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Comparison of a new rapid method for the determination of adalimumab serum levels with two established ELISA kits

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Abstract

Background: Therapeutic drug monitoring (TDM) of adalimumab (ADA) in inflammatory bowel diseases (IBDs) has gained increased attention since several studies showed a correlation between drug levels and mucosal healing. The limitations of routine usage of enzyme-linked immunoabsorbent assay (ELISA) kits for measuring serum ADA concentrations have prompted the development of rapid methods, such as Quantum Blue (QB). We evaluated the interchangeability and agreement between the QB method and two established ELISA kits, Promonitor (PM) and Lisa-Tracker (LT).
Methods: Fifty samples from patients with IBD were included. Quantitative analysis was performed using the ANOVA test for repeated measures, Deming regression and the Bland-Altman plot. Clinical implications were evaluated by concordance in classifying patients into therapeutic windows according to the proposed cut-off levels for subtherapeutic (either <5 or <7.5 μg/mL) and supratherapeutic (>12 μg/mL) ranges.
Results: Statistical differences were detected between the QB method and the two ELISA kits, with QB overestimating ADA serum values compared to them. A lack of interchangeability was observed between methods, with greater differences as ADA levels increased. An analysis of a sub-set of samples with ADA values below 9 μg/mL (n = 25) showed that QB fulfilled the criteria to be interchangeable with the LT assay. Concordance for patient classification into ADA therapeutic windows was better for QB vs. LT than for QB vs. PM, with high agreement (>75%) for subtherapeutic levels among the three methods.
Conclusions: Although quantitative differences existed between the rapid method and ELISA kits that hampered their interchangeability, the agreement for identifying patients with subtherapeutic values of ADA was high.

Keywords: adalimumab; drug monitoring; ELISA; inflammatory bowel diseases; point-of-care testing.

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Introduction

The treatment of the more severe presentations of inflammatory bowel diseases (IBDs) changed remarkably with the introduction of anti-tumor necrosis factor (TNF)-α therapy [1]. The employment of anti-TNFα biological agents has proven efficacy for the induction and maintenance of remission in patients with IBDs [2]. Among those agents, infliximab (IFX) and adalimumab (ADA) are the most commonly used drugs for IBD treatment. IFX, a chimeric mouse/human immunoglobulin G1 monoclonal antibody, was approved to treat moderate to severe IBD prior to ADA, a fully human monoclonal antibody [3].

Unfortunately, loss of response to anti-TNFα therapy occurs during treatment due to a low concentration of the

agent or the development of antibodies against the drug [4]. In the first instance, response could be regained by dose escalation, while the presence of antidrug antibodies necessitates a change of treatment to other anti-TNFα agents or biological drugs (such as vedolizumab or ustikenumab).

Therapeutic drug monitoring (TDM) emerged as a tool to optimize anti-TNFα therapy, as loss of clinical response to anti-TNFα treatment leads to disease relapse and increased health costs. An association between serum levels of anti-TNFα agents and the level of mucosal healing has been described [5]. Recent recommendations on TDM of anti-TNFα drugs have been proposed by outlining the risks and benefits of this approach in IBD management [6, 7].

In the particular case of ADA treatment in IBD, several studies have shown that high ADA serum levels correlated with clinical response and mucosal healing, while the presence of anti-ADA antibodies were positively associated with disease activity [8–10]. Although the cut-off value to predict therapeutic response to ADA varied among studies, the most accepted lower limits are 5 and 7.5 μg/mL, so serum levels above these cut-off points would indicate a high probability of sustained response to ADA [11–13]. Regarding the upper limit, a plateau effect in the relationship between ADA serum levels and mucosal healing was observed above 12 μg/mL [5].

Several methodologies have been developed to measure serum concentrations of anti-TNFα drugs, including different types of enzyme-linked immunoabsorbent assays (ELISA), radioimmunoassay, homogeneous mobility shift assay based on liquid chromatography, reporter gene assay and liquid chromatography (LC) coupled with mass spectrometry (MS) [14]. Among them, the kits based on ELISA are the most widely used in clinical laboratories, with the sandwich ELISA being the most used in the determination of ADA serum levels [15]. In addition, point-of-care tests (POCT) based on lateral flow immunochromatography are commercially available (since 2015 for IFX and since early 2018 for ADA) with the aim of overcoming ELISA limitations.

