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Comparison of a new rapid method for determination of serum anti-adalimumab and anti-infliximab antibodies with two established ELISA kits

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ABSTRACT

Background: Adalimumab (ADL), infliximab (IFX) and their biosimilars are widely used biological drugs. Some patients, however, generate neutralizing antibodies that hamper the effectiveness of these drugs. Evidence shows therapeutic drug monitoring of serum levels ADL/IFX and anti-drug antibodies (ADA) is useful to improve treatment effectiveness. We evaluated a new rapid quantitative method, Quantum Blue (QB), for determining serum anti-ADL and anti-IFX antibodies (Research Use Only labelling) by comparing it with two established ELISA kits, Promonitor (PM) and Lisa-Tracker (LT).
Methods: Eighty samples (40 for each drug type) were analysed. Percentage of agreement and kappa statistic were used to compare positive/negative ADA results. Clinical implications for drug treatment in the patients with discordant results were evaluated. The Chi-square test was used to analyze differences for ADA detection in patients with disease flare and without concomitant immunosuppressant treatment.
Results: Agreement exceeded 80 % among anti-ADL methods. Although LT ELISA showed a lower capacity in detecting anti-ADL antibodies, discrepancies were found for levels close to the cut-off concentration, thus having minimal impact on clinical decisions. Conversely, QB anti-IFX displayed low agreement with PM and LT ELISA kits (67.5 % and 50 %, respectively), and was unable to detect high levels of antibodies, therefore having major clinical implications. Agreement between PM and LT ELISA anti-IFX kits was 82.5 % with all discordant results being undetected for PM and slightly positive for LT.
Conclusion: QB anti-ADL shows similar performance to ELISA kits while QB anti-IFX needs further improvements to achieve reliable antibody detection.

1. Introduction

Anti-tumor necrosis factor (TNF)-α therapy was introduced several years ago to treat moderate to severe forms of certain immune-mediated diseases, mainly inflammatory bowel diseases (IBD), psoriasis, ankylosing spondylitis and rheumatoid arthritis [1]. Among TNFα inhibitors, adalimumab (ADL) and infliximab (IFX) are the two most commonly employed drugs in clinical practice. Apart from originator ADL (Humira) and IFX (Remicade), some biosimilar drugs of ADL (Amgevita, Hulio, Hymiroz, Imraldi, among

Abbreviations: ADA, anti-drug antibodies; ADL, adalimumab; ELISA, enzyme-linked immunoabsorbent assay; IBD, inflammatory bowel diseases; IFX, infliximab; POCT, point-of-care testing; TDM, therapeutic drug monitoring; TNF, tumor necrosis factor.

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others) and IFX (Flixabi, Inflectra, Remsima and Zessly) have been approved.

Although anti-TNFα treatment is highly effective in inducing disease remission, loss of response could occur during maintenance therapy, causing disease progression and increased health expenses [2]. When this happens due to sub-therapeutic drug concentrations, response can be regained after dose increase or shortening the dosing interval. However, loss of response due to drug immunogenicity with the presence of persistently high concentrations of neutralizing anti-drug antibodies (ADA) should be managed by switching treatment to a different drug, either another anti-TNFα agent or one targeting different molecules (such as vedolizumab or ustekinumab) [3]. Concomitant therapy with immunomodulators (azathioprine and methotrexate) is effective in reducing the development of ADA [4].

Therapeutic drug monitoring (TDM) of IFX/ADL and ADA serum levels based on laboratory assays is accepted as a useful and cost-effective tool to support clinical decisions [5]. While current evidence clearly sustains reactive TDM, in which only patients suggestive of loss of response are tested, the role of proactive TDM, in which levels are periodically measured to maintain a target drug concentration in absence of ADA, is in debate [6].

Different methods to measure IFX/ADL and ADA concentrations in blood have been developed, including enzyme-linked immunoabsorbent assays (ELISA), radioimmunoassays, homogeneous mobility shift assays and reporter gene assays [7]. Amongst these, the most commonly used in clinical laboratories for ADA detection are ELISA-based methods. In addition, the assays used for TDM of originator anti-TNFα are also used for biosimilars. A negligible bias was observed in comparative studies, and shared immunodominant epitopes were suspected to be responsible for the formation of ADA against both originator and biosimilar drugs [8].

