-TRACKER Premium® [Theradiag, France] at CHU Saint-Étienne; and Promonitor® [Grifols, Spain] for the remaining 46% of samples from Liverpool Hospital. Measurement of anti-drug antibodies was limited by uniform drug-sensitive ELISAs.

CRP serum levels were measured using an in vitro diagnostic assay on Architect ci16200 analyser [Abbott Laboratories, Abbott Park, IL, USA]. Faecal calprotectin was measured in duplicate on extracts of 50 mg of homogenised stool by ELISA [Bühlmann Laboratories, Switzerland] as per manufacturer's instructions.

2.4. Statistical analyses

Categorical variables are presented as number and percentage, and quantitative data as mean with standard deviation [SD] or median with interquartile range [IQR], as specified. Inter-group comparisons were carried out using independent sample t test or Mann-Whitney U test, as appropriate. Multisite comparisons were carried out using one-way analysis of variance [ANOVA] with Tukey's correction, or Kruskal-Wallis with Dunn's correction. Categorical variables were evaluated using chi square or Fisher's exact test, where appropriate. We performed multiple regression with ADA level as the dependent variable, and binary logistic regression with patients dichotomised according to therapeutic or sub-therapeutic drug levels alongside independent patient and disease characteristic covariates, as specified. Multiple regression sought to determine the individual [expressed as β] and combined

[expressed as R^2] contribution of delivery device, with a range of other independent covariates, to the overall variance in drug level. Binary logistic regression was used to determine the combined and individual [expressed as odds ratios] contribution to predicting therapeutic drug level. All reported *p*- values were two-sided, and *p*-values <0.05 were considered statistically significant. Statistical analysis was performed using IBM SPSS 22.0.0 [IBM, Somers, NY].

3. Results

3.1. Patient characteristics

The demographics and clinical information on the 218 patients [113 male, 52%] included in the analysis are shown in Table 1. Patient characteristics across investigating sites are also presented. Heterogeneity of the patients across the sites was observed for several covariates. Liverpool Hospital was characterised by a low usage of pen delivery and low rates of previous anti-TNF exposure. A greater proportion of patients at Alfred Health had a penetrating disease phenotype. Monash Health tended to have patients with greater disease activity [HBI and CRP] and lower albumin. At CHU Saint-Étienne, disease duration was shorter, body mass index [BMI] was lower, and there was a higher proportion of upper gastrointestinal [GI] disease, yet a smaller burden of perianal disease and minor FCP activity. In addition, the combination of immunomodulators with ADA was seldom used [4%] at CHU Saint-Étienne, in contrast to >50% at each Australian site [Table 1]. Drug levels at CHU Saint-Étienne, measured using the LISA-TRACKER Premium® assay [median 7.6, IQR 4.2–10.3 µg/ml], were higher than those measured

Table 1. Patient and disease characteristics according to investigating site

	All patients	Alfred	Liverpool	St Vincent's	Monash	St Étienne	*p*-value
Number of patients	218	61	50	46	15	46	-
Male	52%	49%	60%	39%	79%	52%	$p = 0.075$
Age [years]	40 [13]	43 [13]	37 [13]	40 [11]	38 [11]	38 [13]	$p = 0.247$
Disease duration [years]	12 [8]	16 [8]	10 [8]	14 [8]	12 [8]	8 [6]	$p < 0.001$
Weight [kg]	74 [17]	74 [16]	81 [17]	72 [18]	77 [15]	65 [13]	$p < 0.001$
Body mass index [kg/m^2]	25 [6]	26 [5]	27 [7]	25 [6]	25 [4]	23 [3]	$p = 0.013$
Montreal L1	27%	31%	22%	20%	33%	32%	$p = 0.504$
Montreal L2	21%	11%	22%	29%	33%	20%	$p = 0.175$
Montreal L3	50%	57%	50%	51%	33%	48%	$p = 0.568$
Upper GI involvement	9%	3%	6%	7%	7%	24%	$p = 0.004$
Perianal disease	40%	41%	48%	50%	27%	22%	$p = 0.035$
Montreal B1	38%	14%	40%	45%	47%	48%	$p = 0.007$
Montreal B2	39%	46%	32%	50%	40%	28%	$p = 0.163$
Montreal B3	23%	40%	28%	5%	13%	24%	$p = 0.002$
Previous anti-TNF exposure	39%	49%	14%	52%	47%	35%	$p < 0.001$
Combination therapy[a]	60% TP 77%; 98/127	72% TP 81%; 34/42	88% TP 80%; 35/44	59% TP 85%; 23/27	80% TP 42%; 5/12	4% TP 50%; 1/2	$p < 0.001$
Current smokers	13%	15%	16%	10%	7%	13%	$p = 0.821$
Harvey-Bradshaw Index [HBI]							
Score, median [IQR]	2 [0–5]	1 [0–2]	0 [0–3]	5 [2–6]	5 [2–9]	NA	$p < 0.001$
Active disease [>4] [%; *n/N*]	27%; 41/152	14%; 6/44	4%; 2/49	58%; 26/45	50%; 7/14		
C-reactive protein [CRP]							
Serum level [mg/L], median [IQR]	4 [2–8]	3 [3–6]	3 [1–9]	5 [5–13]	10 [1–37]	2 [1–3]	$p < 0.001$
Active disease [>5 mg/ml] [%; *n/N*]	31%; 59/193	29%; 17/59	33%; 15/46	36%; 16/44	64%; 7/11	12%; 4/33	
Faecal calprotectin [FCP]							
Faecal level [µg/g], median [IQR]	110 [30–330]	134 [30–300]	70 [22–185]	339 [78–417]	NA	30 [0–288]	$p = 0.027$
Active disease [>150 µg/g] [%; *n/N*]	39%; 44/112	44%; 17/39	27%; 9/33	69%; 9/13		33%; 9/27	
Albumin [g/L], median [IQR]	39 [36–44]	37 [34–40]	45 [42–47]	38 [34–41]	35 [22–41]	44 [41–46]	$p < 0.001$
Pen users	64%	74%	18%	83%	93%	74%	$p < 0.001$
Time to drug level [months], median [IQR]	14 [5–33]	16 [6–29]	14 [4–38]	31 [11–49]	24 [11–51]	6.5 [4–16]	$p < 0.001$
Drug level [µg/ml], median [IQR]	5.2 [3–8.1]	5.3 [3.1–7]	5.2 [2.5–8.6]	4.6 [3.1–7.4]	4.3 [2.4–6.4]	7.6 [4.2–10.3]	$p = 0.052$

