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Research Article

Correlation between Therapeutic Drug Monitoring of Infliximab Serum Trough Levels and other Biomarkers in Iraqi Patients with Crohn's Disease

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Abstract

Background: Inflammatory bowel disease (IBD) is a collection of chronic, recurrent inflammatory illnesses of the gastrointestinal system, including Crohn's disease (CD). Infliximab is one of the biological medications used to treat CD. Therapeutic drug monitoring has evolved as a treatment in IBD, aiming to optimize benefit while meeting more demanding, objective end criteria. ***Objective***: To determine the achievement of target trough level (TL), develop anti-drug antibodies (ADAs) to infliximab, assess response to therapy, and study TL relations with different variables. ***Methods***: The present study was cross-sectional and conducted from May 2022 to November 2022. It included 40 CD patients allotted into 2 groups: group 1 patients achieved the TL target and group 2 patients did not achieve the TL target. ***Results***: Twenty-two patients achieved target TL, while 18 patients did not. Dose escalation is recommended for 11 patients, switching therapy for 15 patients, and continuing the same dosage regimen for 14 patients. In addition, erythrocyte sedimentation rate, C-reactive protein, serum calprotectin and ADAs were significantly lower in patients who achieved target infliximab TL. Only serum calprotectin can be used to predict the achievement of the target TL of infliximab. ***Conclusions***: Therapeutic drug monitoring of infliximab to determine the TL and ADAs can help to explain why some patients do not respond to this drug. Serum calprotectin may be used as a novel marker to predict the TL and response to infliximab.

Keywords: Antidrug antibodies, Crohn's disease, Inflammatory bowel disease, Infliximab, Iraqi patients, Therapeutic drug monitoring.

العلاقة بين المناطرة الدوائية لمستويات إنفليكسيماب في المصل والمؤشرات الحيوية الأخرى لدى المرضى العراقيين المصابين بداء كرون

الخلاصة

الخلفية: يمثل التهاب الأمعاء مجموعة من الأمراض الالتهابية المزمنة والمتكررة في الجهاز الهضمي، بما في ذلك مرض كرون (CD). الأنفليكسيماب هو أحد الأدوية البيولوجية المستخدمة لعلاج CD. تطورت مناطرة الأدوية اثناء علاج مرض التهاب الأمعاء، بهدف تحسين الفائدة مع تلبية معايير نهائية أكثر تطلبا وموضوعية. **الهدف:** لتحديد مدى الوصول لمستوى العلاج المستهدف TL، وتكون الأجسام المضادة لعلاج إنفليكسيماب، وتقييم الاستجابة للعلاج ودراسة علاقة مستوى العلاج مع عدة متغيرات في مرضى مرض كرون. **الطريقة**: الدراسة الحالية مستعرضة وأجريت في الفترة من مايو 2022 إلى نوفمبر 2022. وشملت 40 مريضا CD تم توزيعهم على مجموعتين: حقق مرضى المجموعة 1 هدف TL ولم يحقق مرضى المجموعة 2 هدف TL. **النتائج**: حقق اثنان وعشرون مريضا الهدف TL، بينما لم يحقق 18 مريضا. يوصى بتصعيد الجرعة ل 11 مريضا، وتبديل العلاج ل 15 مريضا، والاستمرار في نفس نظام الجرعة ل 14 مريضا. بالإضافة إلى ذلك، كان معدل ترسيب كرات الدم الحمراء، والبروتين التفاعلي C، وكالبروتيكتين في المصل، و ADAs أقل بشكل ملحوظ في المرضى الذين حققوا هدف إنفليكسيماب TL. يمكن استخدام كالبروتيكتين المصل فقط للتنبؤ بتحقيق TL المستهدف من إنفليكسيماب. **الاستنتاجات**: يمكن أن تساعد مناطرة الأدوية العلاجية للأنفليكسيماب لتحديد TL و ADAs في تفسير سبب عدم استجابة بعض المرضى لهذا الدواء. يمكن استخدام مستوى مصل من كالبروتيكتين كعلامة جديدة للتنبؤ ب TL والاستجابة للأنفليكسيماب.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic, relapsing inflammatory disorders of the gastrointestinal tract (GIT) with an unknown etiology [1]. It is believed that genetic predisposition, environmental or antigenic factors, and dysregulation of the inflammatory response inside the GIT are involved in the etiology [2]. The two primary disorders that comprise IBD are ulcerative colitis (UC) and Crohn's disease (CD) [3]. The inflammation in CD is characterized by granulomatous

