Clinical performance of an infliximab rapid quantification assay

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Abstract

Background: Therapeutic drug monitoring (TDM)-based algorithms can be used to guide infliximab (IFX) adjustments in inflammatory bowel disease (IBD) patients. This study aimed to explore a rapid IFX-quantification test from a clinical perspective.

Methods: This manuscript describes a prospective cohort study involving 110 ulcerative colitis (UC) patients on the maintenance phase of IFX. IFX trough levels were quantified using a rapid quantification assay and a commonly-used reference kit.

Results: Irrespective of the assay used to measure IFX, its through levels were statistically different between patients with and without endoscopic remission (Mayo endoscopic score = 0), as well as between patients stratified by their faecal calprotectin (FC) levels. Despite the fact that the two methods correlated well with each other [Spearman's rank correlation coefficient = 0.843, $p < 0.001$; intraclass correlation coefficients = 0.857, 95% confidence interval (CI): 0.791–0.903], there was a discernible systematic variation; values obtained with the reference kit were on average 2.62 units higher than those obtained with the rapid assay. Notwithstanding, 3 μg/ml was shown to be an acceptable cut-off to assess endoscopic status and inflammatory burden levels using both assays. The percentage of patients that had a positive outcome when the IFX concentration measured by the rapid assay ranked above 3 μg/ml was 88% both for a Mayo endoscopic score ⩽ 1 and for an FC concentration <250 μg/g.

Conclusions: Based on this study, we concluded that using the rapid IFX assessment system with a 3 μg/ml threshold is a reliable alternative to the time-consuming enzyme-linked immunosorbent assays in patients on the maintenance phase of IFX.

Keywords: Infliximab, ulcerative colitis, therapeutic window

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Introduction

The introduction of anti-tumour necrosis factor (TNF)α monoclonal antibodies as therapeutic agents in auto-inflammatory disorders has revolutionized the medical management strategies of these diseases and the health-related quality of life of patients. In the case of inflammatory bowel diseases [IBDs, which include Crohn's disease (CD) and ulcerative colitis (UC)], the use of anti-TNFα agents has led to a decrease in hospitalization rates, risk of surgery and health-related costs.[1]

However, and despite the anti-TNFα success in the treatment of many IBD patients, some of them do not respond to the drug during the induction phase, whereas others experience a loss of response later during treatment.[2] Accumulating evidence from the literature suggests that the outcomes of CD and UC patients on infliximab (IFX) are strongly related

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with the levels of the drug found in the organism.[3–10] From a physician's perspective, understanding the reasons that lead to unresponsiveness is key to delineate future therapeutic strategies, which can include a dose intensification, a switch to another anti-TNFα agent, or adding immunosuppressive drugs or steroids. In this context, the precise and accurate measurement of the circulating drug levels, known as therapeutic drug monitoring (TDM), has a key role. Several TDM-based algorithms and dashboards are being developed to assist the physician in the therapeutic decision-making process.[2,11–13] Moreover, TDM may also be useful to identify cases with supra-therapeutic drug levels (which can be de-escalated to prevent the appearance of adverse effects), and has been proven as a cost-effective strategy when compared with the traditional empirical-based adjustment of drug dosage.[14,15]

Given the importance of TDM in patients on IFX, one can easily find a number of different commercial kits that can measure the concentration of this agent from the patient's serum, most of them relying on an enzyme-linked immunosorbent assay (ELISA) approach. However, these kits have a turnaround time of approximately 8 h, delaying the IFX dose adjustment to the following infusion (usually 6–8 weeks later). A rapid IFX-quantification system, which allows a fast (15 min) assessment of IFX from a patient's serum, has been recently launched in the market by the Bühlmann® company (Schönenbuch, Switzerland). Not only does this system allows an immediate adjustment of the IFX dosage, but it also has the advantage of being a user-friendly desktop device, which can be easily operated by any nurse, technician or physician without the requirement of specific laboratory facilities.

We have recently validated the utilisation of the Bühlmann® rapid assay in a laboratorial context, and concluded that this kit constitutes a reliable and fast alternative to the traditional ELISA kits.[16] In this study we aimed to take a step further and to assess the clinical sensitivity and specificity of this rapid assay, by addressing the existence and interpretability of IFX cut-off values able to guide the therapeutic decision-making process.