Values reflect mean and standard deviation unless otherwise stated. Independent t test [continuous parametric], Mann-Whitney [non-parametric], chi square [categorical] Total missing values: HBI 66 [30%], CRP 25 [12%], FCP 106 [49%], albumin 39 [18%]. Disease location and phenotype presented according to Montreal classification.[23]

GI, gastrointestinal; TNF, tumour necrosis factor; IQR, interquartile range; NA, not available; TP, thiopurine proportion.

[a]Combination of adalimumab and immunomodulator therapy.

with the Shikari® Q-ADA assay in Australian sites overall [median 4.5, IQR 2.8–7.0 µg/ml; $p < 0.001$].

Mean age was 40 [SD 13] years, mean disease duration was 12 [8] years, and 60% received concomitant immunomodulation [77% thiopurine, 23% methotrexate]. At the time of TDM, median FCP was 110 µg/g [IQR 30–330] and CRP 4 mg/L [2-8]. A total of 44/112 [39%] patients and 59/193 [31%] patients had active disease according to the aforementioned FCP and CRP thresholds, respectively. Significant differences in the proportion of pen users were observed across sites. In comparison with pen users, syringe users had higher rates of smoking [21% vs 9%; $p = 0.012$, respectively], a higher albumin [39 vs 42 g/L; $p = 0.016$], lower HBI [2 vs 1; $p = 0.017$], and higher rates of concomitant immunomodulation [54% vs 71%; $p = 0.014$]. There were no significant differences in FCP or CRP across the groups. Further, disease duration, location, phenotype, and rates of previous anti-TNF exposure were similar between pen and syringe users [Table 2]. Regarding indication for TDM, 49% of drug levels were ordered proactively as part of routine care, 47% due to suspected loss of response, 2% for suspected adverse effects, and 2% other [not specified]. There was no difference in proportion of TDM requested proactively or due to suspected loss of response between pen and syringe users [45% vs 55%; $p = 0.204$ and 51% vs 40%; $p = 0.155$, respectively].

3.2. ADA drug levels according to delivery device

Pen delivery device was used in 64% of the cohort. Serum concentrations of ADA were equal between pen and syringe users [median 5.3 vs 5.2 µg/ml; $p = 0.584$, Figure 1a]. Drug levels did not significantly differ between pen and syringe users when dividing the cohort according to active vs inactive disease [using HBI, CRP, and FCP thresholds] or the use of concomitant immunomodulation, as illustrated in Figure 3. Given the differences in patient and disease demographics at CHU Saint-Étienne, a subgroup analysis of drug levels between pen and syringe delivery device was also performed across Australian sites only [median 4.5 vs 5.3 µg/ml; $p = 0.066$, respectively; Figure 2a]. We addressed any potential contribution from assay variability to the differences in drug levels by analysing the 149 samples measured by the Shikari® Q-ADA ELISA kit. The single assay analysis demonstrated no difference in drug levels between pen and syringe delivery [median 4.5 vs 4.7 µg/ml, $p = 0.491$, respectively; Figure 2b].

A total of 15/46 patients at CHU Saint-Étienne had drug level testing whilst using reduced volume, citrate-free ADA. The proportion of patients using each delivery device between the citrate-free and the entire CHU Saint-Étienne cohort were equal [73% vs 74%, respectively; $p > 0.999$]. Drug levels between the citrate-free and standard ADA formulation at CHU Saint-Étienne did not differ [median 7.6 vs 7.7, respectively; $p = 0.660$], and there was no difference

Table 2. Patient and disease characteristics according to delivery device.

	Pen	Syringe	*p*-value
Number	140	78	-
Male	50%	55%	$p = 0.572$
Age [years]	40 [13]	38 [12]	$p = 0.340$
Disease duration [years]	13 [8]	11 [8]	$p = 0.118$
Weight [kg]	74 [17]	74 [16]	$p = 0.866$
Body mass index [BMI] [kg/m^2]	25 [6]	25 [5.9]	$p = 0.732$
Montreal L1	27%	27%	$p > 0.999$
Montreal L2	23%	19%	$p = 0.484$
Montreal L3	50%	53%	$p = 0.670$
Upper gastrointestinal [GI] involvement	9%	9%	$p > 0.999$
Perianal disease	37%	44%	$p = 0.384$
Montreal B1	38%	37%	$p = 0.894$
Montreal B2	42%	34%	$p = 0.181$
Montreal B3	20%	28%	$p = 0.182$
Previous anti-tumour necrosis factor [TNF] exposure	41%	35%	$p = 0.388$
Combination therapy[a]	54% TP 77%; 56/73	71% TP 83%; 53/64	$p = 0.014$
Current smokers	9%	21%	$p = 0.012$
Harvey-Bradshaw Index [HBI]			
Score, median [interquartile range; IQR]	2 [0-6]	1 [0-4]	$p = 0.017$
Active disease [>4] [%; *n*/*N*]	36% [33/91]	13% [8/61]	
C-reactive protein [CRP]			
Serum level [mg/L], median [IQR]	5 [3–8]	3 [1–7]	$p = 0.732$
Active disease [>5 mg/ml] [%; *n*/*N*]	30% [38/126]	31% [21/67]	
Faecal calprotectin [FCP]			
Faecal level [µg/g], median [IQR]	115 [30–377]	109 [29–200]	$p = 0.254$
Active disease [>150 µg/g] [%; *n*/*N*]	43% [29/68]	34% [15/44]	
Albumin [g/L], median [IQR]	39 [35–42]	42 [36–46]	$p = 0.016$
Time to drug level [months], median [IQR]	17 [6–34]	12 [5–31]	$p = 0.319$