While several studies have already compared POCT devices for IFX determination with ELISA kits [16–18], the recently developed rapid methods for ADA measurement have been scarcely evaluated. In the present study, we have carried out a comparison of a new rapid kit for the measurement of ADA serum levels (Quantum Blue) with two ELISA kits (Promonitor and Lisa-Tracker) in samples from patients with IBDs under ADA therapy. To the best of our knowledge, this is the first study published as a full research paper comparing the performance of Quantum Blue rapid method for ADA determination with commercially available ELISA kits. The aim of our study was to assess the interchangeability of these methods and to evaluate the agreement among them for the currently accepted therapeutic ranges.

Materials and methods

Study population

The samples used in the study were obtained from pediatric and adult patients with IBD that underwent routine determination of serum ADA levels at the Hospital General Universitario Gregorio Marañón (Madrid, Spain) or that participated in a research study in the same center (repository C.0003459, Instituto de Salud Carlos III). The serum ADA determination was requested by clinicians or researchers as part of patients' monitoring of either induction or maintenance of ADA treatment. Demographical and clinical characteristics of patients are described in Table 1.

The study was conducted in accordance with the principles of the Declaration of Helsinki, with approval by the corresponding local Ethics Committees.

Measurement of ADA serum levels

Blood samples were collected before the next dose of ADA was injected (trough level), centrifuged and serum samples were kept at −80 °C until processed by Promonitor (PM) ADA ELISA (Grifols-Progenika, Derio, Spain). Fifty samples were selected according to ADA serum concentrations, following the recommendations of the Spanish Society for Clinical Biochemistry (SEQC) for method comparison. SEQC suggests employing 50% of samples out of the normal

Table 1: Summary of demographical and clinical characteristics of patients whose serum samples were selected for the method comparison study.

Number of patients	47
Patients with CD/UC/IC	30/14/3
Age, years	36.0 ± 20.9 (3–74)
Male gender	63.8%
Never smoke	68.1%
Former smokers	12.8%
Current smokers	19.1%
Clinical remission	45.2%
Duration of the disease, years	8.5 ± 8.2 (0–33)
Duration of ADA treatment, years	2.7 ± 3.3 (0–11)
ADA induction phase	17.8%
ADA maintenance at 40 mg every week	20.0%
ADA maintenance at 40 mg every 2 weeks	62.2%
Previous treatment with IFX	27.9%

Results are expressed as mean ± SD. Values in parenthesis stand for range. CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis; ADA, adalimumab; IFX, infliximab.

Table 2: Description of the different assays used for the measurement of adalimumab serum levels according to the information provided by the manufacturers.

Name	Manufacturer	Method	Sample dilution	Range, µg/mL	CV intra-run	CV inter-run
Quantum Blue ADA	Bülhmann	Lateral flow assay	1:20	1.3–35.0	19.1%–29.9%	16.6%–28.6%
Promonitor ADA	Grifols-Progenika	ELISA	1:10 and 1:200	0.02–12.0	6.1%	5.1%
Lisa-Tracker ADA	Theradiag	ELISA	1:201	0.3–16.0	6.1%–13.3%	5.7%–9.7%

range [19], therefore taking 5 µg/mL as reference cut-off point for subtherapeutic values and 12 µg/mL for supratherapeutic levels, we selected 26 samples within the therapeutic range, 12 samples with subtherapeutic serum ADA levels and 12 samples with supratherapeutic concentrations. Six samples were selected from three patients at two different time points, resulting in a final number of 47 patients providing samples for this study.

An aliquot of each selected sample was shipped in dry ice to Hospital General de Tomelloso and Palex Medical for serum ADA determination by Quantum Blue (QB) ADA rapid test (Bühlmann, Schönenbuch, Switzerland) and Lisa-Tracker (LT) ADA ELISA (Theradiag, Marne La Vallee, France), respectively. The researches in both locations were blind to the results obtained with PM ADA ELISA. The maximum time difference between determinations with different methods was 8 months.

The main characteristics of the assays are described in Table 2. All of them were employed according to the manufacturer's instructions. PM ADA was performed in a Triturus ELISA analyzer (Grifols, Barcelona, Spain), LT ADA in an ELISA processing system DSX (Dynex, Chantilly, VA, USA) and QB ADA in a Quantum Blue Reader (Bühlmann). Samples above the upper detection limit were measured again employing a higher dilution factor for PM ADA and extrapolated for LT ADA. Controls provided by manufacturers for each assay were processed for every analytical series.