Material and methods

Cohort

UC patients on the maintenance phase of IFX therapy were prospectively and consecutively recruited from 10 different university and community hospitals. Only patients older than 18 years and with at least 14 weeks of IFX treatment were invited to participate. Exclusion criteria included history of malignancy in the previous 5 years, opportunistic infections or demyelinating diseases; existence of adenomatous polyps or known viral infections; and pregnancy and breastfeeding.

This study was approved by the ethic committee of all hospitals involved and by the Portuguese Data Protection Authority. All patients enrolled signed an informed written consent.

IFX-quantification assays

A total of 110 samples collected from the same number of patients were assayed to determine their serum trough IFX levels using two different commercial kits: Quantum Blue® Infliximab: Quantitative Lateral Flow Assay (Bühlmann, Schönenbuch, Switzerland), hereafter referred to as QB, and Level Infliximab M2920 kit (Sanquin, Amsterdam, the Netherlands), hereafter referred to as Sanquin. Both kits were used strictly following manufacturers' instructions. The lower and upper limits of quantification were 0.4 μg/ml and 20 μg/ml for the QB assay, and 0.08 μg/ml (1:200) and 25 μg/ml (1:1500) for the Sanquin assay, respectively. Whenever the results obtained were below or above these limits of quantification, they were considered to be at those same limits. Sanquin was chosen as the reference test as it is a widely used kit in both laboratorial and clinical contexts. All measurements were carried out by the same researcher.

Assessment of disease outcomes

Disease status, including clinical evaluation, endoscopic and histological activity, and quantification of faecal calprotectin (FC), was assessed at the same time as the IFX concentration (i.e. immediately before an IFX infusion).

Clinical evaluation. Clinical remission was evaluated according to the global Mayo score. Patients were considered to be in clinical remission if their global Mayo score was $\leqslant 2$ and no individual subscore was above 1.

Endoscopic evaluation. Endoscopic activity was evaluated using the Mayo endoscopic subscore:[17] mucosal healing was defined as a Mayo endoscopic subscore equal to 0 or $\leqslant 1$.

Histological evaluation. The presence of histological inflammation was evaluated through the analysis of an average of two biopsy samples from the sigmoid and the rectum. Samples were classified following the Geboes score,[18] and histological remission was defined as a Geboes index $\leqslant 3.0$. All samples were the subject of a central reading by two independent pathologists blinded to the patients' disease status and endoscopic results. Disagreements between pathologists were resolved by a review including a third pathologist (K. Geboes) and using a multiheaded microscope, defining the final score.

Quantification of faecal calprotectin. Stool samples were collected and kept at 4°C (for a maximum of 48 h) until shipment to the central laboratory (Department of Pharmacology and Therapeutics, Faculty of Medicine of University of Porto, Portugal). FC was extracted from stools within a maximum of 7 days after collection using the 'faecal sample preparation kit' (Roche Diagnostics, Germany) according to the instructions provided by the manufacturer, and stored at −80°C until quantification. FC concentration in each sample was determined using the QB kit according to the manufacturer's instructions.

Table 1. Cohort characterization.

	n	%
Sex		
Male	50	45%
Female	60	55%
Smoking status		
Never smoked	74	71%
Former smoker	25	24%
Smoker	5	5%
Location of disease		
Proctitis	2	2%
Left-side colitis	50	49%
Extensive colitis	51	49%
Azathioprine	66	61%
Azathioprine intolerant	23	22%
Steroids	10	9%
Corticodependent	65	59%
Corticoresistant	25	23%
Montreal classification		
Clinical remission	86	80%
Mild UC	16	15%
Moderate UC	5	5%
Severe UC	0	0%

UC, ulcerative colitis.

Statistical analysis

Categorical variables were described through absolute (n) and relative (%) frequencies and continuous variables were described as mean and standard deviation, median, percentiles, and minimum/ maximum values when appropriate. All the reported p-values were two-sided, and p-values <0.05 were considered to be statistically significant. The ability of the measured IFX concentrations to assess the various disease outcomes was evaluated by plotting Receiver Operating Characteristic curves and computing the Area Under the Curve. All data were arranged, processed and analysed with SPSS® v.20.0 data (Statistical Package for Social Sciences, IBM Corp., Armonk, NY). Graphs were computed with Prism 7 (GraphPad Software, Inc., CA, USA).