Values reflect mean and standard deviation unless otherwise stated. Independent t test [continuous parametric], Mann-Whitney [non-parametric], Fisher's exact [categorical]. Total missing values: HBI 66 [30%], CRP 25 [12%], FCP 106 [49%], albumin 39 [18%] Disease location and phenotype presented according to Montreal classification.[23]

TP = thiopurine proportion; NA, not available.

[a]Combination of adalimumab and immunomodulator therapy,

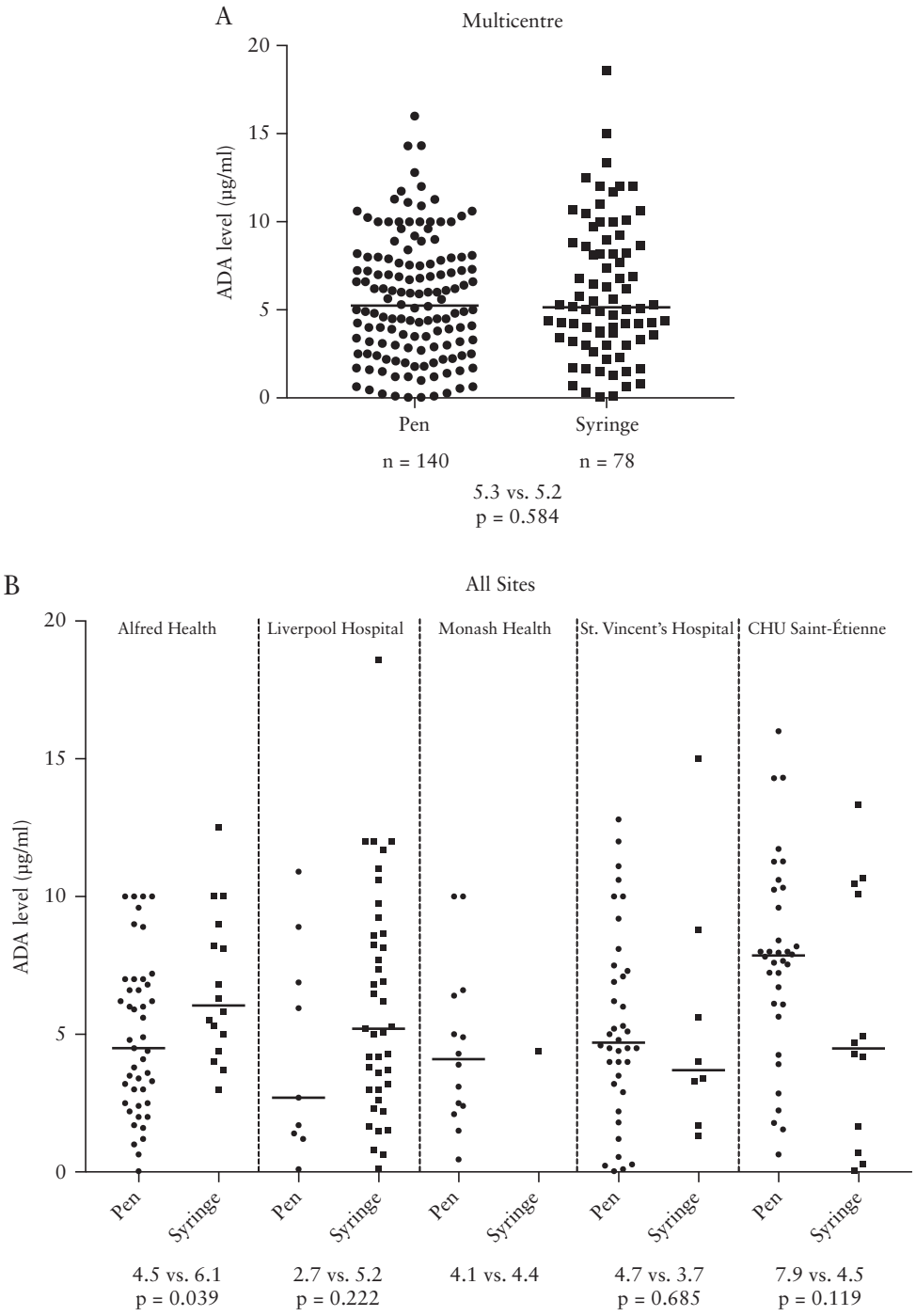

Figure 1. Multicentre [a] and individual site [b] adalimumab [ADA] drug levels according to delivery device. Values and horizontal bars reflect medians. Group comparisons via Mann-Whitney test; *p*-value not calculated at Monash Health due to syringe group size.

between pen and syringe delivery device in the citrate-free cohort [median 7.6 pen vs 7.6 syringe; $p = 0.851$].