inflammation of the bowel wall and may involve any segment of the GIT, from mouth to anus [4,5]. The discontinuous pattern of CD's inflammation, which consists of sections of normal GIT mucosa and inflammation, is what causes the distinctive "skip lesions." Crohn's disease may lead to many complications, such as abscesses, fistulas, and strictures [6,7]. Infliximab is a monoclonal anti-tumor necrosis factor (anti-TNF) antibody used to treat a number of autoimmune conditions, including psoriasis, rheumatoid arthritis, CD, and UC [8,9]. Higher serum trough levels (TL) have been linked to improved clinical outcomes in IBD, according to the exposure-response relationship. This suggests that it may be time to switch from a "treat-to-target" to a "treat-to-trough" therapeutic approach [10]. Clinical studies have confirmed that another factor that affects the level of infliximab causes a loss of response [11–13]. Researchers have found that some factors may be linked to TL or response. These include body weight, C-reactive protein (CRP), serum albumin (ALB), mean platelet volume (MPV), serum oncostatin m (OSM), serum calprotectin (CALP), and platelet (PLT) count [14–19]. Therapeutic drug monitoring (TDM) has emerged as a strategy for treatment optimization in IBD to maximize benefit and reach more stringent, objective end points. Therapeutic drug monitoring involves measuring levels of serum drug concentrations and ADAs to rationalize primary non-response or secondary loss of response, given that low serum drug concentrations or the formation of ADAs are variably associated with treatment failure [20,21]. Three mechanisms could be the cause of medication failure: mechanistic failure occurs when the patient is not responding despite appropriate medication. This kind of failure is probably happening when inflammatory mediators that the particular medication does not block are promoting the disease process. When a patient has a TL of the drug that is below what is needed for therapy and there are no neutralizing ADAs, this is called non-immune-mediated pharmacokinetic failure. Immuno-mediated pharmacokinetic failure happens when the immune system makes ADAs that block the drug. This causes TL to be low or not detectable, which means the therapy stops working [22]. When infliximab TL is maintained between 3 and 7 µg/mL, patients with CD can achieve remission more effectively, experience fewer flare-ups, have higher rates of mucosal healing, and require fewer surgeries and endoscopic procedures in comparison with patients who do not receive TDM [23,24]. It is possible to do therapeutic drug monitoring during an active disease as "reactive TDM" when the disease is not well controlled. This can help with therapy and explain why some patients don't respond to the induction therapy or respond to therapy after an induction regimen but then lose response during maintenance treatment to a biologic drug. While "proactive TDM" can be done in patients with remission in an attempt to prevent future flare-ups and loss of response by optimizing drug concentration at specific times [24,25]. This study aims to determine the achievement of the TL target, the development of ADAs to infliximab, assess response to therapy, and study the TL relationship with different variables in patients with CD.

METHODS

Study design and patient selection

The current study was a cross-sectional study, which took place from May 2022 to November 2022 at the Hepatology Teaching Hospital and involved 40 patients. The patients received treatment in accordance with clinical practice guidelines and the severity of their diseases while being supervised by a gastroenterologist [26,27]. The sample size was calculated according to the equation: Sample size = z2*p(1-p)/d2 Sample size = $z^{2*}p(1-p)/d^2$ Z = standard normal variate (at 5% type 1 error (P<0.05), it is 1.96, and at 1% type 1 error (P<0.01), p = expected proportion in population based on previous studies or pilot studies, and d = absolute error or precision.

Ethical approval

The research proposal specifies the current study's goals, and the suggested data collection methodologies were submitted to the "College of Pharmacy, University of Baghdad," with clearance from the Scientific and Ethical Committee (ID: RECAUBCP2992021A, date: 29-9-2021). The Iraqi Ministry of Health also gave its clearance. Patients provided verbal agreement to participate in the trial.

Inclusion criteria

Patients age > 18 years who are diagnosed with moderate to severe CD according to Crohn's Disease Activity Index (CDAI) [28], and maintained on standard therapy (Infliximab+ azathioprine).

