Received: 18 July 2024 | Accepted: 28 August 2024

DOI: 10.1002/jpn3.12372

JPGN

ORIGINAL ARTICLE

Gastroenterology: Inflammatory Bowel Disease

Lateral flow test versus enzyme-linked immunosorbent assay to measure infliximab trough concentrations: A head-to-head comparison

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Funding information

BÜHLMANN Laboratories AG

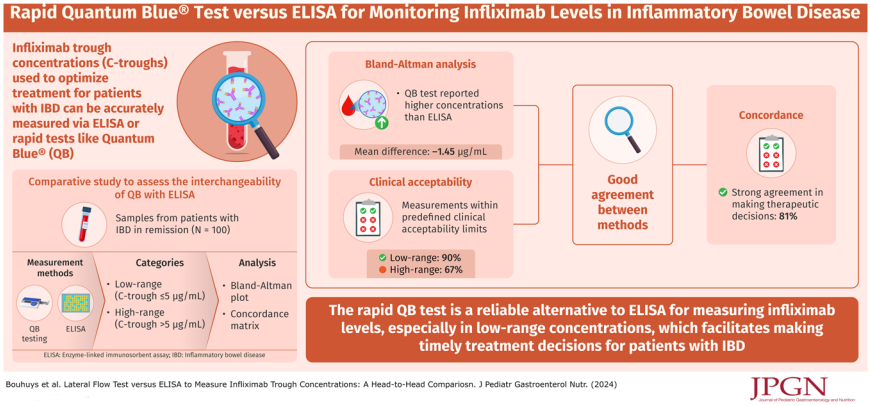
Abstract

Objectives: Infliximab is an antitumour necrosis factor agent used to treat inflammatory bowel disease (IBD). Measurement of infliximab trough concentrations (C-troughs) are used to optimize drug exposure and improve outcomes. Currently, enzyme-linked immunosorbent assays (ELISAs) are used predominantly for this purpose. Novel lateral flow immunoassays provide a rapid result.

Methods: We collected 100 paired serum samples of adolescents and young adults with IBD, who were treated with infliximab maintenance infusions. C-troughs were measured with the Quantum Blue® lateral flow test (QB) with ELISA. Results were categorized as low-range (mean C-trough ≤5 μg/mL) or high-range (>5 μg/mL). A Bland–Altman plot was created with limits of clinical acceptability set at ≤2 μg/mL for low-range and ≤40% for high-range C-troughs. A concordance matrix was created to evaluate the C-trough-based clinical scenario (whether or not to escalate infliximab) using a cutoff value of 5 μg/mL.

Results: Agreement between QB and ELISA was good (intraclass correlation coefficient: 0.85). In the low-range, 90% (95% confidence interval [CI]: 79–96) of measurements were within the limits of clinical acceptability. In the high-range this was 67% (95% CI: 53–79). QB provided higher results than ELISA. The concordance matrix showed 81% agreement (95% CI: 72–88, κ: 0.62).

Conclusions: Lateral flow- and ELISA-based infliximab C-trough measurements were in agreement. The swift establishment of infliximab C-troughs matters for patients experiencing increased disease activity. In the event of a low C-trough, prompt dose escalation can be initiated.



KEYWORDS
ELISA, inflammatory bowel disease, therapeutic drug monitoring

1 | INTRODUCTION

Infliximab is a monoclonal antibody used in the treatment of inflammatory bowel disease (IBD), which acts by binding to tumour necrosis factor (TNF)-α and disrupting the pro-inflammatory cascade signalling. A strong association between infliximab exposure and response to treatment has been established.[1] Due to large inter- and intraindividual variability in the pharmacokinetics of infliximab, standard dosing can result in subtherapeutic drug levels. Several covariates, including inflammatory burden, age, body weight, albumin concentration, concomitant use of immunomodulators and the presence of antidrug antibodies play a role in the variability in infliximab clearance.[2]

Therapeutic drug monitoring (TDM), that is, measuring infliximab trough concentrations (C-troughs, i.e., serum drug concentrations right before the next administration) and interpreting these C-troughs for adjusting further infliximab dosages, helps to optimize drug exposure.[3] TDM of anti-TNF agents was found to be cost-effective,[4] and is associated with an increased likelihood to reach endoscopic healing,[3] which has been accepted as a long-term treatment target in IBD.[5]

Currently, anti-TNF C-troughs are measured with an enzyme-linked immunosorbent assay (ELISA), which often requires transportation of samples to a centralized diagnostic laboratory and usually takes around 1 week or more before the results are out.