Two regression models were applied. First, a multiple regression model for predicting ADA drug level as a continuous variable using delivery device, age, gender, BMI, previous anti-TNF exposure, smoking status, concomitant immunomodulation, CRP, and FCP as predictor variables, was constructed. This model accounted for 20% of the variance in drug levels (R^2 0.20, standard error [SE] 3.40; $p = 0.034$). Previous anti-TNF exposure was the only covariate independently associated with ADA drug level [$\beta = -0.24$; $p = 0.025$].

Second, patients were dichotomised according to sub-therapeutic vs therapeutic drug levels, and binary logistic regression was performed. The full model, including the covariates of delivery device, age, gender, BMI, previous anti-TNF exposure, smoking status, concomitant immunomodulation, CRP, and FCP, explained 33% of drug level variance [$p = 0.009$]. In comparison with anti-TNF naïve patients, those with previous exposure to anti-TNF therapy had 5-fold greater odds of having sub-therapeutic drug levels (odds ratio [OR] 0.20, confidence interval [CI] 0.06–0.61; $p = 0.005$). Drug delivery device was not

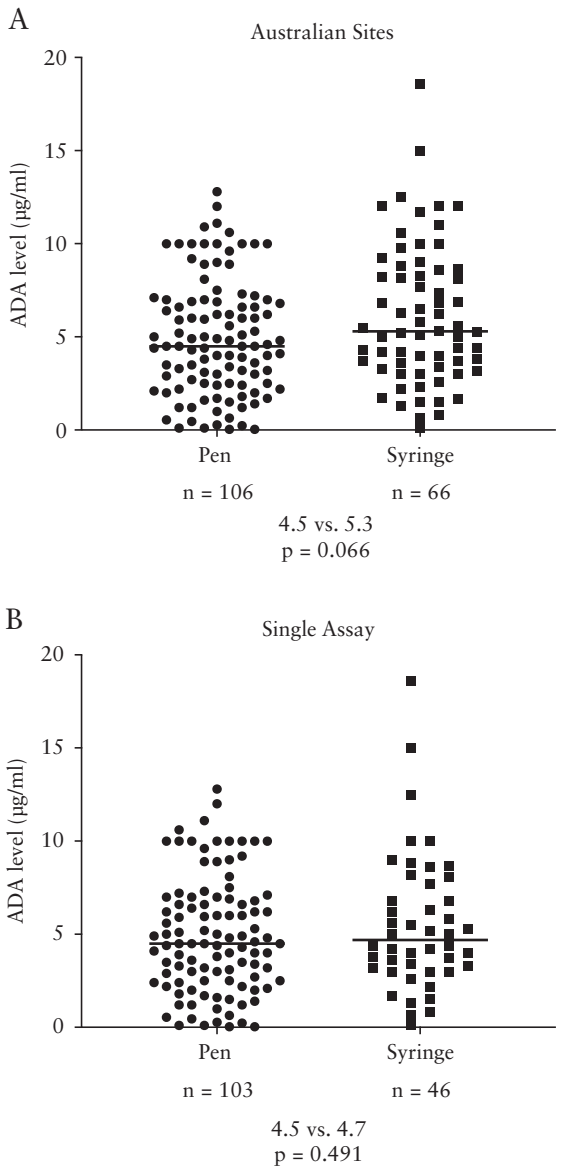

Figure 2. Adalimumab [ADA] drug levels according to delivery device at all Australian sites [a] and all tests performed using the Shikari® Q-ADA drug assay [b]. Values and horizontal bars reflect medians.

independently associated with ADA drug level [OR 2.42, CI 0.68–8.59; $p = 0.17$].

On subgroup analyses by centre, drug levels were significantly higher among syringe users compared with pen users at Alfred Health [median 6.1 vs 4.5 µg/ml; $p = 0.039$; Figure 1b]. In addition, a greater proportion of syringe users had therapeutic drug levels [75% vs 44%; $p = 0.045$]. In contrast, a higher proportion of pen users from CHU Saint-Étienne had therapeutic ADA levels [79% vs 42%; $p = 0.027$], yet no significant difference in absolute drug levels was observed [median 7.9 vs 4.5; $p = 0.119$; Figure 1b]. No differences in drug levels between delivery devices were seen at the remaining sites [Figure 1b].

Similar regression models were applied to data from individual sites. For the data from the Alfred Hospital, drug delivery device was independently associated with 9% of the variance in ADA drug level [$\beta = 0.301$; $p = 0.024$], and both drug delivery device and previous anti-TNF use were independently associated with drug levels when expressed as therapeutic vs sub-therapeutic [OR 0.041, CI 0.002–0.679; $p = 0.026$ and OR 14.23, CI 1.63–124.81; $p = 0.016$, respectively]. No predictive relationships were observed on analysis of data from any of the other sites.

Finally, 48/218 [22%] of patients had undetectable drug levels, permitting measurement of anti-drug antibodies using these drug-sensitive assays. Of these, 22/218 [10%] were positive. Anti-drug antibodies were found in similar proportion between pen and syringe users [11% vs 9%; $p > 0.999$].

3.3. Outcome following adalimumab dose escalation according to delivery device

Follow-up data were available at all Australian centres [172/218 patients, 62% of whom used pen]. Of these, 30 [19%] had secondary loss of response together with sub-therapeutic drug levels, and were escalated to weekly ADA 40 mg dosing. After 12 months of follow-up, a similar proportion of pen and syringe users failed dose escalation (10/20 [50%] vs 6/10 [60%]; $p = 0.709$, respectively). Time to failure was no different according to pen or syringe delivery device [median 169 vs 201 days; $p = 0.836$, respectively].