A distinction between drug-sensitive and drug-tolerant assays for ADA detection is normally specified. Drug-sensitive assays are those incapable of measuring ADA in the presence of the anti-TNFα agent, while drug-tolerant assays are able to detect ADA when the drug is present up to a certain concentration [9]. In most cases the assays are similar, but samples undergo a dissociation step in drug-tolerant ones, which allows ADA release from the drug [10]. Low concentrations of ADA have been associated with transient antibodies that have no clinical relevance and are usually detected only by drug-tolerant assays; in contrast, higher levels of ADA are normally persistent, causing loss of response and are detected also by drug-sensitive assays [9,11].

In addition, point-of-care tests (POCT) based on lateral flow immunochromatography are gaining popularity in TDM. Their main advantages over ELISA are: individual sample analysis, rapid results for immediate treatment adjustment, user-friendly usage, decreased cost per sample when low numbers of samples are measured, and avoidance of transport to centralized laboratories. The first rapid methods were developed for measuring serum drug levels, and several studies compared them with ELISA kits, both for IFX [12,13] and ADL [14,15]. Besides, a qualitative POCT (Promonitor Quick) was developed to detect anti-IFX antibodies using a finger prick sample. Currently, manufacturers are also launching quantitative rapid assays for ADA measurement. One of these new quantitative methods is Quantum Blue, which uses diluted serum samples and therefore requires centrifugation and laboratory processing. Although some studies compared several ADA ELISA kits with each other [16–18] or with other methodologies [19], and the qualitative POCT with its ELISA counterpart [20,21], quantitative rapid assays have not been compared yet to ELISA kits.

This study aims to compare two new rapid kits to measure serum levels of anti-IFX and anti-ADL antibodies (Quantum Blue) with the corresponding ELISA kits from two manufacturers (Promonitor and Lisa-Tracker). To our knowledge, this is the first study comparing the performance of Quantum Blue rapid method for ADA determination, which still has a RUO (Research Use Only) mark, with commercially available ELISA kits. The hypothetical impact of measuring ADA with different assays in clinical decisions was also evaluated, along with ADA detection during IBD flares and when no concomitant therapy with immunomodulators was provided.

Table 1
Summary of demographical and clinical characteristics of patients whose serum samples were selected for the method comparison study. Results are expressed as mean ± SD. Values in parenthesis stand for range.

	Anti-ADL	Anti-IFX
Number of patients	40	40
Patients with CD/UC/other[a]	23/10/7	30/10
Age (years)	47.6 ± 16.0 (22–77)	41.9 ± 19.5 (10–79)
Male gender	57.5 %	45.0 %
Never smoke	41.7 %	60.0 %
Former smokers	30.6 %	20.0 %
Current smokers	27.7 %	20.0 %
Flare at the moment ADA were measured[b]	48.5 %	46.2 %
Duration of the disease (years)	10.7 ± 10.1 (0–36)	9.4 ± 10.2 (0–43)
Duration of ADL/IFX treatment (years)	3.4 ± 3.5 (0–12)	2.9 ± 3.1 (0–10)
Original/biosimilar drug[c]	90 %/10 %	5%/95 %
Maintenance at standard dose[d]	66.7 %	48.7 %
Maintenance with dose intensification	33.3 %	51.3 %
Concomitant treatment with immunosupresants	12.1 %	20.5 %
Previous treatment with other biologics[e]	51.5 %	25.6 %

CD: Crohn's disease; UC: ulcerative colitis; ADA: anti-drug antibodies; ADL: adalimumab; IFX: infliximab.

[a] For anti-ADL method comparison, 7 patients with psoriasis were included. Age, gender, smoking status and original/biosimilar drug included data from these patients but they were excluded for the other categories described in this table.

[b] Information was obtained retrospectively and based on clinical criteria.

[c] Adalimumab: original drug (Humira), biosimilar drug (Amgevita). Infliximab: original drug (Remicade), biosimilar drug (Inflectra).

[d] Adalimumab standard dose: 40 mg every 2 weeks. Infliximab standard dose: 5 mg/Kg every 8 weeks. All other drug treatments consisting in standard dose but in shorter periods or higher doses with standard periodicity were considered as dose intensification.