Exclusion criteria

Patients with other immune system disorders, and those using systemic or rectal steroids in the past 8 weeks.

Study groups

The eligible patients were allocated into two main groups according to TL target achievement: Group 1 includes patients who achieved infliximab TL. Group 2 includes patients who did not achieve infliximab TL. Response assessment is done by using the CDAI score: remission < 150 and active disease ≥ 150 [29].

Sample collection and outcome measurements

Using a disposable plastic syringe, ten milliliters of venous blood were drawn. Six milliliters of blood were stored in a simple disposable tube (gel and clot activator), allowed to clot, and then separated using a centrifuge running at 3000 rpm for ten minutes. Two milliliters were taken in an EDTA tube for the complete blood picture test, and 1.28 milliliters were taken in an erythrocyte sedimentation rate (ESR) tube for the ESR test. The complete blood picture tests, ESR, ALB, and CRP, were performed on the same day of sample collection, while the rest of the serum samples were divided and kept in Eppendorf tubes at

a temperature of -80°C until they could be used for the other assays. Platelet count, mean platelet volume (MPV), packed cell volume (PCV), and hemoglobin level were all assessed using an automated assay with the ADVIA 120 Hematology System. A blood sample of 175 µL was placed in the hematology system to be analyzed. The Mixrate-X20 device was utilized to perform an automated assay in order to measure the erythrocyte sedimentation rate. 1.28 milliliters of the blood sample were placed into the ESR autoanalyzer for examination. The completion time for the results is 30 minutes, which is equivalent to one hour when using the Westergren reference method. For men and women, respectively, the reference range is (≤ 15 mm/hr) and (≤ 20 mm/hr) [30]. The ELISA test is used to assess the concentration of TL (Matriks Biotek, Turkey), ADA (Matriks Biotek, Turkey), OSM (Elabscience Biotechnology/China), and CALP (Elabscience Biotechnology/China). The sandwich type was used as the principle for these tests. The absorbance was calculated at the 450 nm wavelength. The standard curve was then used to determine the proportional measurement of concentration in the samples [31–34].

Statistical analysis

The Shapiro-Wilk test is used to evaluate if a variable follows a normal distribution. Variables that follow a normal distribution are given as mean and standard deviation (SD), while variables that do not follow a normal distribution are stated as median and interquartile range (IQR). When the variables had a normal distribution, the difference between the achieved TL group and the non-achieved TL group was evaluated using an independent t-test (number of infliximab doses, HGB, PCV, TL); if the data did not, a Mann-Whitney U test was used (age, BMI, duration of the disease, CDAI, MPV, ESR, CRP, ALB, OSM, CALP, ADAs, PLT). The Chi-square test is used to determine the difference between categorical variables (gender and response). The forward approach of binary logistic regression analysis was utilized to determine the relationship between various factors and TL attainment. For the goal of forecasting TL accomplishment, the optimal cut-off value (derived by the Youden index = sensitivity + specificity-1), sensitivity, and specificity were found using receiver operating characteristic (ROC) analysis. All statistical analyses were performed using SPSS 27 (Chicago, USA), and p-values less than 0.05 were considered significant.

RESULTS

Table 1 shows the demographic and illness characteristics of patients from both groups. There were no significant differences between patients who met the target level (Group 1) and those who did not meet the target level (Group 2) in terms of all factors. Twenty-two patients (55.0%) met the target TL, while 18 (45.0%) did not.

Table 1: Difference between patients achieved trough level and patients not achieved trough level according to demographic and disease characteristics of the patients

Variables	Group 1	Group 2	*p*-value
Gender *n*(%)			
Female	5(22.7)	9(50.0)	0.072
Male	17(77.3)	9(50.0)	
Age (year) [median (IQR)]	25.00 (22.50-28.0)	29.00 (23.50-39.0)	0.100
BMI (kg/m^2) [median (IQR)]	22.15 (19.97-24.13)	21.16 (20.01-23.03)	0.47
Disease duration (year) [median (IQR)]	4.00 (2.75-7.0)	4.00 (2.75-11.0)	0.882
Infliximab doses (mean±SD)	12.27±6.56	9.83±3.09	0.132
Response			
Remission *n*(%)	14(63.6)	6(33.3)	0.057
Active *n*(%)	8(36.4)	12(66.7)	

BMI: body mass index; IQR: interquartile range; NS: No significant differences ($p>0.05$). Two-sample *t*-test is used for statistical analysis of (no. of dose) Mann Whitney U test is used for statistical analysis of (age, BMI, duration of the disease) Chi-square test is used for statistical analysis of (gender, response).