Results

Characterization of the cohort and disease outcomes

The main baseline characteristics of this study's cohort are depicted in Table 1. Females constituted 55% of the entire population, and only 5% of all patients were current smokers. A minority of patients (2%) had a proctitis diagnosis, whereas 49% of them had left-side colitis and an equal percentage had extensive colitis. Overall 22% of the patients were azathioprine (AZA) intolerant, whereas 59% and 23% were classified as corticodependent and corticoresistant, respectively. At the time of study inclusion, 61% and 9% of all patients were on AZA and steroids, respectively.

The disease outcomes addressed during this study are listed in Table 2. Regarding clinical evaluation, the majority of patients (72%) had a global Mayo score $\leqslant 2$, and 69% of the entire population were considered to be in clinical remission (i.e. had a global Mayo score $\leqslant 2$ and no individual subscore >1). Moreover, 58% or 81% of all patients were considered to be in mucosal healing (endoscopic Mayo score = 0 or $\leqslant 1$, respectively). Regarding FC levels, 66% of the population were below the threshold of 250 μg/g. Finally, the overall median [interquartile range (IQR)] of the IFX trough levels was 6.59 μg/ml (3.03–14.66) using the Sanquin kit, and 5.25 μg/ml (1.70–9.58) using the rapid QB assay.

Analytical comparison between the two different IFX-quantification methods

IFX through levels measured by the Sanquin and QB levels were highly correlated [Spearman's rank correlation coefficient = 0.843, $p < 0.001$; intraclass correlation coefficient (ICC) = 0.857, 95% CI: 0.791–0.903], as shown in Supplementary Figure 1. However, the mean difference and its CI show that the concentrations obtained with the Sanquin kit were, on average, higher than those obtained with the QB (average difference = 2.62 µg/ml, 95% CI: 1.64–3.60). Finally, the Bland–Altman plot shows that the difference between values measured with both kits increases with the increase in IFX concentrations, but is close to 0 for concentrations below 5 µg/ml (Supplementary Figure 2).

Association between IFX trough levels and outcomes

The medians of serum trough IFX concentrations detected with each method for contrasting disease outcomes (concerning clinical remission, endoscopic Mayo score and FC levels) are represented in Figure 1. The results show that IFX trough levels were higher in patients who had positive outcomes irrespective of the assay used, and these results were significant for endoscopic remission (using endoscopic Mayo score = 0 as the criterion for remission) and FC.

We then applied different IFX cut-offs (from 1–10) to the results obtained from each kit, and assessed their ability to predict patient outcomes. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Kappa for each case are depicted in Supplementary Table 1. A positive test was defined as having an IFX level below the cut-off, whereas the disease status was defined as having a negative outcome (not being in clinical remission, having an endoscopic Mayo score >0 or >1, or having an FC level >250 µg/g). NPV represents the percentage of patients who have a positive outcome (no disease) among those who have an IFX above the defined cut-off (negative test result), whereas PPV represents the percentage of patients who have a negative outcome (disease) among those that have an IFX below the defined cut-off (positive test result).

Perceptively, the performance values vary widely with the cut-off chosen and the outcome evaluated, but are considerably similar for both kits when the conditions mentioned are kept stable (i.e. same cut-off and outcome). Figure 2 represents the accuracy (i.e. the sum of true positives and negatives) of the results obtained with either QB or Sanquin in terms of clinical status, endoscopic score and FC level using different cut-offs. The results show that Sanquin and QB have a very similar variation of the accuracy along the different cut-offs. Overall, a value of 3 µg/ml seems to be an acceptable cut-off for QB, although lower values could be considered in a few situations.

NPV has an important role in this context, as it represents the percentage of patients who have an IFX concentration above the cut-off and would not benefit from a drug adjustment. And in fact, 74, 62, 83 and 86% of patients with an IFX trough level >3 µg/ml measured by the Sanquin

Table 2. Disease outcomes.

	n	%
Global Mayo score (*n*, %)		
1	60	57%
2	16	15%
3	8	7%
4	3	3%
5	6	6%
6	3	3%
7	4	4%
8	3	3%
9	1	1%
11	2	2%
Clinical remission = no	34	31%
Clinical remission = yes	76	69%
Endoscopic Mayo score (*n*, %)		
0	63	58%
⩾1	45	42%
⩽1	87	81%
>1	21	19%
FC (µg/g) (*n*, %) QB		
<250	59	66%
⩾250	31	34%
IFX, (median, IQR)		
Sanquin	6.59	3.03–14.66
Quantum Blue	5.25	1.70–9.58

FC, faecal calprotectin; IFX, infliximab; QB, Quantum Blue® Infliximab: Quantitative Lateral Flow Assay (Bühlmann, Schönenbuch, Switzerland).

