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Proactive therapeutic drug monitoring of infliximab: a comparative study of a new point-of-care quantitative test with two established ELISA assays

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SUMMARY

Background
Therapeutic drug monitoring is a powerful strategy known to improve the clinical outcomes and to optimise the healthcare resources in the treatment of autoimmune diseases. Currently, most of the methods commercially available for the quantification of infliximab (IFX) are ELISA-based, with a turnaround time of approximately 8 h, and delaying the target dosage adjustment to the following infusion.

Aim
To validate the first point-of-care IFX quantification device available in the market – the Quantum Blue Infliximab assay (Buhlmann, Schonenbuch, Switzerland) – by comparing it with two well-established methods.

Methods
The three methods were used to assay the IFX concentration of spiked samples and of the serum of 299 inflammatory bowel diseases (IBD) patients undergoing IFX therapy.

Results
The point-of-care assay had an average IFX recovery of 92%, being the most precise among the tested methods. The Intraclass Correlation Coefficients of the point-of-care IFX assay vs. the two ELISA-based established methods were 0.889 and 0.939. Moreover, the accuracy of the point-of-care IFX compared with each of the two reference methods was 77% and 83%, and the kappa statistics revealed a substantial agreement (0.648 and 0.738).

Conclusions
The Quantum Blue IFX assay can successfully replace the commonly used ELISA-based IFX quantification kits. This point-of-care IFX assay is able to deliver the results within 15 min makes it ideal for an immediate target concentration adjusted dosing. Moreover, it is a user-friendly desktop device that does not require specific laboratory facilities or highly specialised personnel.

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INTRODUCTION

Infliximab (IFX), the first anti-tumour necrosis factor alpha (TNFα) to be approved for the treatment of inflammatory bowel disorders, is a chimeric monoclonal IgG1 composed by a murine variable region (25%) and a constant human region (75%).[1–3] Upon binding to TNF, IFX elicits a number of mechanisms that reduce and control the inflammatory response, including the down-regulation of local and systemic pro-inflammatory cytokines, the induction of T-cell apoptosis, and the reduction in leucocytes and lymphocytes migration towards the inflammatory focus.[2, 3]

However, and in spite of the efficacy demonstrated by IFX in the treatment of several autoimmune inflammatory disorders, certain patients do not respond or have a limited response to IFX therapy. In the specific case of inflammatory bowel disorders (IBD), 10–30% of the patients do not respond to IFX induction (primary non-responders), whereas an annual rate of 13% of the initial responders tend to stop due to loss of response.[4–6] It is nowadays increasingly acknowledged that low trough levels of serum IFX can explain the lack of response to therapy. Although the cut-off levels are still debatable, it has been clearly established in the literature that IFX nonresponders have significantly lower serum trough levels of the drug.[4, 7] Moreover, several published studies demonstrate a clear positive correlation between IFX serum levels and rates of endoscopic improvement and remission, whereas undetectable IFX levels place patients at an increased risk of colectomy.[8–11] Overall, IFX serum trough levels between 3 and 7 μg/mL are commonly accepted by physicians and researchers as being the optimal therapeutic window during the maintenance phase.[6, 12–14]

The interindividual variation in IFX serum levels in IBD patients is known to be the result of multiple factors: whereas the immunogenicity of the drug and the consequent formation of antibodies to IFX is widely studied, other factors, such as body mass index, albumin serum concentration, gender, smoking and disease activity/duration are known to impact the pharmacokinetics and pharmacodynamics of IFX, therefore, having a role in the drug availability on the patients' serum.[4, 15, 16] Despite the underlying reasons for this variability, monitoring of serum IFX concentrations and of the formation of anti-drug antibodies during therapy (Therapeutic Drug Monitoring, TDM) is a powerful tool to aid physicians in the therapeutic decision-making process in the case of loss or of suboptimal response.[12–14, 17] Moreover, TDM may also support IFX de-escalation in case of supratherapeutic serum concentrations, enhancing the cost-effectiveness of the therapeutic process and avoiding unnecessary side effects.[12–14, 18]