Table 2 shows the patient classification and recommendations based on TL, ADAs, and disease activity, with recommendations to escalate the dose for 11 (27.5%), switch therapy for 15 (37.5%), and continue therapy for 14 (35%). The TL was substantially higher in group 1 patients than in group 2, although group 2 patients had significantly higher levels of ESR, CRP, CALP, and ADAs.

Table 2: Classification of patients and the recommendations made based on (TL, ADAs, and disease activity) [35]

Patients achieved target level (n=22)			Patients NOT achieved target level (n=18)		
Remission (n=14)	Active disease (n=8)	Remission (n=6)	Active disease (n=12)		
	Mechanistic failure		Negative ADAs (Non-immune pharmacokinetic failure) (n=1)	Low positive ADAs (immune pharmacokinetic failure) (n=4)	High positive ADAs (immune pharmacokinetic failure) (n=7)
			Recommendations made		
Continue therapy	Switching therapy	Escalate the dose	Escalate the dose	Escalate the dose	Switching therapy

ADAs: anti-drug antibodies

Table 3 shows that there were no significant differences among the other biomarkers. Using univariate binary logistic regression, only CALP and ADAs have a significant effect on meeting the goal TL.

Table 3: Difference between patients achieved trough level (Group 1) and patients not achieved trough level (Group 2) according to infliximab TL, CDAI, and other biomarkers

Variable	Group 1	Group 2	*p*-value
CDAI	122.00 (100.75-334.75)	305.50 (120.50-368.00)	0.190[b]
HGB (g/dL)	12.70±2.36	13.27±3.12	0.517[a]
PCV (%)	39.94±5.93	39.08 ±5.60	0.640[a]
MPV (fL)	8.15 (7.30-9.45)	8.85(7.20-9.38)	0.904[b]
ESR (mm/hr)	12.00 (8.00-25.75)	26.50 (14.50-39.25)	0.032[b]
CRP (mg/dL)	2.61 (0.84-6.05)	7.22 (3.18-15.93)	0.042[b]
ALB (g/dL)	4.80 (3.91-5.11)	4.53 (4.07-5.04)	0.798[b]
OSM (pg/ml)	85.67 (57.51-221.95)	135.90 (54.46-538.10)	0.819[b]
CALP (ng/mL)	994.95 (891.50-1058.93)	1373.50 (1075.15-1737.50)	<0.00[b]
TL (µg/mL)	5.25±1.71	1.43±0.86	<0.001[a]
ADAs (ng/mL)	64.31 (30.29-78.54)	86.80 (54.56-212.07)	0.017[b]
PLT (*10^3)	251.00 (210.75-283.75)	295.00 (221.25-342.50)	0.155[b]

ADAs: anti-drug antibodies; ALB: albumin; CALP: calprotectin; CDAI: Crohn's disease activity index; CRP:C-reactive protein; ESR: erythrocyte sedimentation rate; HGB: hemoglobin; MPV: mean platelet volume; OSM: oncostatin-m; PCV: packed cell volume; PLT: platelet; TL: trough level. [a] *t*-test was used; data presented as mean±SD. [b] Mann Whitney U test was used; data presented as median (IQR).

While multivariate binary regression (forward technique) includes ESR, CALP, ADAs, and CDAI variables, only CALP had a significant effect on achieving the goal TL, as illustrated in Table 4.