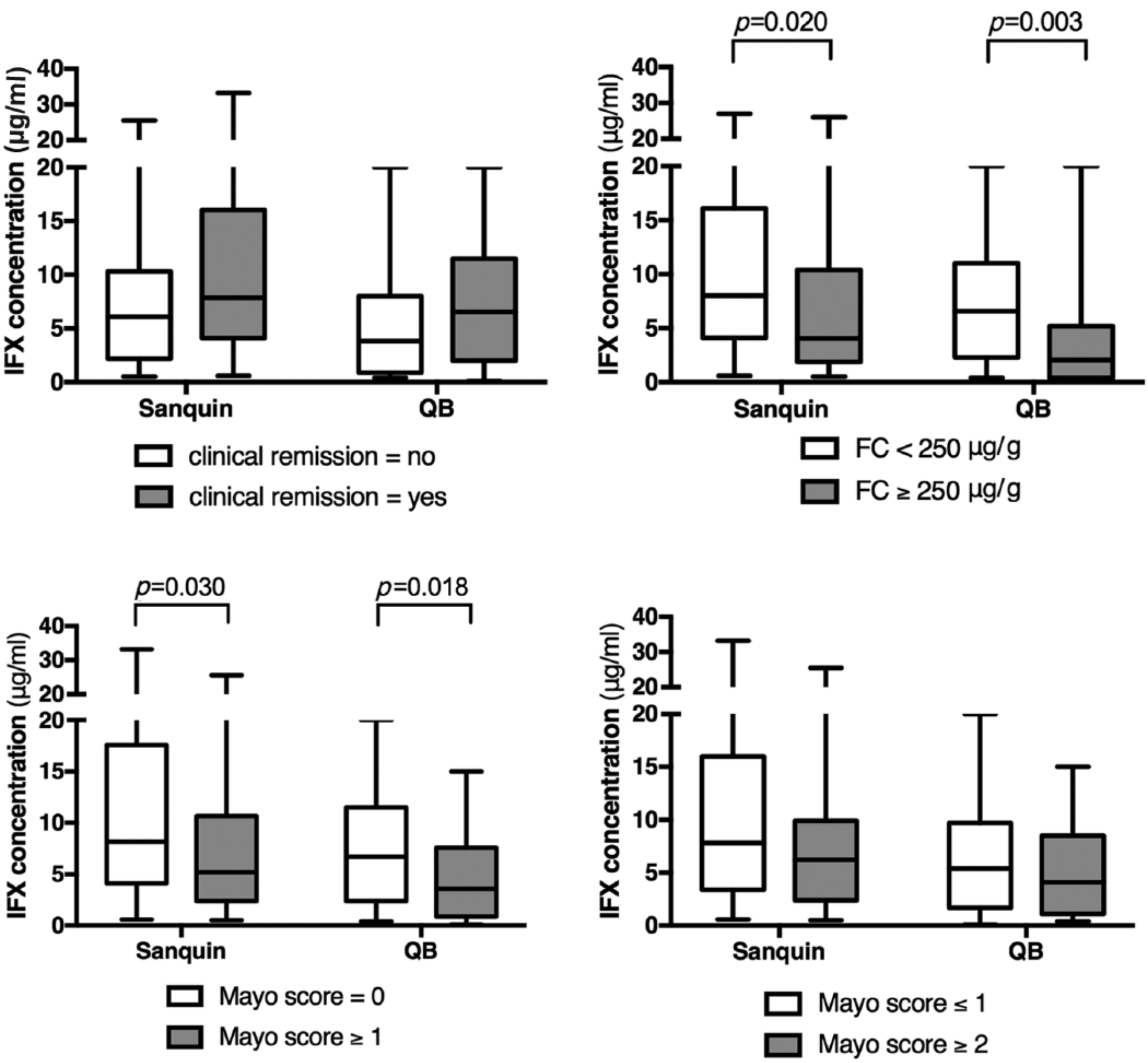

Figure 1. IFX concentrations quantified using the different methods and stratified by disease outcomes.
IFX, infliximab; QB, Quantum Blue® Infliximab: Quantitative Lateral Flow Assay (Bühlmann, Schönenbuch, Switzerland); Sanquin, Level Infliximab M2920 kit (Sanquin, Amsterdam, the Netherlands).

kit are in clinical remission, have a Mayo endoscopic score of 0, have a Mayo endoscopic score ⩽1, and have an FC level <250 µg/g, respectively, whereas these values are 74, 65, 88 and 88% for the QB kit.

