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Short paper

Serum trough infliximab levels: A comparison of three different immunoassays for the monitoring of CT-P13 (infliximab) treatment in patients with inflammatory bowel disease

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ABSTRACT

Background: CT-P13 is a biosimilar drug of reference infliximab and is approved in some countries for use in some indications for which reference infliximab is approved, including inflammatory bowel disease (IBD). The CT-P13 formulation is identical to that of reference infliximab and has similar physiochemical characteristics. However, even a small molecular distinction could lead to different behavior of CT-P13 in immunoanalytical detection systems.
Aim: To determine the correlation between three different enzyme-linked immunosorbent assays for infliximab detection in the measurement of CT-P13 trough serum levels.
Methods: Serum samples (n = 42) from IBD patients (n = 22) treated with CT-P13 Remsima™ (Celltrion, Korea) were evaluated in a blinded way in infliximab assays manufactured by (A) Matriks Biotek (Turkey), (B) Theradiag (France), and (C) R-Biopharm (Germany).
Results: All assays showed excellent qualitative correlation (Cohen's kappa = 0.90 for A vs. B, 0.76 for A vs. C, and 0.83 for B vs. C). A linear quantitative correlation was satisfactory as well (Spearman's r = 0.91 for A vs. B, 0.86 for A vs. C and 0.92 for B vs. C). Assay C did not detect CT-P13 in 6 samples detected by A and/or B.
Conclusion: There is a good correlation of CT-P13 serum level detection between these assays.

1. Introduction

CT-P13 is a biosimilar monoclonal antibody infliximab for the treatment of patients with autoimmune diseases such as inflammatory bowel disease (IBD) [1–3]. Generally, biosimilars are biological products showing high resemblance to the reference biological products, and they exhibit no clinically meaningful differences in terms of safety and effectiveness [4]. Only minor differences in clinically inactive components are allowable in biosimilars compared to reference products. It is expected that the spread of biosimilar monoclonal antibodies will lead to cost savings in healthcare budgets and might also improve the availability of the biological treatment for patients [5–8].

However, even a small difference in the molecular structure can lead to different behaviors of the biosimilar drug in analytical systems for the detection of serum drug levels [9]. Today, the main commercially available assays for determining serum trough levels of infliximab (IFX) are enzyme-linked immunosorbent assays (ELISAs), from those that are relatively inexpensive and easy to perform to more sophisticated and costly detection systems. The main objective of this study was to compare three ELISAs for the detection of serum infliximab trough levels in patients with CT-P13 (Remsima) treatment: SHIKARI Q-Inflixi (Matriks Biotek, Turkey), LISA-Tracker Duo Infliximab (Theradiag, France), and RIDASCREEN

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IFX (R-Biopharm, Germany). Moreover, a technical evaluation of CT-P13 levels' measurement on SHIKARI Q-Inflixi analytical system was performed.

2. Material and methods

2.1. Patients and samples

Patient serum samples were collected at the ISCARE Clinical and Research Centre for Inflammatory Bowel Disease and Charles University in Prague, Czech Republic. The study included sera from 22 IBD patients whose diagnosis of IBD was established according to the guidelines of the European Crohn's and Colitis Organization (ECCO) [10]. Twenty patients (20; 91%) were previously infliximab-naive. All patients were treated with CT-P13 [11,12] in the induction phase. From this cohort of patients, 22 serum samples were measured at week 2 of the induction treatment with CT-P13 (W2), 15 serum samples at week 6 (W6), and 5 samples at week 14 (W14) of the induction treatment; therefore, 42 serum samples were tested. Aliquots of serum samples were stored at −80 °C.

Trough drug concentrations were determined from blood drawn immediately before the next intravenous infusion of CT-P13.

Technical performance analyses were realized with CT-P13 Remsima™ (Celltrion, Korea).

This study was approved by the Ethics Committee of ISCARE (Nr 2015/Ia), and patient data were anonymously used according to the latest version of the Helsinki Declaration of Human Research Ethics.

2.2. Immunoassays

All serum samples were tested by SHIKARI Q-Inflixi (Matriks Biotek, Turkey), LISA-Tracker Duo Infliximab (Theradiag, France), and RIDASCREEN IFX (R-Biopharm, Germany) according to each manufacturer's protocols.