As TDM relies on an efficient and accurate quantification of serum IFX levels and anti-drug antibodies, several methods have been developed, validated and made commercially available for use in hospitals and reference laboratories. Some of them are able to measure both IFX and antibodies to IFX, whereas others are specific for one of these quantifications. Most of the available methods rely on an Enzyme-Linked Immunosorbent Assay (ELISA) technique, whereas alternative ones, based on, for instance, immunoaffinity magnetic purification coupled with high-temperature reversed-phase liquid chromatography, fluid-phase radioimmunoassays (RIA), reporter gene assays and liquid chromatography linked with mass spectrometry are becoming increasingly acknowledged.[19–25]

This study aimed to evaluate the performance of the first point-of-care IFX-quantification assay available in the market by comparing it to two established ELISA methods, using spiked samples and a large and wide-ranged set of clinical samples.

MATERIAL AND METHODS

Samples and patients

Spiked samples of known IFX concentrations (0.5, 1, 1.5, 3, 5, 7, 10, 15, 20, 30 and 40 μg/mL) were generated by diluting the appropriate amount of exogenous IFX (Schering Plough, New Jersey, USA) into a pool of serum from control donors (after signing consent forms). Serum samples for IFX quantification were prospectively and consecutively obtained from a multicentric cohort of IBD out-patients in the maintenance phase of IFX therapy during routine consultations and immediately before the IFX infusion. The Ethics Committees of all involved institutions approved this study, and all patients were required to sign a written informed consent prior to their participation. Blood samples were collected, centrifuged and serum samples were kept at −80 °C until being processed.

IFX-quantification assays

The assay being tested was the Quantum Blue® Infliximab: Quantitative Lateral Flow Assay (Buhlmann, Schönenbuch, Switzerland) – hereafter referred to as point-of-care QB – and the sample quantification was carried out strictly respecting the manufacturers' instructions in an open-label fashion. A chip card, provided with each

test kit, supplied the point-of-care QB reader with the test information and calibration curve for each specific cartridge lot. Briefly, serum samples were diluted 1:20 and a 70 μL aliquot was loaded into the port of the test cartridge. After a 15 min reaction, the cartridge was read and the results were shown on the point-of-care QB reader display. According to the manufacturer, this kit has the following analytical characteristics: the limit of detection is 0.15 μg/mL, and the lower and upper limits of quantification are 0.4 μg/mL and 20 μg/mL respectively.

The point-of-care QB was compared with two well-established ELISA-based IFX-quantification tests: the Level Infliximab M2920 kit (Sanquin, Amsterdam, the Netherlands) – hereafter referred to as Sanquin – and an 'in house' validated procedure. The Sanquin was performed according to the manufacturers' instructions, whereas the 'in house' procedure has been carried out as described elsewhere.[26–29] Briefly, regarding the 'in house' procedure, serum samples were diluted (1:100) and added to a plate pre-coated with TNFα (Peprotech, Rocky Hill, NJ, USA). After 60 min of incubation and an appropriate number of washes, a horseradish peroxidase (HRP)-labelled goat anti-human Fc fragment antibody (MP Biomedicals, Solon, OH, USA) was added and the plate was incubated for 60 min. Afterwards, tetramethylbenzidine (Millipore, MA, USA) substrate was added, and the reaction was stopped 3 min later with 2M H_2SO_4. Finally, the samples' absorbance was read at 450/540 nm, and the IFX was quantified by interpolating the absorbance values in a standard curve built with known concentrations of exogenous IFX (Schering Plough, NJ, USA). According to the manufacturer, the Sanquin kit has the following analytical characteristics: the lower and upper limits of quantification are 0.08 μg/mL (1:200) and 47 μg/mL (1:2000) respectively. For the 'in house' procedure, the upper limit of quantification was calculated as the highest concentration of the standard curve × the sample dilution factor used.

Statistical analyses

Continuous variables were described using the median and interquartile range (IQR) or the average and the standard deviation (when appropriate). The level of statistical significance was set at 0.05. For statistical purposes, the results of all patients' samples for which the concentration measured by the point-of-care QB was below or above the limits of quantification (0.4 μg/mL and 20 μg/mL, respectively) were considered to be those same limits. The accuracy and kappa statistics were computed based on a pre-established therapeutic window of IFX. Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA) and GraphPad Prism version 6.0 for Windows (GraphPad Software, La Jolla California USA) were used for the statistical analysis and plots' design.

