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Short Communication

Comparison of the Quantum Blue® reader Point-of-Care system versus ELISA technique for therapeutic drug monitoring of Infliximab levels

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ABSTRACT

Background: Infliximab (IFX) is a monoclonal antibody used to treat patients with inflammatory bowel disease (IBD). For IFX therapeutic drug monitoring (TDM), the most commonly used analysis is enzyme-linked immunosorbent assays (ELISA) which do not allow results to be provided in real-time. The aim of this study was to compare the in-house ELISA (Promonitor IFX) with the much faster assay Quantum Blue® IFX (QB) for quantification of serum IFX concentration among IBD patients in maintenance IFX therapy.

Methods: We studied 30 serum samples from outpatients in IFX maintenance therapy at Copenhagen University Hospital Hvidovre, Denmark. Samples were used to compare IFX measurements from Promonitor IFX with QB. Therapeutic intervals of < 3 μg/mL, 3–7 μg/mL and > 7 μg/mL were equally covered. Differences were evaluated using Bland-Altman plots and Student *t*-test. Correlation was evaluated using x,y-plot and Pearson's correlation coefficient. The intermediate imprecision (CV%) of QB was measured at two levels (3 μg/mL and 7 μg/mL). For qualitative comparison, weighted kappa statistics (κ) were determined after stratification of results by therapeutic interval.

Results: Promonitor IFX and QB were strongly correlated ($r = 0.92$, $p < 0.001$). The mean difference between Promonitor IFX and QB was −0.57 μg/mL ($p = 0.2$). The CV% of QB was 16.3% at 3 μg/mL and 16.7% at 7 μg/mL. Classification of results according to therapeutic interval showed almost perfect agreement ($\kappa = 0.81$).

Conclusions: QB is a suitable alternative to Promonitor IFX for TDM in patients treated with IFX for IBD. The results revealed a strong correlation between methods, in particular at lower IFX concentrations, representing the most interesting clinical range. When the samples were stratified according to the therapeutic interval, an almost perfect agreement between the methods was observed.

1. Introduction

Twenty years ago, the launching of Infliximab (IFX) changed the therapeutic strategies for a broad range of immune mediated inflammatory diseases [1]. IFX is a monoclonal antibody, targeting tumour necrosis factor (TNF)-α. IFX has proven highly effective in chronic inflammatory disease such as inflammatory bowel disease (IBD) [2]. Despite its demonstrated effect in the treatment of IBD patients, some will not respond to the drug or loose response over time [3,4]. Low IFX levels and presence of IFX antibodies represent the most frequent causes of treatment failure in IBD patients, while adequate drug levels have been demonstrated to be associated with persistent remission [5–8]. The optimal therapeutic window between in IBD patients in maintenance therapy is 3 and 7 μg/mL [9,10]. Given the importance of therapeutic drug monitoring (TDM) in IBD patients, precise and accurate measurements of IFX levels are warranted.

The enzyme-linked immunosorbent assays (ELISA) represent the most commonly used assays for determining IFX TDM in clinical practice [11]. Nevertheless, the ELISA assay requires approximately 4–8 h to be performed. This limits the effectiveness of TDM in clinical practice, delaying the IFX dose adjustment to the following infusion, usually 6–8 weeks later. Point-of-care (POC) tests have been made available, reducing turnaround time to 15–20 min.

This study aimed to compare the POC test Quantum Blue® IFX (Bühlmann) for quantification of IFX with the in-house ELISA assay (Promonitor IFX). Prior studies have demonstrated good agreement

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between POC IFX tests and ELISA assays [12–16]. This study contributes to prior evidence with a comparison of ELISA (Promonitor IFX) with QB among IBD patients receiving IFX maintenance therapy.

2. Material and methods

Venous blood samples were collected into clot activator tubes (Vacuette®, Greiner Bio One International, Germany) from consecutive IBD outpatients in IFX maintenance therapy at Copenhagen University Hospital Hvidovre, Denmark. Samples was centrifuged at 3580 *g* for 4 min within 0.5 to 2 h after sample draw and stored at −20 °C. Serum samples were analyzed using the in-house ELISA kit (Promonitor IFX – Orion Diagnostica, Denmark), hereafter referred to as Promonitor IFX, and stored at −20 °C until further analyse (within of a maximum period of three month). The POC device being tested was the Quantum Blue ® IFX (Bühlmann Laboratories, Schönenbuch, Switzerland), hereafter referred to as QB.