Table 4: Trough level target achievement prediction by different variables

Variable	Univariate analysis[a]	*p*-value	Multivariate analysis[b]	*p*-value
	OR (95% CI)		OR (95% CI)	
CDAI	1.004 (0.999-1.009)	0.102		
ADA (ng/mL)	1.022 (1.001-1.043)	0.040		
HGB (g/dL)	1.084 (0.854-1.374)	0.508		
PCV (%)	0.973 (0.871-1.087)	0.632		
MPV (fL)	0.955 (0.615-1.481)	0.836		
ESR (mm/hr)	1.029 (0.989-1.071)	0.151		
CRP (mg/dL)	1.002 (0.968-1.038)	0.890		
ALB (g/dL)	0.909 (0.340-2.430)	0.850		
OSM (pg/ml)	1.000 (0.998-1.001)	0.908		
CALP (ng/mL)	1.003 (1.000-1.005)	0.022	1.003 (1.000-1.005)	0.022
PLT (x10^3)	1.003 (0.997-1.010)	0.315		

ADA: anti-drug antibody; ALB: albumin; BMI; body mass index; CALP: calprotectin; CDAI: Crohn's disease activity index; CRP:C-reactive protein; ESR: erythrocyte sedimentation rate; HGB: hemoglobin; MPV: mean platelet volume; OR: Odds Ratio; OSM: oncastatin-m; PCV: packed cell volume; PLT: platelet. [a] Binary logistic regression; [b] Binary logistic regression (forward method).

Using receiver operating characteristic (ROC) analysis to find the appropriate cut-off value (defined by the Youden index) that can forecast target TL accomplishment, the cut-off values for ESR were ≤15.5 mm/hr (sensitivity = 72.2%, specificity= 77.8%, and AUC = 0.699), CRP was ≤3.51 mg/dL (sensitivity= 72.7%, specificity = 77.8%, and AUC = 0.688), CALP was ≤1081.95 ng/mL (sensitivity = 81.8%, specificity = 77.8%, and AUC = 0.806), and ADAs were ≤ 102.14 ng/mL (Table 5).

Table 5: Receiver operating characteristic (ROC) analysis

Variable	ROC Curve Analysis					
	Cut-off	Sensitivity (%)	Specificity (%)	AUC	*p*-value	CI 95%
CDAI	202.00	68.2	66.7	0.624	0.183	0.444-0.804
HGB (g/dL)	12.40	68.2	55.6	0.494	0.946	0.306-0.682
PCV (%)	37.45	81.8	44.4	0.581	0.384	0.397-0.764
MPV (fL)	7.65	86.2	27.8	0.511	0.903	0.327-0.696
ESR (mm/hr)	≤15.50	72.2	77.8	0.699	0.032	0.531-0.868
CRP (mg/dL)	≤3.51	72.7	77.8	0.688	0.043	0.505-0.871
ALB (g/dL)	4.60	68.2	55.6	0.525	0.786	0.340-0.710
OSM (pg/ml)	216.48	77.3	44.4	0.523	0.807	0.334-0.711
CALP (ng/mL)	≤1081.95	81.8	77.8	0.806	0.001	0.660-0.951
ADA (ng/mL)	≤102.14	100	44.4	0.720	0.018	0.552-0.887
PLT (x10^3)	281.50	77.3	61.1	0.634	0.150	0.452-0.816

ADA: anti-drug antibody; ALB: albumin; BMI; body mass index; CALP: calprotectin; CDAI: Crohn's disease activity index; CRP:C-reactive protein; ESR: erythrocyte sedimentation rate; HGB: hemoglobin; MPV: mean platelet volume; OR: Odds Ratio; OSM: oncastatin-m; PCV: packed cell volume; PLT: platelet; PMS: Partial mayo score.

DISCUSSION

In the current study, 40 CD patients receiving infliximab were included. Therapeutic drug monitoring was used to assess infliximab TL and the existence of ADAs to infliximab, which can help with therapy optimization such as increasing infliximab dosage, decreasing intervals between doses, or