[e] Including: IFX (for current ADL), ADL (for current IFX), golimumab, ustekinumab and vedolizumab.

2. Methods

2.1. Study population

The sera employed for this study were obtained from a collection of samples from pediatric and adult patients that were requested for routine determination of ADA levels at the *Hospital General Universitario Gregorio Marañón* (HGUGM, Madrid, Spain) as part of their clinical follow-up for maintenance of remission with either IFX- or ADL-based treatments. Demographical and clinical characteristics of the 80 selected patients are shown in Table 1. Most of the patients (73 out of 80) selected presented IBD. Patients were monitored based on a proactive approach. Blood samples were collected just before the next dose of the drug was injected (trough level), which happened just immediately before infusion for IFX and the day before of next injection for ADL. After centrifugation, aliquots of serum samples were stored at -80 °C for research purposes.

The study was conducted in accordance with the principles of the declaration of Helsinki, and approved by the corresponding local ethic committees (internal code 130-C). Informed consent was provided by all patients or their legal guardians before blood sampling.

2.2. Sample selection and measurement of serum levels of anti-drug antibodies

All samples were measured for the corresponding ADA following the manufacturer's instructions of the six assays used (Table 2). All the assays were kept refrigerated at 4 °C before use. Controls provided by manufacturers for each assay were processed before every analytical series and their values checked to be within the expected range. The interpretation of results was done according to the cut-off provided by the manufacturers.

First, samples were measured with Promonitor (PM) ELISA for anti-ADL and anti-IFX antibodies (Grifols-Progenika, Derio, Spain) at HGUGM using a Triturus ELISA analyzer (Grifols, Barcelona, Spain). Serum levels of the drugs were also determined using the corresponding ADL or IFX PM ELISA. Next, 80 samples were selected to achieve four groups: 20 samples with positive ADA for IFX, 20 samples with negative ADA for IFX, 20 samples with positive ADA for ADL, and 20 samples with negative ADA for ADL. This criterion was based on the recommendations of the Spanish Society for Clinical Biochemistry (SEQC) for method comparison, which suggests employing 50 % of samples out of the normal range [22].

An aliquot of each selected sample was shipped in dry ice to *Hospital General de Tomelloso* (HGT, Tomelloso, Spain) for determination of anti-ADL and anti-IFX serum antibodies by Quantum Blue (QB) rapid test using a Quantum Blue Reader (Bühlmann, Schönenbuch, Switzerland). Likewise, another aliquot was shipped to an external laboratory to perform the same determinations by LisaTracker (LT) ELISA (Theradiag, Marne La Vallee, France) in a DSX ELISA system (Dynex, Chantilly, VA, USA). The researchers in both locations were blind to the results obtained with PM ELISA.

Regarding marking, both ELISA kits had a CE-mark, indicating compliance with 98/79/EC directive, while QB had only RUO labelling.

2.3. Statistical analysis

The overall, positive, and negative percentage agreement between methods and the kappa (κ) statistic were computed based on a dichotomous interpretation of ADA concentrations (positive/negative) according to the manufacturers' recommended cut-offs. The Chi-square test was used to analyze differences among assays for detection of positive ADA in patients in flare and without concomitant immunosuppressant treatment at the point of measuring ADA.

GraphPad Prism version 5.0 for Windows and QuickCalcs web application (GraphPad Software, San Diego, CA, USA) were used to perform statistical analyses. The level of statistical significance was set at 0.05.

3. Results

3.1. Agreement among methods for measurement of anti-adalimumab antibodies

Overall agreement between methods was good, with all comparisons showing a percentage of agreement higher than 80 % (Table 3). The highest concordance was observed between PM ELISA and QB (92.5 %; κ = 0.85), superior even to that observed between the two ELISA kits (90 %; κ = 0.80). The lowest agreement was detected for the comparison between LT ELISA and QB (82.5 %; κ = 0.65).