The main characteristics of assays used are included in Table 1.

2.3. Statistical analysis

The data were statistically evaluated using STATISTICA CZ (Version 12; StatSoft, USA). Chi-square, Spearman's correlation, and Cohen's kappa agreement tests were carried out to analyze the agreement between data, and P values < 0.05 were considered significant.

3. Results

3.1. Qualitative and quantitative agreements

Using the in-lab cut-off value of 3 μg/mL as a detected trough level, excellent qualitative agreements were found; see Table 2.

Table 2
Qualitative agreement between different assays.

		SHIKARI Q-INFLIXI			Percent agreement
		POS	NEG	TOT	
LISA-TRACKER	POS	35	1	36	Positive agreement = 97%
	NEG	0	6	6	Negative agreement = 100%
	TOT	35	7	42	Total agreement = 98%
Kappa = 0.909 (95% CI 0.73–1.00)					
		RIDASCREEN IFX			**Percent agreement**
		POS	NEG	TOT	
LISA-TRACKER	POS	33	3	36	Positive agreement = 92%
	NEG	0	6	6	Negative agreement = 100%
	TOT	33	9	42	Total agreement = 93%
Kappa = 0.76 (95% CI 0.50–1.00)					
		RIDASCREEN IFX			**Percent agreement**
		POS	NEG	TOT	
SHIKARI Q-INFLIXI	POS	33	2	35	Positive agreement = 94%
	NEG	0	7	7	Negative agreement = 100%
	TOT	33	9	42	Total agreement = 93%
Kappa = 0.83 (95% CI 0.60–1.00)					

POS positive value, serum trough level of infliximab was detected.
NEG negative value, serum trough level of infliximab was not detected.
TOT total.

Nevertheless, it should be mentioned that many samples could have concentration of therapeutic below the level of 3 μg/mL.

Satisfactory quantitative agreements were also observed. The Spearman's coefficient values were r = 0.96 for SHIKARI *versus* LISA-Tracker, r = 0.86 for SHIKARI *versus* RIDASCREEN, and r = 0.92 for RIDASCREEN *versus* LISA-Tracker; see Fig. 1A–C.

3.2. Technical performance of the SHIKARI Q-INFLIXI assay in the measurement of CT-P13 concentrations

The lower limit of detection (LLOD) was determined from 20 zero samples (normal human sera from infliximab-naive healthy individuals). These normal human sera were used because pre-treatment diseased sera were unavailable. LLOD was calculated as the mean + 2SD (standard deviations). LLOD for SHIKARI Q-INFLIXI assay was calculated as 0.025 μg/mL, which is in accordance with the manufacturer's data.

Intra-assay variability was calculated from five replicates of five CT-P13 solutions in normal human sera containing different concentrations of the CT-P13. Inter-assay variability was evaluated by conducting five determinations of five CT-P13 solutions containing different concentrations of the drug on two different plates with different technicians and at different days.

Dilution recovery was calculated as a percentage of recovery of diluted CT-P13 samples from the 100% sample. Spike recovery was calculated by comparing different concentrations of CT-P13 in

Table 1
Three different immunoassays for the monitoring of CT-P13 (infliximab) serum levels: main characteristics.

Name	Detects	Results interpretation	Limit of detection	Assay range
SHIKARI Q-INFLIXI (Matriks Biotek)	Free infliximab	Quantitative. Generation of standard curve and determination of drug level in μg/mL	0.02 μg/mL	0.02–3 μg/mL (for the standard dilution 1:20 using assay buffer recommended by manufacturer)
LISA-Tracker Duo Infliximab (Theradiag)	Free infliximab	Quantitative. Generation of standard curve and determination of drug level in μg/mL	0.1 μg/mL	0.1–5 μg/mL (for the standard dilution 1:100 using assay buffer recommended by manufacturer)
RIDASCREEN IFX (R-Biopharm)	Free infliximab	Quantitative. Generation of standard curve and determination of drug level in μg/mL	<0.001 μg/mL	2–48 μg/mL (for the standard dilution 1:400 using assay buffer recommended by manufacturer for the measurements in induction therapy phase)