switching to a different class of medication. To the best of our knowledge, this is the first study conducted in Iraq to ascertain the TL and ADAs for infliximab in patients with CD. Out of 40 patients, the current study's results indicated that 6 patients had low TL and were in remission, 1 patient had low TL and no ADAs (non-immune pharmacokinetics failure), and 4 patients had low TL and low positive ADAs (immune pharmacokinetics failure). It is therefore advised that these patients receive higher doses of their medications or shorter intervals between doses [35]. Furthermore, there were 8 patients in active disease despite achieving target TL (need to switch therapy), 7 patients in active disease with high positive ADAs (need to switch therapy), and 14 patients in remission state with the achievement of target TL (need to continue therapy) [35]. Additionally, routine inflammatory markers (ESR and CRP), routine blood tests (HGB, PCV, MPV, and PLT), and albumin were examined in the current study. It was found that ESR and CRP were higher in patients who did not achieve the TL, and no association was found with achievement of the of the TL. A study published in 2019 showed that while ESR, albumin, HGB, and PLT were not associated with TL, higher CRP was associated with a decrease in TL [36]. Another study by Hibi *et al*. supports the idea that CRP may serve as a blood infliximab level indicator for predicting response loss. Specifically, it was found that an increase in CRP could clearly detect the decrease in serum TL that accompanied loss of response [37]. According to a study by Roblin *et al*., a combination of CRP, TL, and stable ADAs can be used to accurately predict a loss of response to infliximab [38]. After 6 months of starting infliximab therapy, Ferreira *et al*. discovered in 2023 that a lower TL of infliximab and a higher ESR can predict the development of ADAs and therapy responses in CD patients [39]. Moreover, a positive correlation between high baseline CRP and increased clearance of the drug and, therefore, lower TL has been reported [40]. To the best of our knowledge, this is the first study on the association between serum CALP and TL and found that serum CALP was significantly higher in patients who didn't achieve the target level of TL. Serum CALP is a new marker that can be used in evaluating and predicting how well CD patients will respond to infliximab and achieve target TL. Fecal calprotectin testing has limits in clinical practice, in contrast to serum CALP testing. Patients may find fecal collection difficult, and delays in sample delivery and processing may reduce the usefulness of the sample in therapeutic settings [41]. Fecal calprotectin exhibits high daily and intraday fluctuation; the best time to sample is unclear [42]. In routine practice, a blood-based biomarker such as serum CALP would be more practical and patient-acceptable [43]. Oncostatin-M was another biomarker used in the current study. The level of OSM did not demonstrate a significant difference between groups 1 and 2 or an association with TL. In CD and UC, high OSM concentrations were linked to stopping anti-TNF therapy and using rescue steroids [44]. According to a recent study, OSM is a promising marker for IBD patient diagnosis and follow-up, but its predictive power for predicting how well an IBD patient would respond to treatment is limited [45]. Interestingly, a study published in 2021 reported significantly higher mucosal OSM gene expression in IBD patients who did not achieve endoscopic remission after starting medication with an anti-TNF; however, the study did not find a significant association between serum OSM concentrations and endoscopic remission [46]. Additionally, the current study shows that ADAs were higher in patients who did not achieve the target TL when compared to those who did. In 2017, a study in South Korean patients with CD showed that there was an inverse correlation between TL and ADA levels [13]. Also, in 2019, a study by Gomes *et al*. found that undetectable levels of infliximab correlated with the detection of ADAs and were independent of disease activity [47]. In addition, a recent study by Reinhold *et al*. found that positive ADAs were correlated with subtherapeutic TL in 15 out of 16 patients, reflecting the known drug-neutralizing effect of ADAs. The last patient who had positive ADAs had TL levels that were above the recommended levels. This could be an example of a temporary ADA development that had no clinical impact [48].

Study limitations

This study had some limitations, which should be noted. First, patients were gathered from a single center in Iraq. Further research is needed to determine whether they accurately represent the overall number of CD patients in Iraq who are on infliximab. Second, the sample size here is quite small. Future research should involve large sample and multicenter investigations in different areas of Iraq to determine whether the findings here can be replicated in other CD patients. Third, TDM results (TL and ADAs) were delayed because samples were obtained from patients and stored for 6 months before lab measurements were completed, delaying therapy recommendations to physicians and patients.

Conclusion

Therapeutic drug monitoring for infliximab (TL and ADAs) is an important tool for optimizing CD treatment and explaining the potential causes of non-responsiveness to this medicine, with following recommendations based on these findings. Serum CALP could be utilized as a novel measure to predict TL accomplishment and response to therapy.

Recommendations

First, future research should involve a large sample size and multicenter investigations in other parts of Iraq to see if the results here can be replicated in other CD patients. Also, cost-effectiveness studies for TDM patients receiving infliximab therapy may be required. Second, TL and ADA tests should be available in

hospitals that treat CD patients with infliximab. Third, to avoid a delay in the lab measurement (the ELISA test), it is recommended to employ the new quick tests for the detection of TL and ADAs.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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