RESULTS

The point-of-care QB assay and the two reference methods were used to measure the IFX concentrations in known IFX-spiked samples to compare their intravariability and recovery rates (Figure 1). The point-of-care QB assay had the smaller intravariability and the better (i.e. closer to real concentrations) recovery rates. In fact, the average recovery rate of the point-of-care QB assay was 92% (ranging from 68% to 108%), compared to 151% (ranging from 57% to 225%) and 114% (ranging from 79% to 160%) for the 'in house' and Sanquin methods respectively.

Moreover, IFX was quantified in a total of 299 serum samples using the three different tested assays. These samples were collected from the same number of IBD patients, a multicentric cohort with a median (IQR) age of 34 (24–45) years and composed of 50% males. All these patients were in the maintenance phase of IFX therapy. The correlation between the IFX levels obtained with each of the three assays is shown in Figure 2. All correlations are significant, with the highest correlation coefficient being found for the Sanquin and the point-of-care QB assay (0.952). Considering the correction made for samples which results were above the limit of detection with the point-of-care QB, these samples were

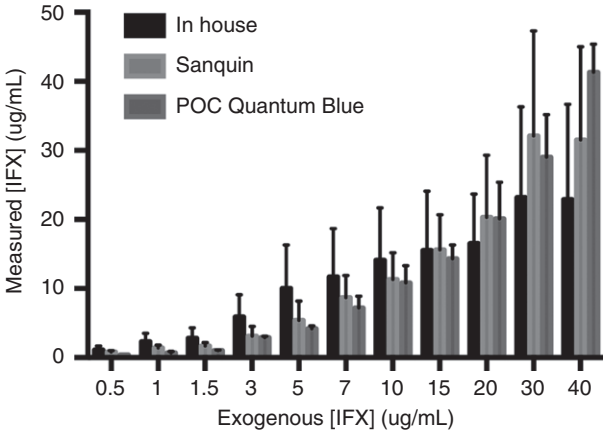

Figure 1 | IFX quantification of exogenously spiked IFX samples of known concentrations. The bars indicate the mean concentration obtained with each assay in the different concentrations and the error bars refer to the standard deviation.

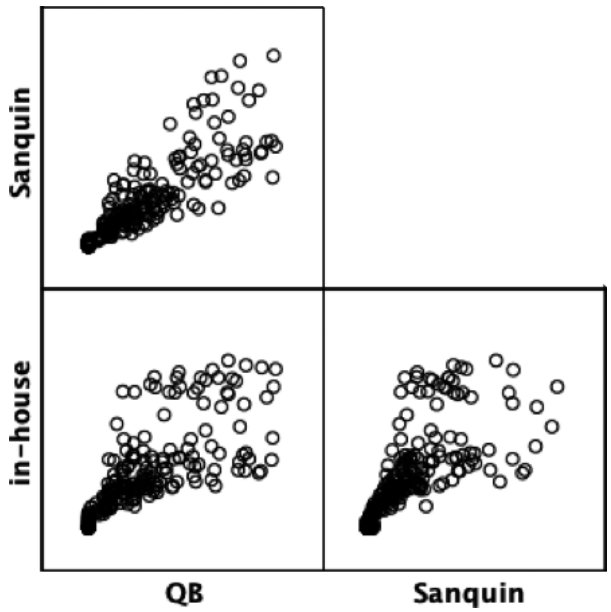

Figure 2 | Dispersion matrix of the IFX quantification in the 299 patients' samples considering the three used methods (μg/mL). The Spearman correlation coefficients are the following: 0.919 for the 'in-house' method vs. the Sanquin kit ($P < 0.001$); 0.913 for the 'in-house' method vs. the point-of-care Quantum Blue kit ($P < 0.001$); and 0.952 for the Sanquin kit vs. the point-of-care QB kit ($P < 0.001$).

excluded and a new correlation analysis was made (Table S1). The pattern of correlations and their significance are similar to those using the entire set of samples.