Point-of-care tests based on the lateral flow technique have become widespread since the coronavirus disease 2019 pandemic and infliximab C-troughs measured with this method may provide a rapid alternative with results available after ~15 min.[6]

We aim to compare infliximab C-troughs measured with the Quantum Blue® test (QB) and the established ELISA method,[7] to determine whether these tests agree sufficiently for QB to replace the ELISA, or to use the two interchangeably.

What is Known

- Measurement of infliximab trough concentrations and subsequent dose adjustment can optimize the effectiveness of infliximab therapy.
- Lateral flow tests may offer a rapid alternative for the established enzyme-linked immunosorbent assay (ELISA).

What is New

- We found good agreement in infliximab trough levels measured with a lateral flow test and ELISA in a real-world clinical setting.
- Rapid measurement of infliximab trough concentration may especially be beneficial for patients that experience increased disease activity while under infliximab maintenance therapy. In the event of a subtherapeutic trough concentration, prompt dose escalation can be initiated.

2 | METHODS

2.1 | Design

For this head-to-head comparison samples prospectively collected for the FREE-study (ClinicalTrials.gov NCT04646187, registration date 20 November 2020) at the University Medical Centre Groningen and Rijnstate Hospital were used. The FREE-study is an ongoing partially randomized patient preference trial comparing a lengthened infliximab interval with an unchanged interval in patients aged 12–25 years with IBD in sustained remission. The FREE-study protocol has previously been published.[8] Serum samples are routinely collected for trough level measurement every 24 weeks (intervention

group) or 48 weeks (control group). The first patient was enrolled in the FREE-study on 11 March 2021. For the current study, data were collected until 8 December 2023.

2.2 | Pre-analytical sample handling

Serum samples were collected routinely for infliximab trough level measurement. At each timepoint, two serum samples were collected from each patient right before infliximab administration. One serum sample was analysed immediately after collection at the local laboratory with QB. The other sample was sent to a diagnostic service facility for ELISA-based testing within 24 h after collection. If transportation within 24 h was not possible, the samples were frozen until the next sending.

2.3 | Measurement of infliximab concentration with lateral flow test

The commercially available QB Infliximab Quantitative Lateral Flow Assay (LF-TLIF10/–25, BÜHLMANN Laboratories AG) was used, hereafter referred to as QB. In short, the radiofrequency identification chip card was inserted into the QB reader (BI-POCTR-ABS) to load lot-specific test parameters. At room temperature, 10 µL serum was mixed with 190 µL chase buffer and vortexed. The test cassette was inserted into the QB reader and 80 µL of the diluted serum was added onto the loading port. After incubating for 15 min, the concentration of free infliximab was displayed. According to the instructions for use, the measuring range of QB is 0.4–20 µg/mL, which can be extended by dilution. The list price of the QB reader is €3000 and the list price of the infliximab test kits is €750 for 25 tests (i.e., €30 per measurement), value-added tax excluded.

2.4 | Measurement of infliximab concentration with ELISA

ELISA was performed by Sanquin Diagnostic Services (Level Infliximab M2920 kit),[9] with a detection range of 0.03–20 µg/mL. Free infliximab in serum was measured. If a trough level <1 µg/mL was measured, the presence and concentration of anti-infliximab antibodies was measured using a radioimmunoassay.[7] The laboratory technicians involved in the QB method were blinded for the ELISA result and vice versa. The costs for ELISA by Sanquin was €63.81 per measurement.

2.5 | Outcome measures

Agreement between the paired measurements was assessed by creating a Bland–Altman plot.[10] The Bland–Altman plot assigns the mean of the ELISA and QB measurements on the *x* axis and the difference between both on the *y* axis. Agreement was compared for both low- (<5 µg/mL) and high- (≥5 µg/mL) range C-troughs. We reasoned that disagreement in the lower range of the test could lead more easily to misclassification of the infliximab concentration than in the higher range. We therefore considered an agreement of ≤2 µg/mL in the low range and ≤40% in the high range as clinically acceptable.

We also performed a scenario analysis of therapeutic consequences. In accordance with the European guideline on the medical management of pediatric Crohn's disease, we considered a C-trough <5 µg/mL (in the absence of anti-TNF-antibodies) to be a reason to increase the dose or shorten the interval. A C-trough ≥5 µg/mL (in patients in remission) was considered to be in the target range, which should lead to an unchanged continuation of the infliximab treatment.