When discordant results were compared, concentrations of anti-ADL antibodies were within a range close to the cut-off levels for all samples except one (Table 4). LT ELISA provided negative anti-ADL antibodies for samples with concentrations between 10–28 UA/mL in PM ELISA and 0.2–0.9 μg/mL in the QB rapid method (patients #2, #4, #5 and #6). Contrarily, QB provided no antibody detection in a sample with low concentrations in the two ELISA kits (18 UA/mL in PM ELISA and 21 ng/mL in LT ELISA) (patient #3). There was only one discrepant sample with high levels of anti-ADL antibodies in both PM (127 UA/mL) and LT (317 ng/mL) ELISA kits, but no antibody detection in QB (patient #1). This sample was re-analysed to check for mistakes, but results were confirmed in this second determination.

3.2. Clinical implications of detection of anti-adalimumab antibodies with different assays

Four patients were being treated with the biosimilar Amgevita instead of the originator drug Humira when anti-ADL antibodies were measured, and no discrepant results among methods were observed. Of the four patients, three had positive anti-ADL antibodies in the 3 assays compared and the remaining patient had no antibodies detected by any method.

The samples from 7 patients with IBD presented discordant results in their levels of anti-ADL antibodies (Table 4). All of these patients, except one, subsequently stopped treatment with ADL. The remaining patient continued to receive ADL at the same dose due to irregular follow-up and the patient's rejection of the switch, despite presenting positive ADA in the PM and QB assays (patient #2).

A patient with high levels of anti-ADL antibodies in both ELISA kits but undetected for the rapid QB assay required surgery and ADL withdrawal (patient #1). Another patient with low antibody levels measured by ELISA but below threshold in the QB method was switched to IFX (patient #3). The same decision was taken for a patient with a positive result in the QB assay and no antibody detection in ELISA kits (patient #7). Four patients with negative antibodies in LT assay but positive values in both or either PM ELISA and QB had their treatment changed to another biological drug (patients #2, #4, #5 and #6), demonstrating a slightly lower capacity of LT ELISA to identify patients with anti-ADL antibodies who would benefit from therapy switching.

The rate of positive anti-ADL antibodies in patients with IBD flare (n = 16) was higher in QB and PM methods (50 % and 43.8 %, respectively) compared to the LT kit (31.3 %), although the difference did not reach statistical significance (p = 0.549). The same trend was found among IBD patients without concomitant treatment with immunomodulators (n = 29), among whom antibody detection rate was higher in QB (51.7 %) and PM (55.2 %) compared to LT (41.4 %), but again the difference was not statistically significant (p = 0.550).

3.3. Agreement among methods for measurement of anti-infliximab antibodies

The agreement was good only for the comparison between the two ELISA kits (82.5 %; κ = 0.65), with 7 samples providing positive anti-IFX antibodies in LT ELISA but negative ones according to PM ELISA. The QB rapid method showed a fair agreement with PM ELISA (67.5 %; κ = 0.35) and poor agreement with LT ELISA (50 %; κ = 0.19) as anti-IFX antibodies were above the QB cut-off in only 7 samples and below the detection limit in the remaining 33 samples (Table 3).

High levels of anti-IFX antibodies in both ELISA kits were missed by the QB rapid method, and the lowest concentrations that provided positive results in QB were 55 UA/mL in PM and 400 ng/mL in LT assays. All discordant results between PM and LT ELISA kits were in a range of values slightly higher than LT cut-off (10–50 ng/mL; patients #21–27) (Table 5).

Table 2
Description of the different assays used for the measurement of concentrations of anti-drug antibodies in serum according to the information provided by the manufacturers.

Name	Manufacturer	Method (units)	Sample dilution	Anti-ADA type	Range	CV intra-run	CV inter-run	Cut-off point
Quantum Blue	Bülhmann (Switzerland)	Lateral flow assay (μg/mL)	1:10	Anti-ADL	0.2–10.0	TBD	TBD	0.2
				Anti-IFX	0.6–12.0	8.2–18.9%	18.5–22.9%	0.6
Promonitor	Grifols-Progenika (Spain)	ELISA (UA/mL)	Undiluted & 1:10	Anti-ADL	3.1–185.0	6.6 %	6.6 %	10.0
				Anti-IFX	2.0–144.0	10.0%	8.0 %	5.0
Lisa-Tracker	Theradiag (France)	ELISA (ng/mL)	1:2	Anti-ADL	0–160	2.8–4.1%	5.5–11.1%	10.0
				Anti-IFX	0–200	4.1–8.5%	10.2–15.8%	10.0

ADA: anti-drug antibodies. ADL: adalimumab; IFX: infliximab; CV: coefficient of variation; TBD: to be determined.