Quantitative analysis

The Intraclass Correlation Coefficients (ICC) for the paired-comparisons of the IFX-quantification assays used in this study are listed in Table 1. The highest ICC was found when comparing the Sanquin and point-of-care QB assays (0.939). Interestingly, the highest mean difference was also found between these two assays (0.92), and the 95% IC did not include 0, showing that the concentrations measured by the Sanquin kit were consistently higher than those measured by the point-of-care QB. Moreover, these analyses were repeated excluding all samples that had a result higher than 20 μg/mL with the point-of-care QB kit, and the results were similar (Table S2). The only difference was the highest mean difference being found for the 'in house'-QB comparison, and its 95% CI also excluding 0.

The Bland–Altman plots show a greater dispersion in the comparison of the 'in house' with the point-of-care QB assay (Figure 3). It should be noticed that this

Table 1 | Intraclass correlation coefficients (ICC) and differences found upon comparing the different IFX-quantification assays

	ICC		Differences	
	ICC	95% CI	Average	95% CI
In house point-of-care QB	0.889	0.861–0.911	0.37	−0.07–0.81
Sanquin point-of-care QB	0.939	0.924–0.952	0.92	0.54–1.30

dispersion is mainly localised in the 10–15 μg/mL range of concentrations, whereas the dispersion in the comparison of the Sanquin with the point-of-care QB assay seems to increase gradually with the increase in the measured concentrations. These analyses were repeated excluding all samples that had a result higher than 20 μg/mL with the point-of-care QB kit and the results were similar (Figure S1).

Qualitative analysis

For the qualitative analyses, the results for each method were stratified according to a commonly accepted IFX therapeutic window – lower than 3 μg/mL, 3–7 μg/mL and higher than 7 μg/mL. The agreement, that is, the percentage of patients that were classified in the same group using the point-of-care QB and one of the reference assays – was then analysed (Table 2). The accuracies between the point-of-care QB and the 'in house' and Sanquin assays were high (77% and 83% respectively). The kappa statistic revealed that the point-of-care QB assay had a substantial agreement with both reference assays, according to the kappa's level of agreement published by Landis and Koch.[30]

DISCUSSION

This study aimed to evaluate the performance of a new point-of-care assay to determine serum IFX concentrations that has been recently launched in the market. To do so, this new test was used to quantify IFX in spiked samples and in the serum of 299 IBD patients under IFX maintenance therapy, and the results were compared with those obtained using two well-established ELISA methods (the Sanquin and an 'in house' assay). The results were very promising, as the new point-of-care QB assay revealed an excellent performance when measuring the spiked samples with known IFX concentrations. In fact, the point-of-care QB assay performed better than