When adjusting the IFX cut-off to evaluate clinical status to 1 (with Sanquin) or 2 (with QB), the percentage of patients that test above these values and are, indeed, in clinical remission, is 71% and 73%, respectively. This shows that although accuracy can be higher, the NPV is slightly smaller for these lower cut-offs. The same thing occurs when one addresses endoscopic remission (using endoscopic Mayo score ⩽1 as the remission criterion) using IFX cut-offs <3 µg/ml: the accuracy is higher, but the NPV is lower. On the other hand, the PPV (percentage of patients that are below the IFX cut-off and could benefit from an IFX dose adjustment) are consistently lower than the NPVs, and for a cut-off of 3 µg/ml vary from 23–50% with the Sanquin kit, and from 23–55% with the rapid QB kit.

To test whether the IFX values measured by these kits could also be used to assess deep remission, the Geboes index was considered as a criterion to establish histological remission, and the sensitivity, specificity, PPV, NPV, accuracy and Kappa for each cut-off concerning the occurrence of

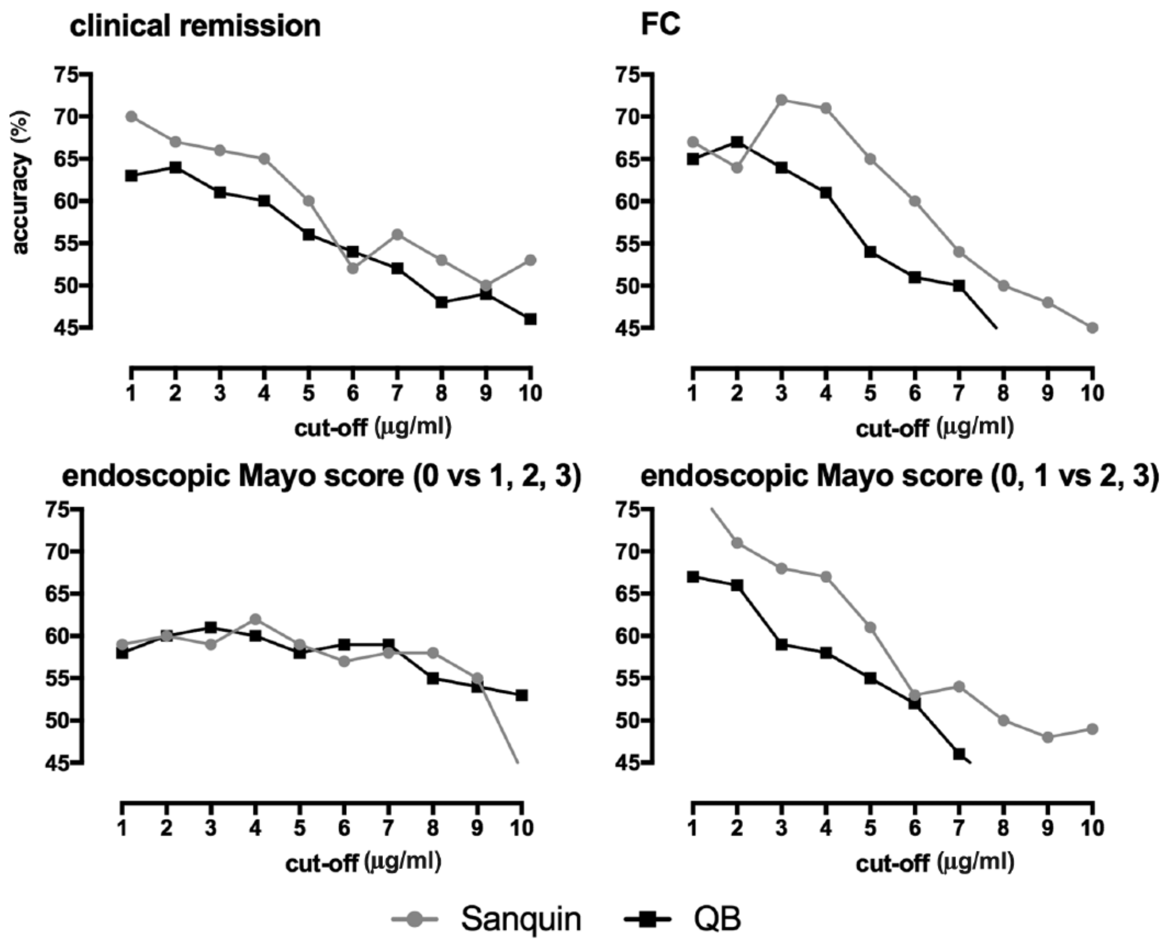

Figure 2. Accuracy values of the different cut-off values (only those between 45–75% represented).
FC, faecal calprotectin; QB, Quantum Blue® Infliximab: Quantitative Lateral Flow Assay (Bühlmann, Schönenbuch, Switzerland); Sanquin, Level Infliximab M2920 kit (Sanquin, Amsterdam, the Netherlands); vs, versus.

deep remission (with or without the histological criterion) are depicted in Supplementary Table 2. Although a cut-off of ⩽3 µg/ml seems to be acceptable to assess histological remission irrespective of the kit used, the identification of one specific cut-off in what concerns deep remission is hampered by the overall stability of accuracy across the different cut-offs.

Qualitative comparison between the two different IFX-quantification methods

A qualitative comparison of the assays for a cut-off of 3 µg/ml is depicted in Table 3, and shows an accuracy of 88% and a Kappa (standard error of the mean) of 0.718 (0.070). In fact, the distribution of patients according to a 3 µg/ml cut-off is rather similar between both methods, with only 13 patients (12.0%) being placed differently (they have IFX levels <3 µg/ml when using the rapid QB test, but above that cut-off when using the Sanquin test).

Discussion