Table 3
Qualitative comparison (positive *vs.* negative) between the rapid test Quantum Blue and the two ELISA kits for ADA determination. The cut-off values used for ADA positiveness were described in Table 2. The agreement between the two ELISA kits was 90.0 % (PPA = 80 %, NPA = 100 %) with a kappa of 0.800 (0.618-0.982) for anti-ADL antibodies, and 82.5 % (PPA = 100 %, NPA = 65 %) with a kappa of 0.650 (0.429-0.871) for anti-IFX antibodies.

ADA type	ELISA kit results	QB negative	QB positive	Overall agreement	PPA	NPA	Kappa (95 % CI)
Anti-ADL	PM negative	19	1	92.5 %	90 %	95 %	0.850 (0.687–1.000)
	PM positive	2	18				
	LT negative	19	5	82.5 %	87.5 %	79.2 %	0.646 (0.411–0.881)
	LT positive	2	14				
Anti-IFX	PM negative	20	0	67.5 %	35 %	100 %	0.350 (0.129–0.571)
	PM positive	13	7				
	LT negative	13	0	50.0%	25.9 %	100 %	0.185 (0.039-0.332)
	LT positive	20	7				

ADA: anti-drug antibodies; ADL: adalimumab; IFX: infliximab; CI: confidence interval; PPA: positive percentage agreement; NPA: negative percentage agreement; QB: Quantum Blue; PM: Promonitor; LT: Lisa-Tracker.

Table 4
Demographic data, clinical characteristic and laboratory data for patients with discordant results among methods for serum anti-adalimumab (ADL) antibodies. Patients were ordered from higher to lower concentration of anti-adalimumab antibodies measured by Promonitor ELISA. Concentrations of serum ADL were measured with Promonitor ELISA.

ID	IBD	Gender	Age	Drug	Dose (mg /week)	Active disease	IS	Prev BD	SerumADL	AAA PM	AAA LT	AAA QB	Clinical decision
1	CD	Male	31	Humira	40/2w	Yes	No	No	<0.62	127.0	316.9	<0.2	Stop ADL and surgery
2	UC	Female	24	Humira	40/1w	Flare	No	IFX	<0.62	27.6	4.6	0.7	Irregular follow-up, clinicians planned change to UST but patient refused
3	UC	Female	35	Humira	40/1w	Yes	No	No	<0.62	18.0	20.8	<0.2	Change to IFX
4	CD	Male	60	Humira	40/2w	Yes	No	No	<0.62	14.1	4.1	0.9	Change to UST
5	CD	Male	27	Humira	40/1w	Yes	No	IFX	<0.62	11.5	3.4	0.5	Change to UST
6	UC	Female	58	Humira	40/2w	Flare	No	IFX	<0.62	11.3	ND	0.2	Change to VDL
7	CD	Female	30	Humira	40/1w	Flare	No	No	<0.62	ND	ND	0.2	Change to IFX

IBD: inflammatory bowel disease; CD: Crohn's disease; UC: ulcerative colitis; IS: concomitant use of immunosupresants; Prev BD: previous treatament with other biological drugs; AAA: anti-adalimumab antibodies; PM: Promonitor; LT: Lisa-Tracker; QB: Quantum Blue; ADL: adalimumab; IFX: infliximab; UST: ustekinumab; VDL: vedolizumab; ND: not detected.

3.4. Clinical implications of detection of anti-infliximab antibodies with different assays

Most patients treated with IFX were receiving the biosimilar Inflectra (95 %); thus a good concordance between both ELISA kits for detecting ADA against this biosimilar drug was found. These assays also agreed in detecting antibodies in the only 2 patients treated with the originator drug Remicade.

All except one of the 20 patients with positive anti-IFX antibodies in both or either ELISA kits, but undetectable levels in the QB method, experienced a change in their IFX treatment (Table 5). The only patient that continued with the same IFX regimen had an irregular follow-up and antibodies were detected exclusively by LT ELISA (patient #22). IFX therapy was stopped in all patients, except one, who had positive antibodies in both ELISA kits (n = 13); this single patient was successfully managed with IFX dose escalation and had a low concentration of anti-IFX antibodies (patient #20). Regarding the 7 patients with positive antibodies in LT ELISA that were not detected in PM ELISA (patients #21–27), only 2 were managed with switching to ADL (patients #21 and #27), showing a limited clinical impact of anti-IFX antibodies in the range of 10–50 ng/mL in LT ELISA.