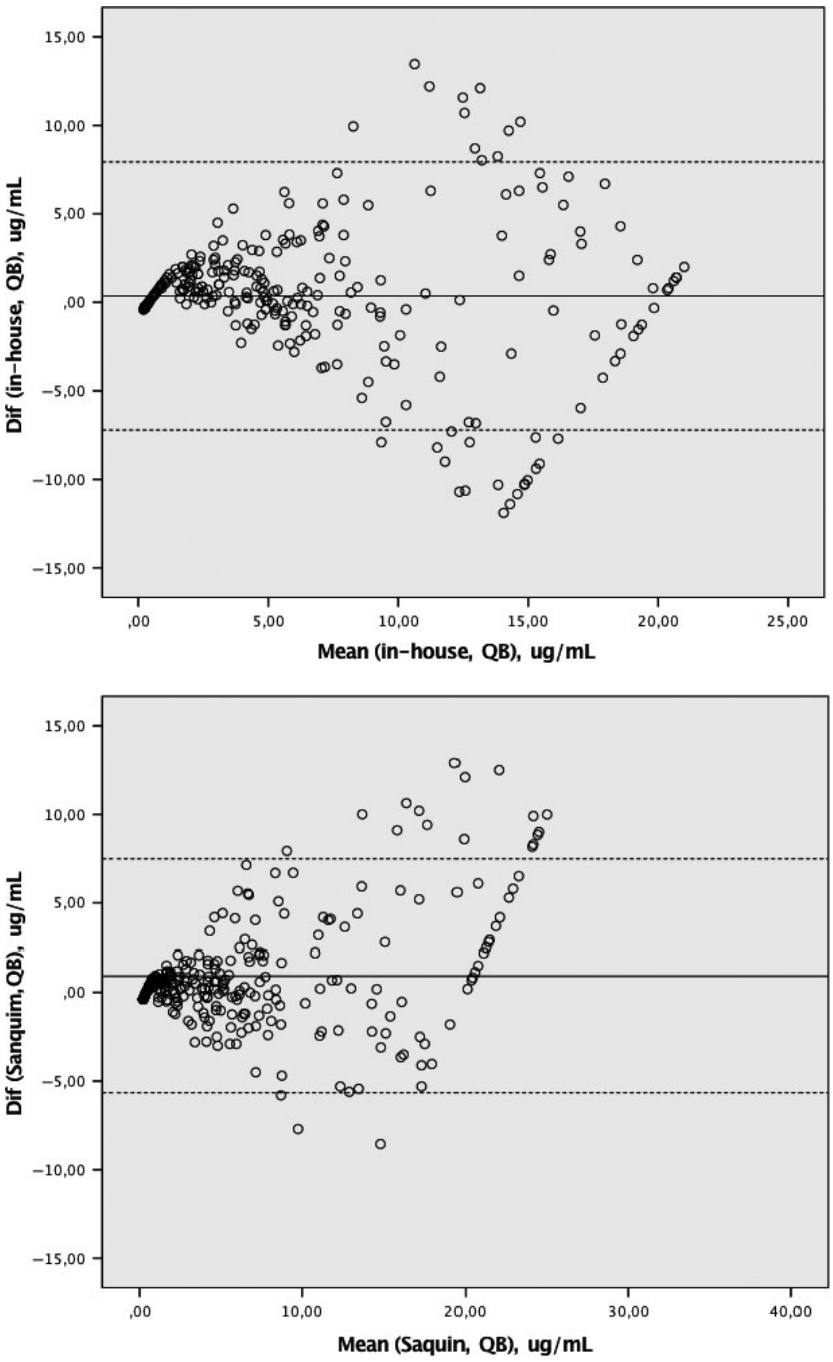

Figure 3 | Bland–Altman plots comparing the point-of-care Quantum Blue with the *in house* and Sanquin assays.

Table 2 | Qualitative comparison between the point-of-care Quantum Blue and the reference assays

	QB < 3 μg/mL *n* (%)	3 μg/mL < QB > 7 μg/mL *n* (%)	QB ≥ 7 μg/mL *n* (%)	Accuracy	Kappa (95% CI)
In house <3 μg/mL	100 (75.8)	1 (1.4)	0 (0.0)	77%	0.648 (0.577–0.719)
3 μg/mL ≤ in house > 7 μg/mL	31 (23.5)	48 (66.7)	14 (14.7)		
In house ≥ 7 μg/mL	1 (0.8)	23 (31.9)	81 (85.3)		
Sanquin < 3 μg/mL	119 (90.2)	4 (5.6)	0 (0.0)	83%	0.738 (0.673–0.803)
3 μg/mL ≤ sanquin > 7 μg/mL	12 (9.1)	48 (66.7)	14 (14.7)		
Sanquin ≥ 7 μg/mL	1 (0.8)	20 (27.8)	81 (85.3)		

the two reference methods both in terms of intravariability and recovery rates. Regarding the quantitative analysis of the results obtained from the patients' serum, both reference methods had a significant correlation with the point-of-care QB, and the ICCs were consistently high (0.889 and 0.939). Interestingly, although the Sanquin assay was the closest to the point-of-care QB, it also had a bias of 0.92, revealing that the former kit measures consistently higher values than the latter. This result finds support at the concentrations obtained from the spiked samples, in which in all but the 40 μg/mL sample higher values were obtained when using Sanquin instead of point-of-care QB. Moreover, this IFX overestimation of the Sanquin assay has been noticed elsewhere, where the average percentage of IFX recovery for this kit was shown to be 139%.[21] The differences found upon comparing the different methods, as well as those between measured and nominal values, are noteworthy. However, one should keep in mind that these differences are inherent to the nature of IFX itself and that of the ELISA method. In fact, IFX is a monoclonal antibody, and therefore, affinity variation is an issue in ELISA assays.[31] Moreover, being a solid-phase capture assay, ELISA techniques may present some problems in the anti-TNF-antibody ligation. Steenholdt *et al.* have recently published a revision in which the technical biases of measuring IFX are well described and documented.[32]

Interestingly, the Bland–Altman plots of the patient's measurements locate the differences between the point-of-care QB and the other two assays in different ranges of concentrations. In fact, and concerning the 'in house' assay, most differences that fall out of the 95% CI are located in the 10–15 μg/mL range of concentrations, whereas concerning the Sanquin assay the differences increase as the IFX concentrations raise.