2.6 | Sample size calculation

For the current study, the first 100 collected serum samples were used for paired measurement. This sample size was based on the recommendation by Martin Bland, stating that 100 is a good sample size to accurately estimate the limits of agreement (LOA).[11]

2.7 | Statistical analysis

Study data were collected and managed using REDCap electronic data capture tool hosted at the University Medical Centre Groningen.[12,13] Statistical analyses were performed using IBM SPSS Statistics (Version 28). For the Bland–Altman plot, the mean difference between the two methods (bias), the upper and lower LOA (±1.96 SD) and the 95% confidence interval (CI) of the LOAs were calculated and displayed in the plot. Additionally, agreement was quantified by calculating the intraclass correlation coefficient (ICC) and its 95% CIs based on single measurements, absolute-agreement, two-way mixed-effects model.[14,15] A value < 0.5 was considered poor, 0.5–0.75 moderate, 0.75–0.9 good and >0.9 excellent.[14] The therapeutic consequence of the ELISA and the QB were determined individually and displayed in a concordance matrix. Other outcome measures included the κ statistic to quantify agreement. The strength of agreement was interpreted as poor for $\kappa < 0.0$, slight for 0.0–0.2, fair for 0.2–0.4, moderate for 0.4–0.6, substantial for 0.6–0.8 and almost perfect for 0.8–1.0.[16]

2.8 | Ethical consideration

The study protocol of the FREE-study has been approved by the Institutional Review Board (IRB)

UMCG (METc 2020/340). Written informed consent was obtained from all patients and parents/legal guardians of minor patients before enrolment. The IRB Committee issued an exemption for additional ethical review of the current study (METc 2021/131).

3 | RESULTS

Between 11 March 2021 and 8 December 2023, we collected 100 samples on all of which a paired measurement was performed. The number of paired measurements per subject ranged from 1 to 8. Anti-infliximab antibodies were detected in one sample. In Table 1 we present the patient characteristics.

3.1 | Bland–Altman analysis

In Figure 1 we show the agreement between QB and ELISA with a Bland-Altman plot. None of the measurements was above the upper limit of detection. The mean difference between infliximab measurements across the whole range was −1.45 µg/mL (lower LOA −5.49 – upper LOA 2.59), indicating that QB provides higher results than ELISA. The mean difference was more pronounced for high-range infliximab concentrations (>5 µg/mL) compared to low range (≤5 µg/mL) with −2.23 µg/mL (LOA between −7.11 and 2.64) and −0.69 (LOA between −2.91 and 1.53), respectively. In the low range, 46 of 51 (90%) paired measurements were within the predefined limits of clinical acceptability. In the high range, 33 of 49 (67%) paired measurements were within the predefined limits of clinical acceptability.

TABLE 1 Patient characteristics.

Characteristic	*n* = 21
Gender	
– Male	11 (52%)
– Female	10 (48%)
Age at study baseline (median [range])	16 (12–25)
Diagnosis	
– Crohn's disease	16 (76%)
– Ulcerative colitis	5 (24%)
Age at diagnosis (median [range])	11 (6–16)
Infliximab dose per administration	
– 5 mg/kg	14 (67%)
– 10 mg/kg	7 (33%)
Fecal calprotectin at study baseline (median [range])	47 (30–234)

Note: Presented as *n* (%) unless stated otherwise.

3.2 | ICC

The ICC including all measurements is 0.85 (95% CI: 0.60–0.93), indicating good agreement.

3.3 | Concordance

Table 2 shows the concordance matrix of therapeutic consequences (either escalating the infliximab dose or continuing the same dose) in both the low and high-range infliximab concentrations. Overall, 81 of 100 paired measurements (81%; 95% CI: 72–88) were concordant. Discordance between the ELISA and QB, leading to infliximab underdosing on the one hand and overdosing on the other hand, was observed in 19 of 100 paired measurements (19%; 95% CI: 13–28). The κ statistic is 0.62, indicating substantial agreement. Subanalysis results in concordance in 86% (95% CI: 74–93) of measurements in the low range and 80% (95% CI: 66–89) in the high range.