Of all samples, 30 were included in the comparison of IFX measurements between Promonitor IFX and QB. Samples were consecutively chosen to cover the therapeutic interval [9,10]; 10 samples < 3 μg/mL, 10 samples within 3–7 μg/mL and 10 samples > 7 μg/mL. No ethnical permission was needed for the study, as the purpose was quality assurance, and no further patient samples was requested.

Prior to analysis on the QB, serum was diluted 1:20 with Chase Buffer (Bühlmann Laboratories, Schönenbuch, Switzerland) in a test tube. This suspension was mixed and loaded into the port of the test cartridge according to the manufacture's instruction. After 15 min, the result is visualized on a display.

Quantitative comparison of QB and Promonitor IFX was assessed with Bland-Altman plots and Student *t*-test. A p-value of < 0.05 was considered statistically significant. The 95% limits of agreement were calculated using $\bar{x} \pm 1.96$*SD. Correlation was evaluated using x,y plot and Pearson's correlation coefficient (r). For qualitative comparison, weighted kappa statistics (κ) were determined after stratification of results by therapeutic interval (< 3 μg/mL, 3–7 μg/mL and > 7 μg/mL) [9,10]. The intermediate imprecision (CV%) of QB was investigated at two levels (3 μg/mL and 7 μg/mL). A high control suspension from Bühlmann Laboratories were used to assess the CV% at the high limit. As no low control was available from Bühlmann Laboratories, a patient sample with IFX concentration of 3 μg/mL was considered suitable as the low control and used to evaluate the CV% at the low level (3 μg/mL). Statistical analyses were performed using STATA/SE 15.1.

3. Results

The overall median concentration of IFX was 4.3 μg/mL (measuring range 0.9–14.6 μg/mL) using Promonitor IFX and 4.25 μg/mL (measuring range 0.5–20.0 μg/mL) using QB. The QB showed high correlation with Promonitor IFX (r = 0.92, p < 0.001) (Fig. 1).

The mean difference between Promonitor IFX and QB was −0.57 μg/mL (p = 0.20). The Bland-Altman plot (Fig. 2) showed that the differences was close to zero at IFX concentrations within the therapeutic interval, but generally increased with IFX concentration above 7 μg/mL. Two of the paired samples with concentrations above 7 μg/mL had differences outside the 95% limits of agreement. The CV% for QB for low control was 16.3% and 16.7% for the high control.

Dashed lines represent the lower and upper 95% limits of agreement, and mean difference of Infliximab (IFX) μg/mL.

When stratifying by therapeutic interval, the kappa statistic revealed that QB had an almost perfect agreement with Promonitor IFX (κ-value of 0.81) (Table 1), according to the kappa's level of agreement published by Landis and Koch [17]. Overall, QB was in agreement with results from Promonitor IFX in 25 cases (83.3%), For the 10 samples with IFX < 3 μg/mL, the agreement with the Promonitor IFX was 9/10 (90.0%), for the 10 samples with IFX 3–7 μg/mL, the agreement was 7/10 (70.0%), and for the 10 samples > 7 μg/mL, the agreement was 9/10 (90.0%). However, discordance with influence on treatment strategy was revealed in five cases.

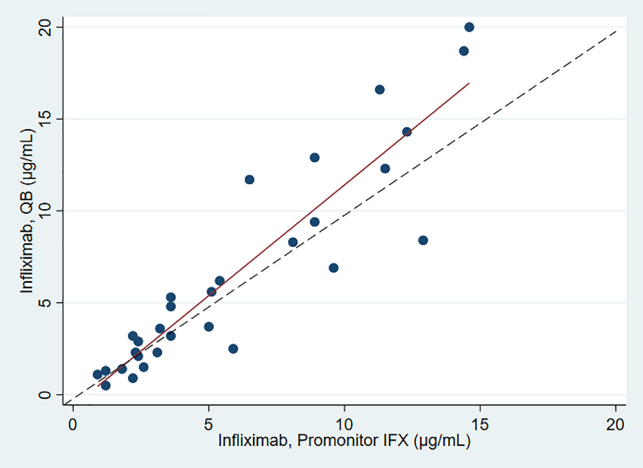

Fig. 1. Association of Infliximab (IFX) concentrations measured on Promonitor IFX and QB.