The qualitative analysis of the results is more important in this context, as the placement of a patient within a certain range of IFX concentrations will be reflected in the clinical decisions made if TDM is applied. In this regard, the samples were stratified according to one possible IFX therapeutic window for the maintenance phase of IFX treatment: lower than 3 μg/mL, within the range of 3–7 μg/mL, and higher than 7 μg/mL. The groups formed by the point-of-care QB and each of the reference assays based on this particular criteria were rather similar: indeed, the kappa statistics indicate a substantial agreement between them. From a clinical point of view, the quantification of IFX coupled with the assessment of the anti-drug antibodies is a powerful tool to guide the physicians' therapeutic decisions: using this particular IFX therapeutic window, a patient with IFX levels lower than 3 μg/mL should escalate the dosage (either increasing the amount of IFX in the infusions or decreasing the interval between them) or switch to a different intra- or interclass drug – depending on the presence and concentration of antibodies to IFX; a patient with IFX levels within the range of 3–7 μg/mL does not need dosage adaptation; and a patient with IFX levels higher than 7 μg/mL can safely de-escalate the dosage.[6, 12–14] According to our results, the clinical decision based on the IFX quantification only (but prone to further adjustments should the concentration of antibodies to IFX be known) would be of a similar nature in 77% of the patients – using the point-of-care QB instead of the 'in house' assay – or in 83% of the patients – using the point-of-care QB instead of the Sanquin assay. Conversely, 24% and 10% of the patients considered to have an IFX concentration below the optimal therapeutic window when evaluated by the point-of-care QB were actually within or above this window when their serum was assayed with the 'in house' or Sanquin methods respectively. Moreover, approximately 33% of the patients considered within the therapeutic window by the point-of-care QB assay were actually above or below it according to the tests used as reference, whereas 15% of the patients placed by the QB point-of-care test above the therapeutic window were considered to be within that window when assayed by the Sanquin and 'in house' methods. Although these percentages may seem noteworthy, it is important to highlight that the decisions made following a TDM approach must be integrated in the patients' clinical context. As so, it is likely that considering the presence of symptomatology and other disease markers would dilute these differences, particularly in the cases where patients are placed in different groups using two different assays, but the measured IFX concentrations are actually borderline the therapeutic window. Moreover, the 3–7 μg/mL therapeutic window used in our study is a commonly used reference, but different thresholds have been established for different methods by different authors. Our results are only indicative of an overall qualitative agreement during IFX maintenance therapy, but the specific percentages would obviously vary should a different therapeutic interval be used. Further studies are needed to integrate the clinical status and disease progression of the patients with the performance of the point-of-care QB assay in a TDM context, as well as to define specific IFX cut-offs that should be applied with this method in the different therapeutic phases.

The clinical advantage of using a point-of-care assay for the monitoring of IFX (and other variables) in the IBD patients is rather evident: the turnaround time of the point-of-care QB assay is 15 min, compared to the approximately 8 hours taken by a common ELISA-based kit. This allows the physician to optimise the IFX treatment immediately when using the point-of-care QB, as opposed to optimise the treatment in the following infusion (usually 6–8 weeks later) when using one of the commercially available ELISA-based kits. The benefits of optimising the IFX therapy in IBD and other inflammatory diseases' treatment are amply acknowledged in the literature. In fact, adjusting the IFX doses (either escalating or de-escalating) and the infusion intervals has been proved to be a clinically powerful tool and a cost-effective strategy.[12–14, 16–18, 33] For those reasons, a serious effort of the medical and research community has been recently applied to the development of novel point-of-care assays concerning the IBD patients monitoring.[34–37] However, most of these tests are focused on disease activity. To the best of our knowledge, there are only a couple of pilot studies describing other lateral flow based assays for the on-site monitoring of serum IFX levels.[38, 39] The Quantum Blue® Infliximab: Quantitative Lateral Flow Assay from Buhlmann assay is the first test already developed and launched in the market for this purpose, and our analysis is the first extensive evaluation of its performance by comparing it with other two already established methods.