4. Discussion

A recent study of factors that influence drug levels in patients on adalimumab therapy unexpectedly suggested that the mode of delivery—whether by syringe or by pen—influenced the serum concentrations of the drug.[17] If that were the case, it raises serious concerns about the choice of mode of delivery. This observation prompted the current retrospective multicentre study in patients with Crohn's disease treated with ADA. There were several important findings. First, no significant differences were found in drug levels between pen and syringe users overall. Second, drug levels did not differ between devices after controlling for clinical and biochemical makers of disease activity, the use of concomitant immunomodulators, and previous anti-TNF exposure. Third, site-specific differences in absolute and relative drug levels between pen and syringe users were observed. Finally, there was no difference between delivery devices in the frequency of drug failure over a 12-month follow-up of a small cohort of patients who received dose-intensified ADA.

ADA is commonly prescribed for the induction and maintenance of remission in luminal CD. The early success following initiation of ADA therapy is limited by the rate of secondary loss of response. Although the definition varies, secondary loss of response is observed in patients whom initially experience improvements in clinical scoring indices but later suffer from clinical relapse during maintenance therapy.[8] Strategies to optimise ADA therapy and prevent drug failure are of great clinical and economic interest, given the relative paucity of alternative, efficacious biological agents for the management of CD and the high cost of these therapies. Simple approaches such as informed recommendations regarding mode of delivery are attractive techniques that require further exploration.

Intuitively, inconsistencies with patient factors such as administration technique and depth of injection might lead to variability in drug bioavailability. The finding that syringe delivery was associated with higher trough drug levels in the previous study[17] were of even more relevance, given that four of five sites in the current study have at least 74% of subjects preferring pen delivery. Reassuringly, the present multicentre analysis found no difference in drug levels between the devices on direct group comparison. Furthermore, after employing the same statistical techniques used in our earlier study, delivery device was not predictive of ADA drug level across all sites. As

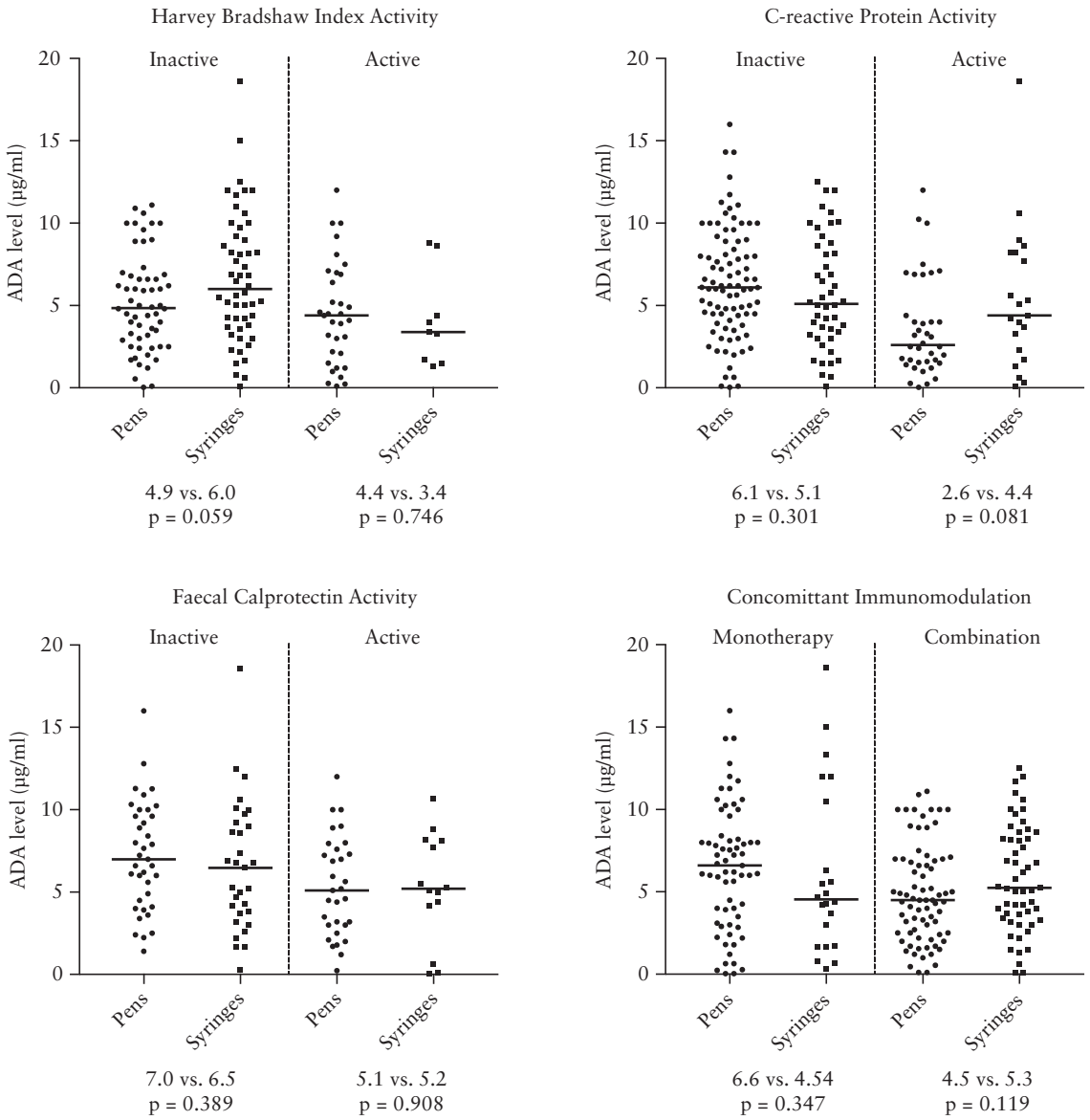

Figure 3. Adalimumab [ADA] drug levels according to delivery device dichotomised into disease activity or concomitant immunomodulation. Values and horizontal bars reflect medians. Group comparisons via Mann-Whitney test. Threshold for activity as follows: Harvey-Bradshaw Index >4; C-reactive protein >5 mg/L; faecal calprotectin >150 μg/g.