Statistical analysis

For calculations, samples with ADA serum levels below the limit of quantification were considered to be equal to that lower limit in each particular method. The normality of ADA serum concentration distribution for each method was checked using the Kolmogorov-Smirnov test. Statistical comparison was performed using an ANOVA test (repeated measures) with a Bonferroni's *post hoc* test. A method comparison was carried out by Deming regression analysis [20] and the Bland-Altman plot [21]. The agreement between methods and kappa (κ) statistics were computed based on previously recommended concentrations for a therapeutic range of ADA [5]. GraphPad Prism version 5.0 for Windows and QuickCalcs web application (GraphPad Software, San Diego, CA, USA) were used to perform statistical and method comparison analyses and to generate plots. The level of statistical significance was set at 0.05.

Results

The ADA serum concentrations measured in 50 samples for each method were first checked for normality, with all of them showing a normal distribution. The mean value and standard deviation obtained for each method were 13.2 ± 8.9 µg/mL for QB, 9.3 ± 5.9 µg/mL for PM and 10.1 ± 5.3 µg/mL for LT. The statistical analysis of the ADA serum levels obtained with the three methods showed a significant difference between QB and the two ELISA kits, with a positive bias for QB, while the *post hoc* test displayed no differences between PM and LT assays (Figure 1 and Table 3).

To assess method interchangeability, Deming regression and Bland-Altman test were performed. A proportional difference was observed for QB when compared with the two ELISA kits and a systematic difference was detected between QB and LT methods also (Table 3). In addition, a slight systematic bias was noticed between the two ELISA assays. Bland-Altman plots showed that QB displayed higher ADA values than the two ELISA assays, with increased differences between methods for greater ADA levels (Figure 2).

Given that Bland-Altman plots showed smaller differences for QB with respect to the two ELISA assays in samples with lower ADA concentrations, a quantitative analysis was carried out in a sub-set of samples. We

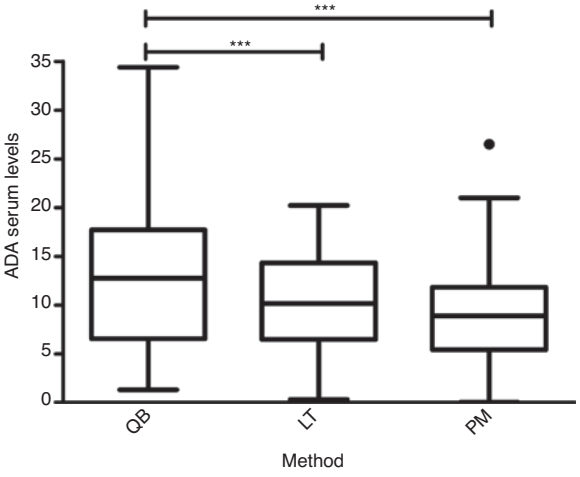

Figure 1: Graphic representation of serum ADA concentrations measured by the three different assays using Tukey box plot.
QB, Quantum Blue ADA; PM, Promonitor ADA; LT, Lisa-Tracker ADA.
***$p < 0.001$.

Table 3: Quantitative comparison of the three kits employed for measurement of adalimumab serum levels.

Methods	Bias of the mean	p-Value *post hoc* test	DR slope	DR Y-intercept	Bland-Altman limits of agreement
QB vs. PM	3.9 (2.5–5.2)	<0.001	1.59 (1.33–1.84)	−1.54 (−4.39 to 1.30)	3.9 (−5.4 to 13.2)
QB vs. LT	3.1 (1.7–4.5)	<0.001	1.78 (1.50–2.06)	−4.76 (−7.96 to −1.56)	3.1 (−6.6 to 12.8)
LT vs. PM	0.8 (0.1–1.5)	ns	1.13 (0.98–1.27)	−2.04 (−3.66 to −0.43)	0.8 (−3.9 to 5.4)

Values in parenthesis correspond to 95% confidence interval. DR, Deming regression; QB, Quantum Blue ADA; PM, Promonitor ADA; LT, Lisa-Tracker ADA; ns, not significant.