Several commercial kits and different protocols have been optimized for an accurate determination of IFX levels from patient serum, but the recent development of a rapid IFX assessment test holds the promise of revolutionizing the TDM-based therapeutic algorithms, by allowing an immediate adjustment of the IFX dosage (as opposed to delaying this intervention to the following infusion cycle). This study aimed to assess the clinical sensitivity and specificity of this rapid assay, by using it to measure samples from 110 patients, fully characterized regarding their clinical, endoscopic and inflammatory burden status.

Table 3. Qualitative comparison between Sanquin and QB assays.

			QB		Total
			⩾3 µg/ml	<3 µg/ml	
Sanquin	**⩾3 µg/ml**	*n*	68	13	81
		%	84.0%	16.0%	100.0%
	<3 µg/ml	*n*	0	26	26
		%	0.0%	100.0%	100.0%
Total			68	39	107

QB, Quantum Blue® Infliximab: Quantitative Lateral Flow Assay (Bühlmann, Schönenbuch, Switzerland); Sanquin, Level Infliximab M2920 kit (Sanquin, Amsterdam, the Netherlands).

The overall results and the clinical stratification obtained using different cut-offs were compared with those obtained using an already validated and widely used IFX-quantification kit (Sanquin).

The results reported here show that although the concentrations obtained by the different methods are strongly correlated, there is a systematic variation: the concentrations measured by the Sanquin kit were, on average, 2.62 units higher than those measured by the rapid QB test, which is consistent with the median IFX values obtained with each method for the entire population. The Sanquin kit's bias towards measuring higher values when compared with other kits has been noticed before.[16,19] Overall, other methodological comparisons involving two or more IFX-quantification assays show that, most of the times, the assays compare quite well against each other (even when they are not ELISA-based), but systematic deviations are rather common and are likely to result from the fact that different assays use different antibodies with varying IFX affinities.[19–25]

The association of IFX serum levels with disease outcomes or inflammatory markers such as clinical response, clinical remission, mucosal healing, endoscopic improvement and C-reactive protein levels have been often reported.[3,5,6,8–10,26] Accordingly, our results show that IFX trough levels were significantly lower when patients had an endoscopic Mayo score ⩾1 or an FC concentration ⩾250 µg/g. A similar pattern was found for clinical remission and for an endoscopic Mayo score >0 (i.e. patients who have a negative outcome had lower IFX trough levels), although in this case the results were not significant. This might be due to the small size of the cohort, or to the fact that the patients analysed were very stable, most of them (80%) in clinical remission according to the Montreal classification and with over 14 weeks of IFX therapy (primary nonresponders were excluded).