previously demonstrated, previous anti-TNF exposure had a modest inverse association with both absolute and therapeutic drug levels.[24] Whereas drug levels were equivalent between the two groups, syringe users had a milder disease activity clinically, as measured by the HBI, and a higher proportion of smokers compared with pen users. However, the significance of this is uncertain given that HBI correlates poorly with both biomarker and endoscopic disease activity, and no significant differences in FCP were found in our cohort between devices.[25–29] In addition, despite a deleterious effect on disease activity, the relationship between cigarette smoking and anti-TNF drug levels is unclear.[13,30,31] Of note, syringe users were found to have higher concentrations of albumin and a greater proportion of concomitant immunomodulation—two factors associated with better disease control and favourable anti-TNF pharmacokinetics.[32,33]

We performed follow-up in a cohort of 30 patients who were intensified to weekly ADA, on the basis of secondary loss of response and sub-therapeutic ADA drug levels, to see if there was a difference in rates of response according to delivery device. No difference was found in outcomes between these two groups. In addition to absolute drug level comparison, evaluating clinical outcomes between delivery device is important given the inconsistent relationship between ADA drug level and disease activity previously described.[12,13]

Of interest, subgroup analysis by centre revealed site-specific differences in drug level between delivery devices. Syringe users at Alfred Health had both significantly higher serum drug levels and a higher proportion of patients in the therapeutic range. After controlling for age, gender, and BMI, delivery device accounted for 9% of the variance in drug level at this site. Such findings were not observed at other participating sites. In fact, a higher proportion of pen compared with syringe users at CHU Saint-Étienne achieved therapeutic ADA levels. The reason for these discordant results is unclear. Comparing these two sites, patients from Alfred Health had higher

BMI, significantly longer disease duration, higher FCP, and a greater proportion of penetrating and perianal disease, than those at CHU Saint-Étienne. Disease duration and activity may affect drug levels, but such factors were similar between pen and syringe users at both sites and therefore are unlikely to account for the observed findings.

One unexplored potential explanation for drug level variance between delivery devices is patient administration technique. At CHU Saint-Étienne, only patients delivering ADA via pen underwent formal administration education by IBD nurses. Evaluation of self-administration technique has not been previously investigated in IBD patients. Data from the diabetes literature indicate a clear relationship between insulin administration technique and drug efficacy. Numerous studies have demonstrated that insulin administration education, evaluation, and re-training influence glycaemic control.[34,35] Accordingly, we hypothesise that the site-specific differences in observed drug levels between pen and syringe users at Alfred Health and CHU Saint-Étienne may relate to differences in administration technique. Both pen and syringe delivery have shared and individual weaknesses, such as tissue micro-aspiration and obstruction of flow, altered angle and depth of administration, insufficient duration in the subcutaneous compartment leading to drug retention within the delivery device, and peripheral leakage.[36,37] Although injection site pain may impact on successful administration, and despite reports of improved tolerability,[38] we did not find any significant impact of citrate-free ADA on drug level, although numbers were small. Site variance in ADA drug level may arise due to the lack of guiding international recommendations for correct administration, education, and assessment, similar to those observed in the diabetes literature.[36]

The current study has several limitations. First, the inability to prove causality and the longitudinal effect of delivery device on drug levels is inherent in any cross-sectional study design. Larger, prospective controlled trials, that incorporate supervised administration with random allocation to either pen or syringe delivery, would be useful to confirm bioequivalence. Despite a study size of 218 patients, a larger population with a greater spectrum of disease activity and a higher number of syringe users would allow for more generalisable findings. Furthermore, serial trough drug levels in each patient would allow for greater confidence in approximating steady state.

Second, variance in drug level assays between sites may limit the ability to analyse the entire cohort without adjustment. However, a high correlation between two ADA ELISA kits has been previously demonstrated,[39] and data presented in abstract form show excellent agreement between all three ELISA assays used in the current study.[40] To further address the possibility of between-site differences in drug levels, we observed no difference in levels between devices in the 149 patients tested using the same Shikari® Q-ADA ELISA assay [Figure 2b].

Third, missing biomarker and assessment of clinical disease activity data across sites may have influenced the strength of associations with drug level found on regression analysis. This was partly addressed by subgroup comparisons according to surrogates of disease activity [Figure 3]. Including biomarkers up to 45 days either side of TDM was necessary to capture sufficient data in a retrospective analysis, but may limit accuracy of estimates of disease activity.

Fourth, we were unable to identify and control for patient adherence between the two devices, rates of spillage, the identity of the administrator [patient or proxy], and the site of administration—all important considerations for future studies. While we did not record site of administration, only one centre offered education in thigh injections. Given this, we expect the number of patients injecting into the thigh to be small and the impact negligible. In addition, studies have not demonstrated a difference in drug level after a single dose of both ADA and a biosimilar into the thigh and abdomen in healthy volunteers.[14,16]

Fifth, all sites instruct patients to undergo TDM the day before ADA administration, but the exact proximity to trough was only recorded at Liverpool Hospital and CHU Saint-Étienne. Although trough confirmation would be useful for standardisation, ADA TDM earlier in the cycle approximates trough levels.[17,41] Furthermore, we do not expect timing of TDM to differ significantly between delivery device, and as such any impact on drug level comparison between pen and syringe is likely to be negligible.