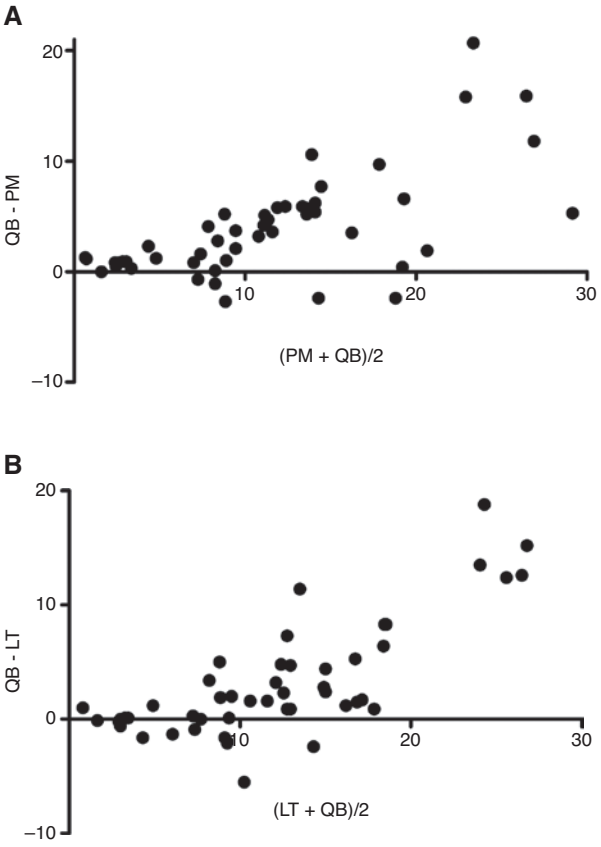

Figure 2: Bland-Altman plots comparing the rapid test QB ADA with the PM ADA (A) and LT ADA (B) ELISA kits.
QB, Quantum Blue ADA; PM, Promonitor ADA; LT, Lisa-Tracker ADA.

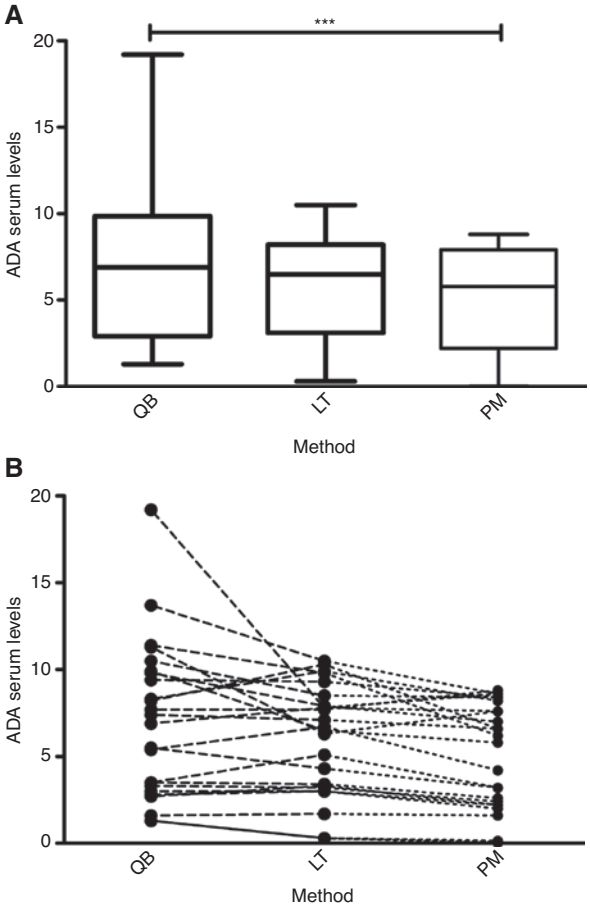

Figure 3: Graphic representation of serum ADA concentrations measured by the three different assays using Turkey box plot (A) and parallel dot plot (B).
Only samples with ADA serum concentrations lower than 9 μg/mL with PM assay were represented. QB, Quantum Blue ADA; PM, Promonitor ADA; LT, Lisa-Tracker ADA. ***$p < 0.001$.

selected all the samples with ADA levels within the subtherapeutic range and part of those within the therapeutic range (<9 μg/mL), resulting in a group of 25 samples. In the statistical analysis, no differences were found between QB and LT in the new comparison, while significant differences still remained between QB and PM (Figure 3). In agreement with these results, criteria for interchangeability were fulfilled between QB and LT in this range of ADA values. Nevertheless, a proportional difference, although smaller than in the complete group of samples, was still observed between QB and PM assays (Table 4).