As expected, patients with an IBD flare (n = 18) had a lower rate of detection of anti-IFX antibodies in the QB method (33.3 %) compared to PM and LT ELISA kits (72.2 % and 83.3 %; p = 0.005). Among patients without concomitant therapy with immunomodulators (n = 31), QB detected positive anti-IFX antibodies in a significantly lower proportion (22.6 %) than ELISA kits (PM = 58.1 %, LT = 67.7 %) (p < 0.001). However, we noticed an unexpected 75 % rate of antibody detection by LT ELISA in patients concomitantly treated with immunomodulators, although 50 % had levels slightly above the cut-off concentration (between 10 and 31 ng/mL).

4. Discussion

TDM of anti-TNFα drugs is routinely performed in clinical practice and many laboratories use ELISA kits for this purpose. However, some aspects of TDM still need to be addressed to obtain high-

Table 5
Demographic data, clinical characteristic and laboratory data for patients with discordant results among methods for serum anti-infliximab (IFX) antibodies. Patients were ordered from higher to lower concentration of anti-IFX antibodies measured by Promonitor ELISA. Concentrations of serum IFX were measured with Promonitor ELISA.

ID	IBD	Gender	Age	Drug	Dose (mg-Kg/week)	Active disease	IS	Prev BD	SerumIFX	AIA PM	AIA LT	AIA QB	Clinical decision
8	CD	Female	10	Inflectra	5/6w	Flare	Yes	No	<0,4	34,5	392,6	<0,6	Change to ADL
9	CD	Female	31	Inflectra	5/8w	Flare	No	ADL	<0,4	32,5	388,1	<0,6	IFX stopped due to infusion reaction, currently only with AZA
10	CD	Female	64	Inflectra	5/8w	No	No	ADL	<0,4	23,5	322,9	<0,6	IS added, but later change to UST
11	CD	Male	52	Remicade	5/6w	No	No	No	<0,4	23,4	241,5	<0,6	Change to ADL
12	CD	Male	63	Inflectra	10/8w	Flare	No	No	<0,4	22,7	339,4	<0,6	Change to ADL
13	CD	Female	59	Inflectra	5/8w	Flare	No	No	<0,4	22,4	265,2	<0,6	Bowel resection
14	UC	Male	78	Remicade	5/8w	Yes	No	No	<0,4	19,4	201,4	<0,6	Change to ADL
15	UC	Female	10	Inflectra	5/4w	Flare	No	No	<0,4	16,3	233,4	<0,6	Change to ADL
16	CD	Female	45	Inflectra	5/8w	Yes	No	No	<0,4	11,9	145,2	<0,6	Change to VDL
17	CD	Female	28	Inflectra	5/8w	Yes	No	No	<0,4	11,1	131,8	<0,6	Change to ADL
18	CD	Female	49	Inflectra	5/4w	No	Yes	No	<0,4	9,9	95,9	<0,6	Change to ADL
19	UC	Female	42	Inflectra	5/4w	Yes	No	No	<0,4	6,4	81,5	<0,6	Change to VDL
20	CD	Male	56	Inflectra	5/8w	Flare	No	UST[a]	<0,4	6,3	73,9	<0,6	Dose escalation
21	CD	Male	21	Inflectra	5/6w	No	No	No	<0,4	2,4	47,7	<0,6	Change to ADL
22	UC	Female	57	Inflectra	5/8w	No	Yes	No	<0,4	ND	31,1	<0,6	Irregular follow-up, no changes
23	CD	Male	30	Inflectra	5/8w	Yes	No	ADL	0,49	ND	18,8	<0,6	Dose escalation
24	CD	Female	29	Inflectra	5/4w	Flare	No	No	<0,4	ND	14,6	<0,6	Dose escalation and IS added
25	CD	Female	18	Inflectra	5/8w	No	Yes	No	<0,4	ND	12,0	<0,6	Dose escalation
26	CD	Female	26	Inflectra	5/8w	Flare	Yes	No	<0,4	ND	11,8	<0,6	Dose escalation
27	UC	Male	79	Inflectra	5/8w	Yes	Yes	No	<0,4	ND	10,4	<0,6	Change to ADL