Finally, the primary outcome of interest in our study was ADA drug level—a factor with inconsistent associations with disease activity. However, consistent with bioequivalence at standard maintenance dosing, our limited 12-month follow-up data demonstrated no temporal difference in the effect of delivery device on disease outcomes after dose intensification. Given numbers were small, this result should be interpreted with caution and requires confirmation in larger, prospective studies with a longer period of follow-up.

In summary, this is the first study to demonstrate equal ADA serum drug levels between pen and syringe delivery in a cohort of patients with CD, after controlling for a range of potentially confounding variables. The significance of the heterogeneity in drug levels across the sites is uncertain, but may reflect discordance in administration technique and highlight an unmet need for investigation of education, evaluation, and re-training in self-administered subcutaneous biologics.

Funding

This work did not require external funding. All data used in this study were drawn from existing health records collected in the routine care of patients with Crohn's disease at each centre. Manuscript submission and publication fees were supported by Alfred Health Gastroenterology Department.

Conflict of Interest

RDL, IEC, EPV, EF, ES have no conflicts of interest to declare; SJB reports personal fees from AbbVie, personal fees from Janssen, all outside the submitted work; PRG reports grants and personal fees from AbbVie, grants and personal fees from Janssen, personal fees from MSD, personal fees from Takeda, grants and personal fees from Ferring, grants from Falk Pharma, grants and personal fees from Danone, grants from A2 Milk Company, personal fees from Merck, personal fees from Nestle Health Science, personal fees from Allergan, personal fees from Celgene, and his department financially benefits from sales of a digital application and booklets on the low FODMAP diet, all outside the submitted work; MPS reports personal fees from AbbVie, personal fees from Janssen, personal fees from Takeda, grants and personal fees from Ferring, personal fees from Pfizer, personal fees from MSD, personal fees from Celgene, grants from Orphan, all outside the submitted work; SJC reports personal fees and non-financial support from AbbVie, personal fees and non-financial support from Janssen, personal fees and non-financial support from MSD, personal fees and non-financial support from Takeda, personal fees and non-financial support from Pfizer, personal fees from Vifor, personal fees from Celgene, personal fees and non-financial support from Ferring, personal fees and non-financial support from Shire, non-financial support from Orphan, grants from the Gastroenterological Society of Australia, all outside the submitted work; XR reports personal fees from AbbVie, personal fees from Janssen, personal fees from MSD, personal fees from Takeda, personal fees from Pfizer, personal fees from Theradiag, all outside the submitted work; MGW reports grants and personal fees from AbbVie, personal fees and

non-financial support from Shire, personal fees and non-financial support from Takeda, non-financial support from Ferring, all outside the submitted work.

Acknowledgments

We would like to acknowledge the work of the clinic nursing, allied health, and administrative staff at each centre.

Authors Contributions

All authors have made substantial contributions to the following: [1] the concept and design of the study, or acquisition of data, or analysis and interpretation of data; [2] drafting the article or revising it critically for important intellectual content; and [3] final approval of the version to be submitted.