A qualitative comparison of methods was performed to check the impact on clinical decisions of determining ADA serum concentrations with different assays. Two subtherapeutic cut-off points (5 and 7.5 μg/mL)

Table 4: Quantitative comparison of the three kits employed for measurement of adalimumab serum levels for those samples with adalimumab concentration lower than 9 μg/mL with Promonitor assay (n = 25).

Methods	Bias of the mean	p-Value *post hoc* test	DR slope	DR Y-intercept	Bland-Altman limits of agreement
QB vs. PM	1.9 (0.9–2.9)	<0.001	1.56 (1.16–1.95)	−0.86 (−3.12 to 1.40)	1.9 (−2.9 to 6.6)
QB vs. LT	1.0 (−0.1 to 2.1)	ns	1.49 (1.00–1.97)	−1.21 (−4.97 to 1.37)	1.0 (−4.4 to 6.4)
LT vs. PM	0.9 (0.4–1.4)	ns	0.93 (0.77–1.09)	−0.47 (−1.53 to 0.58)	0.9 (−1.5 to 3.3)

Values in parenthesis correspond to 95% confidence interval. DR, Deming regression; QB, Quantum Blue ADA; PM, Promonitor ADA; LT, Lisa-Tracker ADA; ns, not significant.

and one supratherapeutic cut-off level (12 μg/mL) were considered. Overall agreement among the three assays was 64% and 58% for 5 and 7.5 μg/mL as subtherapeutic cut-off values, respectively. In both cases, the agreement between QB and LT was similar to the concordance between the ELISA kits, while the disagreement was higher between QB and PM (Table 5). Consequently, the strength of the agreement according to κ values was "moderate" for QB and PM, and "good" for QB and LT. As previously observed in the quantitative analysis, the agreement between methods was better for patient classification within the subtherapeutic range, with a slightly better concordance for the 5 μg/mL cut-off point (83.3% for QB vs. PM; 81.8% for QB vs. LT) than for the 7.5 cut-off level (76.5% for QB vs. PM; 81.3% for QB vs. LT). However, major discrepancies were detected for patient classification into the supratherapeutic range (agreement of 46.2% for QB vs. PM; 70.4% for QB vs. LT).

Discussion

The measurement of ADA trough serum levels, jointly with determination of anti-ADA antibodies, is considered a useful biomarker in detecting loss of response in patients undergoing ADA therapy [22]. ADA serum concentrations are normally measured with ELISA kits in clinical laboratories but new rapid POCT methods have been launched onto the market recently. Therefore, method comparison of POCT and ELISA techniques is necessary in order to assess their interchangeability, as no international reference standard currently exists. In addition, the recently proposed ADA therapeutic ranges (mostly based on ELISA assays) have to be validated for the new rapid tests. In this study, we compared two established ELISA kits (PM and LT) for ADA measurement with a new flow lateral immunochromatographic method (QB). This is the first study comparing the QB rapid assay for ADA serum

Table 5: Qualitative comparison between the rapid test QB ADA and the two ELISA kits when 5 μg/mL (A) or 7.5 μg/mL (B) were used as cut-off point for subtherapeutic levels.

A	QB < 5 μg/mL	QB 5–12 μg/mL	QB > 12 μg/mL	Agreement	κ (95% CI)
PM < 5 μg/mL	10	2	0	68.0%	0.531 (0.351–0.710)
PM = 5–12 μg/mL	0	12	14		
PM > 12 μg/mL	0	0	12		
LT < 5 μg/mL	9	1	0	80.0%	0.688 (0.515–0.860)
LT = 5–12 μg/mL	1	12	7		
LT > 12 μg/mL	0	1	19		
B	**QB < 7.5 μg/mL**	**QB 7.5–12 μg/mL**	**QB > 12 μg/mL**	**Agreement**	**κ (95% CI)**
PM < 7.5 μg/mL	13	4	0	62.0%	0.454 (0.278–0.630)
PM = 7.5–12 μg/mL	1	6	14		
PM > 12 μg/mL	0	0	12		
LT < 7.5 μg/mL	13	2	0	78.0%	0.660 (0.489–0.832)
LT = 7.5–12 μg/mL	1	7	7		
LT > 12 μg/mL	0	1	19		

The agreement between the two ELISA kits was 80.0% with a κ of 0.691 (0.522–0.861) and 76% with a κ of 0.645 (0.474–0.846) for 5 μg/mL and 7.5 μg/mL cut-off points, respectively. CI, confidence interval; QB, Quantum Blue ADA; PM, Promonitor ADA; LT, Lisa-Tracker ADA.

level measurement with other methods published as a full research paper. Although our results showed relevant quantitative differences between methods, the impact on clinical decision-making would be minimal, as the agreement in identifying patients with subtherapeutic ADA levels was high between methods.