4 | DISCUSSION

4.1 | Summary of main findings

We performed a head-to-head comparison of a rapid lateral flow quantitative infliximab C-trough test versus ELISA in 100 paired serum samples of adolescents and young adult patients with IBD in sustained remission. We showed that infliximab concentrations measured with QB agreed sufficiently with the ELISA-based quantification. There was good agreement in the continuous comparison and substantial concordance of therapeutic consequences using a cutoff of 5 µg/mL.

4.2 | Comparison with existing literature

The QB method yielded higher infliximab concentrations than ELISA, indicating a systematic difference. This was more pronounced in the high-range subgroup, where the mean difference was 2.23 µg/mL. Toja-Camba et al.[17] also observed that QB slightly overestimated the infliximab concentration, with a median C-trough of 4.86 µg/mL for ELISA (Promonitor) and 6.15 µg/mL for QB ($p < 0.001$). Two older studies, on the other hand, observed systematic differences in the opposite direction, with ELISA-based measurements (both Sanquin) 2.62 and 0.92 µg/mL higher than QB, respectively.[18,19] The difference between the two older and the two newer studies may reflect a change in measuring procedures over time, such as a change in calibration or in the antibody used for ELISA.

We found three additional studies that specifically compared QB with ELISA by calculating an ICC. The

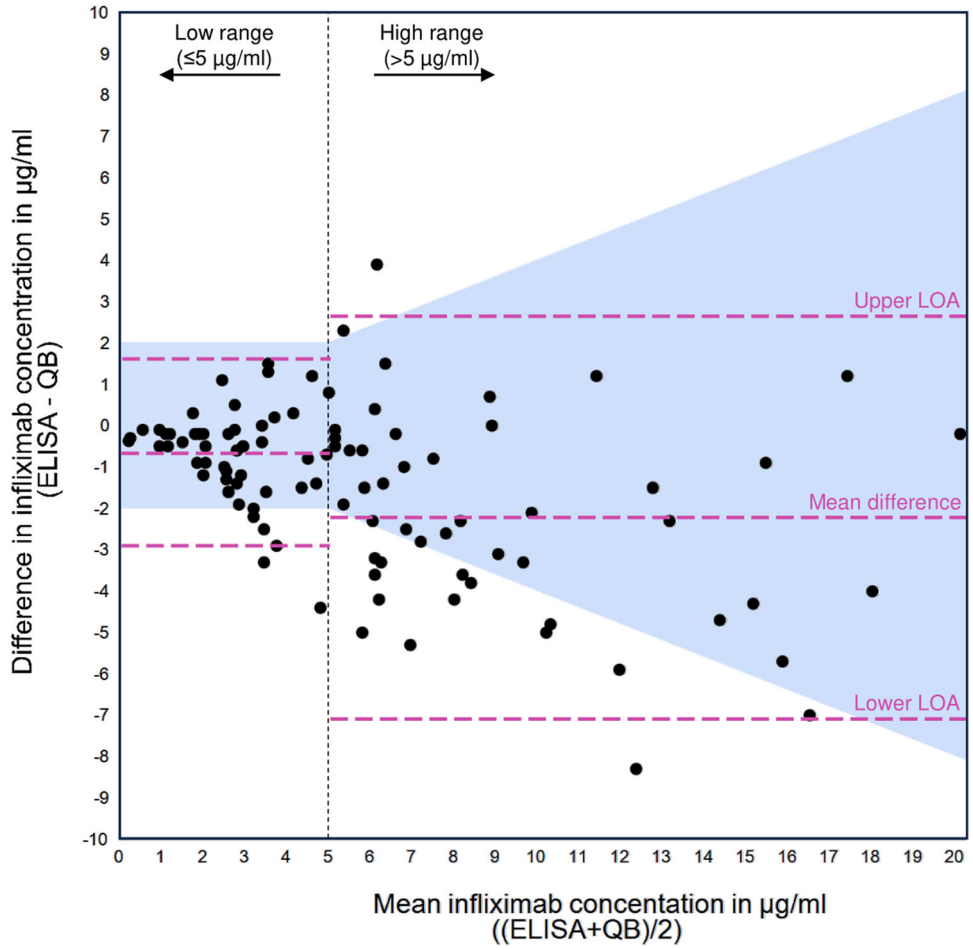


FIGURE 1 Bland–Altman plot showing difference against mean for ELISA versus QB. The shaded area corresponds with the predefined range of clinical acceptability, which were arbitrary set at ≤2 µg/mL in the low range and ≤40% in the high range. The dashed pink lines are the mean difference and the upper and lower 95% LOA. ELISA, enzyme-linked immunosorbent assay; LOA, limits of agreement; QB, Quantum Blue®.