References

1. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet* 2012;**380**:1590–605.
2. Hanauer SB, Sandborn WJ, Rutgeerts P, *et al.* Human anti-tumor necrosis factor monoclonal antibody [adalimumab] in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006;**130**:323–33; quiz 591.
3. Sandborn WJ, Hanauer SB, Rutgeerts P, *et al.* Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. *Gut* 2007;**56**:1232–9.
4. Hanauer SB, Feagan BG, Lichtenstein GR, *et al.*; ACCENT I Study Group. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;**359**:1541–9.
5. Colombel JF, Sandborn WJ, Rutgeerts P, *et al.* Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007;**132**:52–65.
6. Vavricka SR, Bentele N, Scharl M, *et al.*; Swiss IBDnet and Swiss IBD Cohort Study Group. Systematic assessment of factors influencing preferences of Crohn's disease patients in selecting an anti-tumor necrosis factor agent [CHOOSE TNF TRIAL]. *Inflamm Bowel Dis* 2012;**18**:1523–30.
7. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. *Aliment Pharmacol Ther* 2011;**33**:987–95.
8. Allez M, Karmiris K, Louis E, *et al.* Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: definitions, frequency and pharmacological aspects. *J Crohns Colitis* 2010;**4**:355–66.
9. Bodini G, Savarino V, Fazio V, *et al.* Relationship between drug serum concentration and clinical activity in patients with Crohn disease who achieved remission with adalimumab - a prospective study. *Gastroenterology* 2012;**142**:S388–8.
10. Roblin X, Marotte H, Rinaudo M, *et al.* Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2014;**12**:80–4.e2.
11. Mazor Y, Almog R, Kopylov U, *et al.* Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. *Aliment Pharmacol Ther* 2014;**40**:620–8.
12. Chiu YL, Rubin DT, Vermeire S, *et al.* Serum adalimumab concentration and clinical remission in patients with Crohn's disease. *Inflamm Bowel Dis* 2013;**19**:1112–22.
13. Ward MG, Warner B, Unsworth N, *et al.* Infliximab and adalimumab drug levels in Crohn's disease: contrasting associations with disease activity and influencing factors. *Aliment Pharmacol Ther* 2017;**46**:150–61.
14. Paulson S, Noertersheuser P, Garimella T, Doan T. Assessment of relative bioavailability, safety, and tolerability of single doses of adalimumab administered via an autoinjector pen and a prefilled syringe. *J Am Acad Dermatol* 2007;**56**:AB9.
15. Shin D, Lee Y, Kang JW, Ellis-Pegler R. A phase I pharmacokinetic study comparing pre-filled pen and pre-filled syringe of Sb5, an adalimumab biosimilar in healthy subjects. *Ann Rheum Dis* 2016;**75**:1005–6.
16. Ramael S, Van Hoorick B, Tiessen R, *et al.* Similar pharmacokinetics of the adalimumab [Humira®] biosimilar BI 695501 whether administered via subcutaneous autoinjector or prefilled syringe [VOLTAIRE®-AI and VOLTAIRE®-TAI]: phase 1, randomized, open-label, parallel-group trials. *Rheumatol Ther* 2018;**5**:403–21.
17. Ward MG, Thwaites PA, Beswick L, *et al.* Intra-patient variability in adalimumab drug levels within and between cycles in Crohn's disease. *Aliment Pharmacol Ther* 2017;**45**:1135–45.
18. Gomollón F, Dignass A, Annese V, *et al.* 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 1: diagnosis and medical management. *J Crohns Colitis*. 2017;**11**:3–25.
19. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet* 1980;**1**:514.
20. Ward MG, Kariyawasam VC, Mogan SB, *et al.* Clinical utility of measuring adalimumab trough levels and antibodies to adalimumab in patients with inflammatory bowel diseases. *J Gastroenterol Hepatol* 2013;**28**:100–1.
21. Vermeire S, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation between the Crohn's disease activity and Harvey-Bradshaw indices in assessing Crohn's disease severity. *Clin Gastroenterol Hepatol* 2010;**8**:357–63.
22. Gisbert JP, Bermejo F, Pérez-Calle JL, *et al.* Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009;**15**:1190–8.
23. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* 2006;**55**:749–53.
24. Gisbert JP, Marín AC, McNicholl AG, Chaparro M. Systematic review with meta-analysis: the efficacy of a second anti-TNF in patients with inflammatory bowel disease whose previous anti-TNF treatment has failed. *Aliment Pharmacol Ther* 2015;**41**:613–23.
25. Zittan E, Kabakchiev B, Kelly OB, *et al.* Development of the Harvey–Bradshaw Index-pro [HBI-PRO] score to assess endoscopic disease activity in Crohn's disease. *J Crohns Colitis* 2017;**11**:543–8.
26. Baars JE, Nuij VJ, Oldenburg B, Kuipers EJ, van der Woude CJ. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis* 2012;**18**:1634–40.
27. Gomes P, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. *Gut* 1986;**27**:92–5.
28. Jones J, Loftus EV Jr, Panaccione R, *et al.* Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. *Clin Gastroenterol Hepatol* 2008;**6**:1218–24.
29. D'Haens G, Ferrante M, Vermeire S, *et al.* Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis* 2012;**18**:2218–24.
30. Brandse JF, Vos LM, Jansen J, *et al.* Serum concentration of anti-TNF antibodies, adverse effects, and quality of life in patients with inflammatory bowel disease in remission on maintenance treatment. *J Crohns Colitis* 2015;**9**:973–81.
31. Lie MR, Peppelenbosch MP, West RL, Zelinkova Z, van der Woude CJ. Adalimumab in Crohn's disease patients: pharmacokinetics in the first 6 months of treatment. *Aliment Pharmacol Ther* 2014;**40**:1202–8.
32. Ungar B, Levy I, Yavne Y, *et al.* Optimizing anti-TNF-α therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2016;**14**:550–7.e2.
33. Fiorino G, Danese S. Adalimumab and azathioprine combination therapy for Crohn's disease: a shining diamond? *J Crohns Colitis* 2016;**10**:1257–8.
34. Spollett G, Edelman SV, Mehner P, Walter C, Penfornis A. Improvement of insulin injection technique: examination of current issues and recommendations. *Diabetes Educ* 2016;**42**:379–94.
35. Nakatani Y, Matsumura M, Monden T, Aso Y, Nakamoto T. Improvement of glycemic control by re-education in insulin injection technique in patients with diabetes mellitus. *Adv Ther* 2013;**30**:897–906.
36. Frid AH, Kreugel G, Grassi G, *et al.* New insulin delivery recommendations. *Mayo Clin Proc* 2016;**91**:1231–55.
37. Frid A, Hirsch L, Gaspar R, *et al.*; Scientific Advisory Board for the Third Injection Technique Workshop. New injection recommendations for patients with diabetes. *Diabetes Metab* 2010;**36**[Suppl 2]:S3–18.

38. Nash P, Vanhoof J, Hall S, *et al*. Randomized crossover comparison of injection site pain with 40 mg/0.4 or 0.8 mL formulations of adalimumab in patients with rheumatoid arthritis. *Rheumatol Ther* 2016;**3**:257–70.
39. Ruiz-Argüello B, del Agua AR, Torres N, Monasterio A, Martínez A, Nagore D. Comparison study of two commercially available methods for the determination of infliximab, adalimumab, etanercept and anti-drug antibody levels. *Clin Chem Lab Med* 2013;**51**:e287–9.
40. Sam M, Ng W, Connor S, Toong C. Comparative evaluation of four commercially available ELISA kits for therapeutic drug monitoring of adalimumab. Poster session presented at: 16th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology; Sep 16–19, 2018; Brisbane, Australia..
41. Ungar B, Engel T, Yablecovitch D, *et al*. Prospective observational evaluation of time-dependency of adalimumab immunogenicity and drug concentrations: the POETIC study. *Am J Gastroenterol* 2018;**113**:890–8.