Although an initial systematic review on the cost-effectiveness of TDM for anti-TNF drugs reported limited evidence to support this statement [23], a later systematic review showed that TDM strategy lead to cost savings, compared to empiric management, with greater evidence for IFX than for ADA [24]. Therefore, the definition of cut-off points in order to take appropriate decisions based on anti-TNF drug concentrations, is a key aspect of TDM in patients with loss of response. An area under the curve of 0.77 for mucosal healing was found for ADA serum trough levels above 4.9 μg/mL in IBD [11]. Likewise, a cut-off value of 5.0 μg/mL showed the best combination of sensitivity (0.80) and specificity (0.56) for clinical remission in patients with Crohn's disease [12]. Consequently, Mitrev et al. suggested using 5.0 μg/mL as cut-off point to define the subtherapeutic range [7]. However, there is no complete consensus as other authors have reported a higher level of serum adalimumab, 7.5 μg/mL, as a cut-off to predict sustained response [13, 25, 26].

Independently of the subtherapeutic cut-off point employed, the concordance between the QB rapid test and the two ELISA assays was high (>75%) in identifying patients with subtherapeutic levels of ADA. Likewise, no major differences were found between both subtherapeutic cut-off levels regarding method concordance and κ values. Only two patients (out of 12) in QB vs. PM comparison, and one patient (out of 11) in QB vs. LT comparison, would be classified into the therapeutic ADA range with the rapid method although having subtherapeutic levels with ELISA kits. In addition, ADA levels were between 5.1 and 5.5 μg/mL for these patients so they were close to the cut-off point and should be cautiously interpreted before any clinical decision. These figures did not vary greatly when a cut-off point of 7.5 μg/mL was used, as only four patients (out of 17) were misclassified as being into therapeutic range with QB compared to PM assay, and two patients (out of 15) when QB was compared to LT. Anyway, currently no strong evidence exists to support cycling (switch to a second anti-TNF agent) or swapping (change to a drug with a different mechanism of action) strategies in patients with IBD based only in ADA serum levels, so any clinical decision should be taken cautiously and looking upon other factors, such as patient and disease characteristics and the presence of anti-ADA antibodies.

The main differences between tests were found for higher levels of ADA, resulting in a relevant discordance in classifying patients with supratherapeutic values of ADA. This disagreement for elevated ADA concentrations could be due, in part, to the higher upper quantification limit of QB than the two ELISA kits, which implies that an extra dilution or extrapolation out of the calibration range was required in PM and LT assays, respectively. This limitation could affect the capacity of each test to identify patients with clinical response that would benefit from a dose reduction of ADA. However, it should be mentioned that there is less consensus on the supratherapeutic cut-off point (12 μg/mL) and that proactive TDM in patients with quiescent IBD – and therefore likely to be within therapeutic or supratherapeutic ADA levels – is not recommended by the American Gastroenterological Association (AGA) [6]. Consequently, ADA TDM with the QB method should be employed to assess subtherapeutic values in patients with suspicion of loss of response rather than other possibilities such as dose or interval de-escalation.

Among the methods for ADA serum level measurement, ELISA kits are used the most in clinical laboratories, but other options are available. A homogeneous mobility shift assay (HMSA) was developed by Prometheus Laboratories (San Diego, CA, USA) in 2013 [27]. A comparison between HMSA and ELISA methods showed that both of them were useful to monitor ADA serum values in patients with Crohn's disease and loss of response, but HMSA displayed lower values of ADA compared to the ELISA kit, so cut-off points employed were much lower for the HMSA method [28]. Another option is reporter gene-based bioassays, in which detection is based on neutralization of TNF bioactivity. Van Bezooijen et al. performed a comparison between one bioassay and two ELISA kits and showed that there were significant differences in measured concentrations between methods, leading to a lack of interchangeability of ADA therapeutic ranges [29]. In addition, another available method is LC-MS/MS, which demonstrated a good agreement with the LT assay, using a recently developed approach [30].