TABLE 2 Concordance matrix of therapeutic consequences.

	ELISA: adapt treatment (trough level <5 µg/mL)	ELISA: continue treatment (trough level ≥5 µg/mL)	Total
QB: Adapt treatment (trough level <5 µg/mL)	43	4	47
QB: continue treatment (trough level ≥5 µg/mL)	15	38	53
Total	58	42	100

Note: Green: concordant test results. Orange: discordant test results.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; QB, Quantum Blue®.

ICCs ranged between 0.82 and 0.94.[18–20] This is consistent with the ICC of 0.85 that we found, although methods of ICC calculation could differ as this was not specified in the studies.

The concordance between QB and ELISA was evaluated in several studies. We distinguished two categories of C-trough results (<5 mg/L vs. ≥5 mg/L), whereas other groups used three different categories (<3, 3–7 and >7 mg/L).[17,19–22] The κ statistics in these studies ranged from 0.67 to 0.81, corresponding with substantial to almost perfect agreement. Our κ of 0.62 was slightly lower, but an infliximab concentration ≥5 mg/L is internationally accepted as the recommended target C-trough for children in the maintenance phase.[23]

4.3 | Strengths and limitations of the study

In our study, individual blood samples were processed immediately for measuring the C-trough, as is customary in clinical practice. To our knowledge, other studies comparing the lateral flow method with ELISA first stored blood samples and then tested them at once in large batches. We show that even in a less-controlled real-life setting measurements with QB agrees sufficiently with ELISA to use the former in situations where a quick result provides added value for the patient. One might consider the patient who, during the maintenance phase of infliximab treatment, starts experiencing symptoms suggestive of a disease flare, prompting the question of whether there could be a pharmacokinetic loss-of-response or presence of anti-infliximab antibodies. This study's cohort was limited to adolescents and young adults, which may impact the generalizability of our findings to younger