Given the systematic differences encountered in the quantification, one would expect the two different methods to have different clinical cut-offs. However, that is not the case: 3 µg/ml is an acceptable threshold for both assays particularly in what concerns assessment of endoscopic status and inflammatory burden (measured by the FC levels). Regarding clinical status, although 3 µg/ml may be a satisfactory cut-off, values of 1 and 2 µg/ml can be considered for the Sanquin and QB assays, respectively. These cut-offs have a marginally better accuracy and smaller NPV when compared with 3 µg/ml. The same holds true for IFX cut-offs of 1 and 2 µg/ml when addressing endoscopic activity using an endoscopic Mayo score ⩽1 as criterion for remission: the accuracy raises and the NPV drops when compared with those of a cut-off of 3 µg/ml. Concerning deep remission, however, the different cut-offs seem to behave similarly and it is not easy to choose a single value. This is likely related to the fact that deep remission is a composite endpoint, and therefore reflects the different behaviours of its components.

The lack of impact of the systematic bias observed in the optimal clinical cut-off is easily explained by observing the Bland–Altman plot: in fact, this plot shows that the differences encountered in the values measured by both methods are particularly close to 0 for IFX levels <5 µg/ml. In other words, at levels as low as those considered for the clinical threshold, the assays seem to behave in a similar fashion. This is supported by the comparative analysis of the assay's results, which shows that for a threshold of 3 µg/ml, 88% of the patients fall equally above or below the cut-off irrespective of the method used.

In practical terms, a clinical cut-off should help a physician decide whether a patient may benefit from an IFX dose adjustment. A cut-off of 3 μg/ml has considerably high NPVs, which means that it can exclude patients from benefiting of an IFX dose adjustment with a considerable degree of certainty. Conversely, the PPVs are rather low, which means that having an IFX trough concentration below the defined cut-off does not necessarily imply having clinical activity, endoscopic lesions or a high inflammatory burden. In other words, not all patients with IFX levels below the cut-off will benefit from a dose intensification, and such a decision must be contextualized with other indicators (such as symptomatology, presence of antibodies to IFX and biomarkers).

The 3 μg/ml (or closer) cut-off has been often referred to in the literature,[3,26,27] but so have lower[8,27–29] and higher ones,[7,8,10,27] showing that cut-offs are deeply related to the method used and outcome being assessed, and studies such as these are absolutely necessary to validate thresholds and explore their interpretability. One word of caution should be added: our results were derived from a UC patient cohort, and are therefore only applicable in the context of UC. In fact, the literature shows several instances in which the parallel analysis of CD and UC patients yields different cut-offs or different behaviours of the same cut-off.[8,27,28]

This study has several strengths that should be noticed, namely its prospective design with a systematic and inclusive evaluation of the therapeutic response; and the fact that all quantifications were performed by the same researcher, and therefore the user can be excluded as a source of variation. On the other hand, and as a limitation, one should point out that the sample size was relatively small, although similar to that used in analogous studies;[5,6,8,20,28] and that the occurrence and amount of anti-IFX in the clinical samples was not taken into consideration.

In conclusion, we have explored the applicability of IFX trough level cut-offs using a recently launched rapid QB test and comparing it with a widely used ELISA kit. Overall, both assays have a good quantitative and qualitative agreement, and a cut-off of 3 μg/ml seems to be appropriate, namely when one is assessing the endoscopic status (using an endoscopic Mayo score = 0 as the criterion for remission) or the inflammatory burden. Different cut-offs can be considered for specific situations, and this ultimately depends on whether the user wants to optimize the accuracy or the NPV of the results.

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Author contributions were as follows: FM: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; study supervision; critical revision of the manuscript for important intellectual content. JA: IFX and faecal calprotectin assays; analysis and interpretation of data. JL, KG and FC: histological analysis. CCD: statistical analysis. All the other authors: recruitment of patients and collection of samples. All authors read and approved the final version of the manuscript.

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Conflict of interest statement

FM served as speaker and received honoraria from Merck Sharp & Dohme (NJ, USA), Abbvie (IL, USA), Vifor (Glattbrugg, Switzerland), Falk (USA), Laboratorios Vitoria (Amadora, Portugal), Ferring (Saint-Prex, Switzerland), Hospira (IL, USA) and Biogen (MA, USA).

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