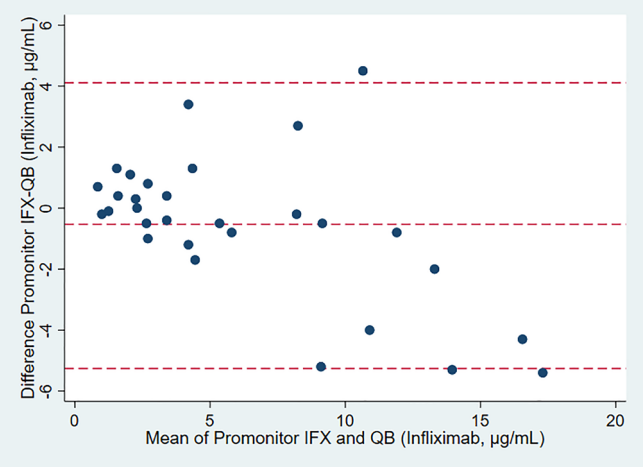

Fig. 2. Bland-Altman plot of difference versus mean, comparing Infliximab concentrations between Promonitor IFX and QB.

Table 1
Infliximab (IFX) measured with Promonitor IFX and QB stratified by therapeutic window (< 3 μg /mL, 3–7 μg /mL and > 7 μg /mL).

	Promonitor IFX			
QB	< 3 μg/mL	3–7 μg/mL	> 7 μg/mL	Total
< 3 μg/mL	9	2	0	11
3–7 μg/mL	1	7	1	9
> 7 μg/mL	0	1	9	10
Total	10	10	10	30
κ-value	0.81			

4. Discussion

This study revealed a strong correlation between IFX concentrations measured by the Promonitor IFX and QB, in particular at lower concentrations, which represent the most interesting clinical testing range. When the samples were stratified according to the therapeutic interval, an almost perfect agreement between methods was observed.

Our results of a good correlation between ELISA assays and POC are in line with results from prior studies [12–16]. We observed that on

average QB measured 0.57 μg/mL higher IFX levels than Promonitor IFX, but that the higher QB levels were particularly observed above the therapeutic level. In contrast, two prior studies [12,15] found, that QB generally showed lower IFX levels compared to two different ELISA assays, of which one was the ELISA (Sanquin). IFX overestimation of the Sanquin kit compared to other ELISA kits have been noticed elsewhere [16,18].

Clinical agreement between the methods is essential, as the placement of a patient within a certain IFX interval will be reflected in the treatment. In this study, the clinical decision based on IFX concentrations only, was similar in 83% of the patients using QB instead of Promonitor IFX. The decision made following a TDM approach should be integrated in the patient's clinical context [16].

Our study contributes to the existing evidence on IFX TDM with a comparison of Promonitor IFX with QB. Despite prior studies showing that IFX quantification assays compare well against each other [11], results may be influenced by the use of different antibodies in different ELISA assays with diverse affinities for IFX [19].

The QB demonstrated acceptable analytical impression, with higher values in comparison to Promonitor IFX, which was estimated to 4% within the study period (data not shown). However, both assays satisfied the manufactures quality objectives. From this point of view, the Promonitor IFX is more precise for application of follow-up of patients. However, POC assays are advantageous with a 15–20 min turnaround time and IFX quantification on a single sample. In comparison, answers are not quickly available from the ELISA assays, and for cost-effectiveness single samples are not run, only as a batch. Based on this, POC assays open up for an increase of effectiveness/efficacy of TDM in clinical practice, as the test result will be available almost immediately. This allows instant modification of the patient's drug dose. However, with POC assays, there comes also disadvantages, including the lack of internal and external quality control results, and the greater difficulty in tracking errors. Further, IFX POC assays requires serum instead of total blood, which adds centrifugation to the process, thus hindering the use by clinicians directly on site.

A limitation to this study was the relatively small sample size, and that the presence of antibodies to IFX that may interfere with the IFX quantification was unaccounted for. However, in a consecutive in-house analysis of more than 200 outpatients less than 1% were tested positive for anti-IFX (data not shown). Therefore, we do not expect occurrence of anti-IFX to interfere with our data.

In conclusion, our data demonstrate that QB is a suitable alternative to Promonitor IFX for TDM in patients treated with IFX for IBD.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.10.010.

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