Regarding the performance of the ELISA kits employed in our study, one of the first assessments of the PM assay was carried out in 2012, demonstrating good performance in terms of precision, linearity and clinical evaluation [31]. In a later study, PM showed better performance than Sanquin ELISA (Amsterdam, The Netherlands) in terms of recovery and bias, and high correlation was found between both ELISA methods [32]. LT also exhibited highly concordant results with the Sanquin assay in regression

and Bland-Altman analyses [29]. In agreement with these results, our comparison of PM and LT kits showed better correlation and agreement between them than the corresponding comparisons with the QB method, although a small systematic difference was detected, with LT providing slightly higher ADA serum values than PM.

Rapid methods for TDM of IFX and ADA have been launched onto the market to overcome the limitations of ELISA kits. Their main advantages are: rapid results, individual sample analysis, user-friendliness, avoidance of accumulating samples for an ELISA batch and not requiring transport to centralized laboratories. Although they are considered as POCTs, these methods still require sample centrifugation to obtain serum. Rapid methods are normally based on lateral flow immunochromatography, although other alternatives have been proposed such as a fiber-optic surface plasmon resonance-based biosensor [33]. Our results showed that the QB rapid method displayed higher values of ADA than ELISA kits, and discordances were mainly due the fact that differences increased with higher levels of ADA. Similar conclusions were reported in previous studies comparing the same POCT method for IFX with commercially available ELISA assays. For example, QB IFX exhibited systematic differences with IFX ELISA kits, with greater divergences for higher values of IFX [16, 17]. Two more studies also reported relevant differences affecting the classification of patients within the supratherapeutic range of IFX, as method comparison analysis revealed an overestimation of IFX levels for POCT compared to ELISA [18, 34]. Finally, differences with an ELISA kit and between the two POCT methods evaluated (QB and RidaQuick, with the first displaying higher values of IFX) were also identified in the latest evaluation of this issue [35]. Therefore, QB ADA seems to have the same lack of concordance with ELISA kits as its IFX counterpart for concentrations close to or above the supratherapeutic range, which demonstrates that greater efforts in standardization between POCT methods and ELISA kits are still required.

As already mentioned, to the nest of our knowledge this is the first study published as a full paper comparing the QB rapid method for ADA serum level determination with established ELISA kits. However, two communications to the United European Gastroenterology Week have reported method comparison analyses between QB and ELISA kits for ADA measurement. The manufacturer of QB compared this method with RidaScreen ELISA (R-Biopharm, Darmstadt, Germany). As with our results, the bias was higher at a cut-off point of 12 μg/mL than at 5 μg/mL (13.8% vs. 0.3%) [36]. Likewise, in a later comparison with three ELISA assays and exogenously-spiked samples, QB showed higher ADA values and systematic differences with two of the ELISA methods and with the spiked samples [37]. Regarding a rapid assay by another manufacturer, a better agreement was reported in the comparison of RidaScreen ADA ELISA and RidaQuick ADA POCT, both from R-Biopharm, but this was to be expected as both methods use the same antibody for ADA detection [38, 39].

There are some limitations of our study. Firstly, we did not perform an analytical evaluation and comparison with spiked samples of the methods, largely because this had already been carried out [29, 32, 36] and was beyond the scope of our study, which was to assess interchangeability of the three assays. Secondly, the number of analytical series was not high enough to evaluate imprecision, however, data from manufacturers and previous analysis of POCT methods for IFX corroborated that rapid techniques normally had less precision than ELISA kits. Finally, we did not measure anti-ADA antibodies in our serum samples and, although this could have helped to assess if they had an effect on sample discrepancies, it is not necessary for statistical and method comparison analyses.

Conclusions

Our study results indicate that QB ADA shows quantitative differences with the two ELISA kits included in our comparison that impede their interchangeability. Those differences were mainly at higher levels of ADA and thus the agreement among the three assays to identify patients with subtherapeutic concentrations of ADA (either below 5 μg/mL or 7.5 μg/mL) was high. Furthermore, QB and LT fulfilled interchangeability criteria for ADA values below 9 μg/mL. Otherwise, classification of patients within the supratherapeutic range at the current cut-off point (12 μg/mL) is highly dependent on the test used. These differences could be explained by the lack of standardization, as different antibodies with varying ADA affinities are employed by each manufacturer. Standardization and cross-validation are required to achieve interchangeability of different assays for TDM of ADA, and will also help to establish reliable therapeutic windows for this anti-TNF drug and to encourage TDM-based clinical decision-making. Given the current situation, we recommend long-term monitoring of ADA trough levels for IBD patients using the same assay throughout the entire follow-up.

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