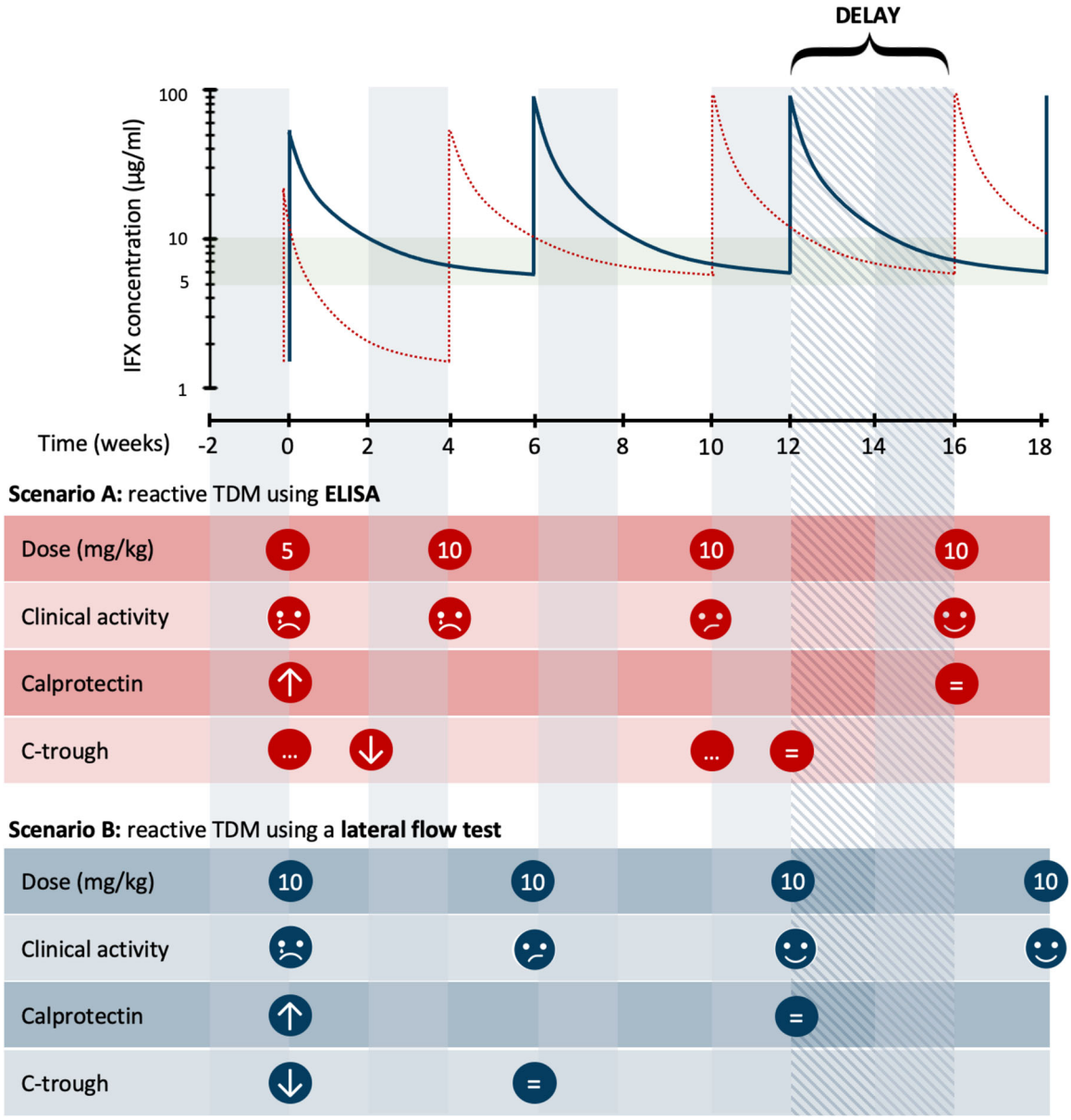


FIGURE 2 Clinical scenarios of an imaginary patient treated with infliximab maintenance (5 mg/kg every 8 weeks) who presents with a disease flare. Scenario A: infliximab C-trough is measured with ELISA in a reference laboratory. The subtherapeutic concentration is reported after 2 weeks and it takes another 2 weeks before the patient receives an increased infliximab dose. Scenario B: infliximab C-trough is measured with a lateral flow test and the subtherapeutic infliximab concentration is recognized immediately. Immediate adjustment of the treatment may limit the duration of the flare by 4 weeks. ↑/↓, above/below target range; =, in-range; C-trough, trough concentration; ELISA, enzyme-linked immunosorbent assay; IFX, infliximab; TDM, therapeutic drug monitoring.

children with potentially different pharmacokinetic profiles. As anti-infliximab antibodies were only present in one paired serum sample, we were not able to evaluate whether QB performs equally well in situations of immunogenic loss-of-response.

4.4 | Implications for clinical practice

We found sufficient agreement between the lateral flow test and the ELISA test in the lower infliximab concentrations to use the lateral flow test for monitoring patients during the maintenance phase, both for proactive and reactive TDM. The lower range (C-trough < 5 mg/L) is exactly where precision is of utmost importance. A delay in the recognition of a subtherapeutic infliximab concentration can lead to a delay in adjusting the treatment plan. Although there is no consensus on how to dose-escalate in case of pharmacokinetic failure (dose increase, interval shortening, or even a new intensified induction scheme), the authors of this article advocate the strategy of doubling the dosage as quickly as possible and shortening the interval if the subtherapeutic trough level is accompanied by a (symptomatic) disease flare. Figure 2 shows how a lateral flow infliximab test and immediate dose escalation can help to limit the duration of a disease flare.

Early pro-active TDM with a lateral flow test during the induction phase would also be desirable. However, QB is less suitable for this purpose, as its test performance is poorer in the higher range. During induction treatment, the target infliximab C-troughs are ≥25 and ≥15 μg/mL at Weeks 2 and 6, respectively.[23]

5 | CONCLUSIONS

We found good agreement in C-troughs between the QB lateral flow test and ELISA in the lower ranges of infliximab. The swift establishment of infliximab C-troughs matters for patients experiencing increased disease activity in the maintenance phase. In the event of a low C-trough, prompt dose escalation can be initiated.