CD: Crohn's disease; UC: ulcerative colitis; IS: concomitant use of immunosupresants; Prev BD: previous treatament with other biological drugs; AIA: anti-infliximab antibodies; PM: Promonitor; LT: Lisa-Tracker; QB: Quantum Blue; AZA: azathioprine; IFX: infliximab; ADL: adalimumab; UST: ustekinumab; VDL: vedolizumab; ND: not detected.
a. This patient also had IFX, ADL, certolizumab and UST (in that order) as previous biologic treatment.

quality evidence [23]. Although the main controversial issue in TDM is related to reactive *vs.* proactive approaches, other questions remain open and affect the methodology used for TDM [6,7]. Among them, the role of POCT has attracted attention as it provides some advantages over ELISA kits, especially its fast results that allow immediate dose adjustments. While POCT assays to measure serum drug levels and to qualitatively detect anti-IFX antibodies are already available and have been compared with ELISA kits, quantitative rapid methods are currently being developed for ADA. Two new rapid tests to measure anti-IFX and anti-ADL antibodies have been launched under the QB brand (based on flow lateral immunochromatography) but still with RUO labelling. Although these are rapid methods, it should be taken into account that they do not fulfil completely the POCT criteria as they still require centrifugation, sample dilution, and reagent refrigeration. In this study, we compare these quantitative rapid methods with the corresponding ELISA kits (PM and LT), which are widely available in clinical laboratories. We found that detection of anti-ADL antibodies by the QB method was highly concordant with ELISA kits, while anti-IFX antibodies with a clinical impact on patient outcomes were not detected by QB, due to the poorer limit of quantification compared to ELISA kits.

Immunogenicity is recognized as a problem in patients treated with IFX and ADL. ADA are generated by the immune system after coming into contact with the foreign immunoglobulin-structures of biological drugs. ADA are thought to interfere with the action of biological agents through several mechanisms, including neutralization of the drug, competition with the target, and formation of immune complexes that increases drug elimination [24]. The appearance of ADA is associated with loss of response to therapy, poor clinical outcomes, hypersensitivity reactions and severe adverse effects [25]. Therefore, expert recommendations currently include ADA determination as an essential component of TDM algorithms for IFX/ADL treatments [26,27].

ADA can be detected by two types of assays: drug-sensitive and drug-tolerant. In this study, we compared drug-sensitive methods without performing any prior treatment of serum samples. Due to the intrinsic design of drug-tolerant assays, they normally detect positive antibodies in more patients than drug-sensitive assays [10,28]. However, it is not clear that a higher detection rate of ADA leads to improved or anticipated identification of patients requiring changes in their biological therapy, since these assays may detect transient ADA with no or little impact in drug effectiveness or concentration [29]. Consequently, drug-tolerant assays are not currently recommended by experts [26]. Drug-sensitive assays however normally detect ADA when they are present at concentrations sufficient to cause loss of drug efficacy and, consequently, have been proved useful in a clinical context [9]. Our results also showed that ADA levels slightly above the decision cut-off in drug-sensitive assays should be interpreted cautiously. For anti-ADL antibodies, discrepancies among the 3 methods were indeed observed for concentrations close to the cut-off point. Regarding anti-IFX antibodies in the comparison between ELISA kits, patients with low levels in LT ELISA but undetected in PM ELISA were managed successfully with IFX dose escalation in most cases and without the need to switch to a different biological drug.

Given the lack of a universal calibrator, it is not surprising to find differences among methods to measure ADA [24]. Despite the efforts made by Gils and collaborators to develop antibodies to harmonize assays for both ADL [30] and IFX [31], ADA commercial assays are not yet standardized. Actually, results are often provided in different units, as is the case for the assays compared in our study. This issue is recognized as a key point to be addressed, as standardization of assays would lead to more accurate results, more reliable clinical thresholds and better comparability between methods [32].