Another important advantage of this point-of-care QB assay is its user friendliness: this desktop device may be operated by any nurse, physician or researcher, as opposed to the current commercially available ELISA-based kits and other IFX-quantification methods, which require highly trained personnel and specific laboratory facilities. Moreover, the ELISA-based assays should be run in batches for the sake of cost-effectiveness, which, depending on the dimension of the IBD population being followed in each care centre, may represent a further delay. The point-of-care QB test, on the other hand, is designed to be individually used for each patient sample. As a limitation, whereas ELISA-based methods are usually able to measure antibodies to IFX in addition to IFX itself, the point-of-care QB assay, for the time being, measures only the IFX concentration.

As the main aim of this study was to assess whether the point-of-care QB could effortlessly replace the time-consuming and difficult to apply ELISA-based kits, we focused our efforts in the comparison between the different assays, and we have not taken into consideration the analytical performance characteristics of the point-of-care QB kit. These have, however, been thoroughly analysed by the manufacturers, have been reported elsewhere,[40] and are clearly indicated in the instructions booklet that accompanies the kit. In this respect, it is worth noticing that the point-of-care QB limits of quantification are 0.4–20 μg/mL. The lower and upper limits of detection may hold a disadvantage when compared to the ELISA-based kits or other IFX-quantification methods. Although the point-of-care QB assay was shown to perform rather well in concentrations above its upper limit after adjusting the dilution factor (Figure 1), we were unable to do so in the patients' samples and we have chosen to strictly respect the indications available in the kit's booklet. As so, and for statistical purposes, we have considered all patients' samples that were below the limit of detection to have an IFX concentration of 0.4 μg/mL, and all patients' samples that were above the limit of detection to have an IFX concentration of 20 μg/mL. This strategy had an obvious impact in the quantitative analyses of the patients' samples, although the overall results remained very satisfactory. Moreover, the quantitative analyses were repeated excluding all values that had a result higher than 20 μg/mL with the point-of-care QB assay, and the results were shown to be similar (Tables S1, S2 and Figure S1). Finally, and as the limits of detection are rather far apart the IFX therapeutic window (3–7 μg/mL), they should have no clinical impact in the physician assessment and decision-making process.

This study involved spiked samples and a large number of serum samples, which were obtained from a multicentric and heterogeneous cohort and are therefore a faithful representation of the reality. Notwithstanding, there were a couple of limitations that should be noticed and taken into account: the IFX quantification was always made by the same researcher, which hampers the assessment of the interassay variability; moreover, the presence of antibodies to IFX and other compounds that may differentially interfere with the IFX quantification in each kit was unaccounted for.

On the basis of this study, we may conclude that the point-of-care Quantum Blue is a reliable alternative to the time-consuming ELISAs, allowing the fast and accurate assessment of IFX levels, which in turn contributes towards a proactive and cost-effective therapeutic managing of IBD patients. In the era of the personalised medicine, the fast implementation of tailored therapeutic solutions is important for the patients' quality of life and the healthcare resources optimisation.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bland–Altman plots excluding samples >20 μg/mL according to the point-of-care QB assay.

Table S1. Spearman correlation coefficients excluding samples >20 μg/mL according to the point-of-care QB assay.

Table S2. Intraclass Correlation Coefficients and differences found upon comparing the different IFX-quantification assays excluding samples >20 μg/mL (according to the point-of-care QB assay).

AUTHORSHIP

Guarantor of the article: Fernando Magro.

Author contributions: J Afonso: study concept and design; acquisition,interpretation, analyses of data and drafting of the manuscript. CC Dias: Statistical analyses. F Magro: Study concept and design; acquisition, interpretation and analyses of data; drafting of the manuscript; study supervision and critical revision of the manuscript for important intellectual content. All the other authors: patients' enrolment and samples collection.

All authors approved the final version of the manuscript.

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