ACKNOWLEDGEMENTS

We thank Hiltjo Kuiper (chief analyst laboratory of clinical pharmacy and pharmacology, UMCG), Albert-Jan Voerman and Justine Hanning-Huisman (analysts laboratory of clinical pharmacy and pharmacology, UMCG) and Miranda Klein Tank (senior analyst laboratory of clinical pharmacy, Rijnstate Hospital) for the lateral flow trough level measurements. We thank Sylvia Arendsen-Meijer (research nurse Rijnstate hospital) for data collection and patient follow-up. BÜHLMANN Laboratories AG (Schönenbuch, Switzerland) donated the QB tests for unrestricted use. The company had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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REFERENCES

1. Moore C, Corbett G, Moss AC. Systematic review and meta-analysis: serum infliximab levels during maintenance therapy and outcomes in inflammatory bowel disease. *J Crohns Colitis*. 2016;10(5):619-625.
2. Hemperly A, Casteele NV. Clinical pharmacokinetics and pharmacodynamics of infliximab in the treatment of inflammatory bowel disease. *Clin Pharmacokinet*. 2018;57(8):929-942.
3. Sethi S, Dias S, Kumar A, Blackwell J, Brookes MJ, Segal JP. Meta-analysis: the efficacy of therapeutic drug monitoring of anti-TNF-therapy in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2023;57(12):1362-1374.
4. Guidi L, Pugliese D, Panici Tonucci T, et al Therapeutic drug monitoring is more cost-effective than a clinically based approach in the management of loss of response to infliximab in inflammatory bowel disease: an observational multicentre study. *J Crohns Colitis*. 2018;12(9):1079-1088.
5. Papamichael K, Cheifetz AS, Melmed GY, et al. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2019;17(9):1655-1668.e3.
6. Guzman NA, Guzman DE, Blanc T. Advancements in portable instruments based on affinity-capture-migration and affinity-capture-separation for use in clinical testing and life science applications. *J Chromatogr A*. 2023;1704:464109.
7. Wolbink GJ, Vis M, Lems W, et al. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum*. 2006;54(3):711-715.
8. Bouhuys M, Lexmond WS, Dijkstra G, et al. Efficacy of anti-TNF dosing interval lengthening in adolescents and young adults with inflammatory bowel disease in sustained remission (FREE-study): protocol for a partially randomised patient preference trial. *BMJ Open*. 2021;11(11):e054154.
9. Wolbink GJ. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2005;64(5):704-707.
10. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;327(8476):307-310.
11. Bland M. How can I decide the sample size for a study of agreement between two methods of measurement? Accessed February 12, 2021. https://www-users.york.ac.uk/~mb55/meas/sizemeth.htm
12. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inf*. 2009;42(2):377-381.
13. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inf*. 2019;95:103208.

14. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med*. 2016;15(2):155-163.
15. Edwards C, Allen H, Chamunyonga C. Correlation does not imply agreement: a cautionary tale for researchers and reviewers. *Sonography*. 2021;8(4):185-190.
16. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159-174.
17. Toja-Camba FJ, García-Quintanilla L, Rodríguez-Martinez L, et al. Enhancing therapeutic drug monitoring in inflammatory bowel disease: a comparative analysis of rapid point-of-care infliximab, adalimumab and anti-drug antibodies' determination against ELISA. *Pharmaceutics*. 2023;15(11):2615.
18. Magro F, Afonso J, Lopes S, et al. Clinical performance of an infliximab rapid quantification assay. *Ther Adv Gastroenterol*. 2017;10(9):651-660.
19. Afonso J, Lopes S, Gonçalves R, et al. Proactive therapeutic drug monitoring of infliximab: a comparative study of a new point-of-care quantitative test with two established ELISA assays. *Aliment Pharmacol Ther*. 2016;44(7):684-692.
20. Curci D, Lucafò M, Cifù A, et al. Determination of serum infliximab concentration by point-of-care devices in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 2019;69(4):474-479.
21. Novakovic V, Abdija S, Larsen PB, Fenger M, Gredal L, Jacobsen KK. Comparison of the Quantum Blue® reader point-of-care system versus ELISA technique for therapeutic drug monitoring of Infliximab levels. *Clin Biochem*. 2019;74:73-75.
22. Gibson DJ, Ward MG, Rentsch C, et al. Review article: determination of the therapeutic range for therapeutic drug monitoring of adalimumab and infliximab in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2020;51(6):612-628.
23. van Rheenen PF, Aloi M, Assa A, et al. The medical management of paediatric Crohn's disease: an ECCO-ESPGHAN guideline update. *J Crohns Colitis*. 2021;15(2):171-194.

How to cite this article: Bouhuys M, Wessels MMS, Vries W, Lambeck AJA, Touw DJ, Rheenen PF. Lateral flow test versus enzyme-linked immunosorbent assay to measure infliximab trough concentrations: a head-to-head comparison. *J Pediatr Gastroenterol Nutr*. 2024;1-8. doi:10.1002/jpn3.12372