So far, several studies, mainly focused on ELISA kits, have compared diverse commercially available assays for ADA measurement. The first study comparing different ELISA kits for anti-IFX antibodies was published in 2016 and reported relevant differences among them that could lead to erroneous therapeutic decisions in patients with double-negative or double-positive results for serum concentrations of IFX and anti-IFX antibodies [16]. Two studies evaluated the concordance between the two ELISA kits for anti-IFX antibodies that we assessed in our study. Nasser et al. compared three assays (PM, LT and the Ridascreen kit from R-Biopharm) in 85 serum samples and they found a best correlation between PM and LT, while the correlation of both with Ridascreen was not as good; furthermore, the correlation between PM and LT was excellent (r = 1) if only samples with undetectable levels of IFX were included [13]. In the second study, concordance was perfect but only 4 patients from a total of 35 had positive anti-IFX antibodies [18]. A recent study identified different cut-off points to interpret results for anti-IFX ELISA assays from first generation, second generation and ready-to-use kit successively developed over time [33]. Regarding assays to measure anti-ADL antibodies, one study described differences among three ELISA kits for low-positive or borderline concentrations [17], and another found minor differences among three methods (ELISA, reporter gene assay, and surface plasmon resonance), but again the number of patients with positive antibodies was low (6 maximum) [19]. Therefore, more comparative studies are needed, especially for anti-ADL assays and among different methodologies.

The differences among assays and also among diverse methodologies could have clinical implications. Although this problem would probably affect a minority of patients, the impact on their clinical outcome could be profound [34]. This was also noticed in our study, especially in the detection of anti-IFX antibodies by the QB rapid method, since no detection of high levels of antibodies would incorrectly lead to continuation of IFX treatment. In contrast, minor differences were detected between the two ELISA kits for anti-IFX antibodies, thus having limited clinical impact. However, low concentrations detected by LT anti-IFX ELISA should be interpreted with caution as we observed them in an unexpectedly high rate of patients with concomitant immunomodulator treatment. This fact could have clinical implications for proactive TDM, as it has been suggested as being useful in eliminating the need for immunomodulator treatment in order to avoid side-effects in long-term therapies [35]. Regarding detection of anti-ADL antibodies, disagreements among methods were observed for concentrations close to the cut-off point, which should always be managed prudently. Therefore, we anticipated restricted clinical impact when using the QB anti-ADL rapid method instead of ELISA kits, but interferences could affect particular samples.

We should acknowledge some limitations in our study. Firstly, the number of analytical series was not high enough to evaluate the precision of the QB assays, which was not specified by the manufacturer in the technical instructions either. Secondly, we did not perform any further analysis to identify the interference causing discrepant results for anti-ADL antibodies in one sample. We suggest that one of the likely causes is a hook effect due to the high concentration of antibodies in that particular sample. Finally, the study was initially designed to include only samples from patients with IBD, but it was necessary to include seven patients with psoriasis for the comparison of methods for anti-ADL antibodies to reach 40 patients in this particular group. This being said, the disease had no influence on ADA measurement or discrepancies among methods.

5. Conclusion

Our study indicates that the QB rapid method for determination of anti-ADL antibodies provided results which agree highly with those provided by two established ELISA kits, despite one of our samples probably being affected by an interference. However, QB was not able to detect anti-IFX antibodies in samples with high levels measured by ELISA kits and consequently missed patients that required changes in their biological treatment. Therefore, QB anti-ADL assay could be employed in a clinical setting when a CE mark is obtained by the manufacturer. The QB anti-IFX test however needs further improvements in its limit of detection before moving from RUO to CE marking. The two ELISA kits showed high agreement in detecting both ADA, although small differences were noticed for concentrations close to the cut-off point. LT ELISA showed negative anti-ADL antibodies when low levels were detected in PM ELISA, while the opposite happened for anti-IFX antibodies. Therefore, low ADA concentrations in drug-sensitive ELISA kits should be interpreted with caution and monitored with the same assay in the long-term.

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CRediT authorship contribution statement

Emilio J. Laserna-Mendieta: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Project administration, Funding acquisition. **Sara Salvador-Martín:** Resources, Data curation. **Ignacio Marín-Jiménez:** Resources, Data curation. **Luis A. Menchén:** Resources, Data curation. **Beatriz López-Cauce:** Resources, Data curation. **Luis A. López-Fernández:** Writing - review & editing, Supervision, Funding acquisition. **Alfredo J. Lucendo:** Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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