Point-of-care measurement of infliximab and adalimumab trough levels by the fluorescence resonance energy transfer (FRET) assay

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Background

Therapeutic drug monitoring (TDM) is the clinical practice of measuring serum drug concentrations to guide clinical decision-making. In IBD patients, it is applied mainly in infliximab (IFX) and adalimumab (ADL) therapy. TDM is either performed reactively in response to disease activity, or proactively in the absence of symptoms. The aim of the present study was to compare two analytical methods for the detection of trough infliximab and adalimumab levels (TL): point-of-care (POCT) fluorescence resonance energy transfer (FRET) method, and the enzyme-linked immunosorbent immunoassay (ELISA).

Methods

TL IFX were measured in n=24 and TL ADL in n= 21 blood samples. For ELISA, standard peripheral venous samples were used, and for POCT FRET measurements, capillary finger puncture was performed. Two assay results were compared evaluating the regression relationship using Spearman's correlation coefficient and the Bland-Altman plots. Cohen's kappa analysis was used to compare the degree of qualitative agreement between the two data sets. Repeatability was assessed by the difference of the repeated measurements, their means and standard deviations.

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ProciseDX is a benchtop analyzer that uses a UV LED to excite a patented terbium cryptophor as a donor that is covalently bound to terbium. For the Procise IFX and Procise ADL assays, the first binding event is the binding of a drug in serum (IFX or ADL) to a TNFa-labeled donor, and the second binding event uses a monoclonal Fab anti-IFX/TNFa or anti-ADL/TNFa acceptor-labeled complex. The intensity of the fluorescence signal emitted from the reaction is converted to relative fluorescence units (RFU), and the resulting drug concentration is reported in units of µg/ml.
Procise IFX and Procise ADL are alidated in accordance with EP Directive 98/79/EC on in vitro diagnostic medical devices. The manufacturer provides data on the reproducibility and comparability of its method (ISO 17511, ISO 18153. Procise IFX calibrators and controls are based on the 1st International Standard of the WHO Expert Committee on Standardization of Biologics NIBSC 16/170, for Procise ADL it is the NIBSC 17/236 standard defined by the same institution.

Results

The high degree of correlation was found between the measured values, both qualitative and quantitative ($p<0.0001$ for both IFX and ADL), either expressed by the Spearman's correlations, or by Bland-Altman analysis. Cohen's weighted kappa 0.903 for IFX and 0.834 for ADL showed substantial/almost perfect agreement of qualitative results. Closeness of the agreement of successive measurements was very acceptable for both TLs of both drugs.

Figure 1 **Correlation of infliximab and adalimumab trough levels measured by FRET (ProciseDx) and by the comparative ELISA assay**

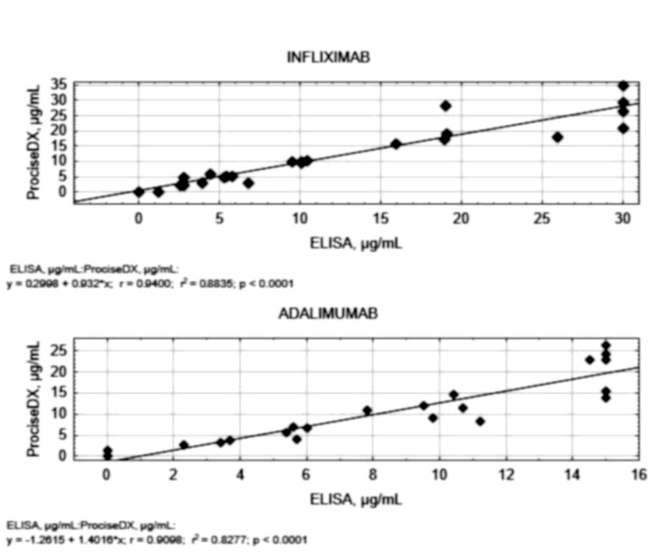

Figure 2 **Bland-Altman difference plots (means and differences of paired measurements) for infliximab and adalimumab trough levels, FRET ProciseDx *versus* comparative ELISA**

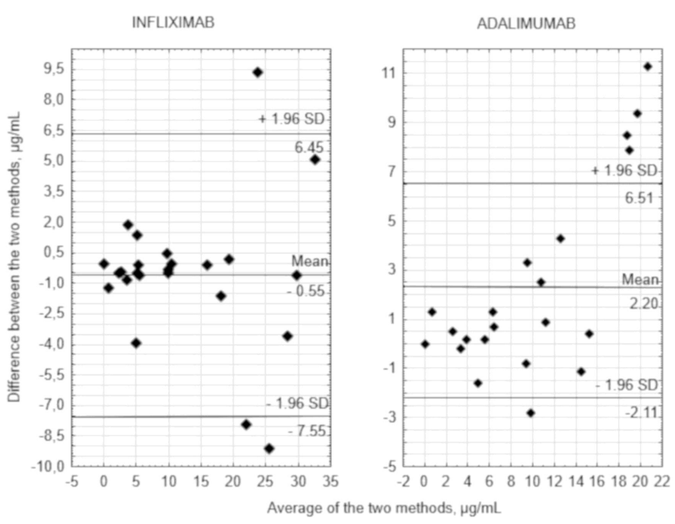

Table 1 **Qualitative agreement between infliximab and adalimumab trough levels measured by FRET (ProciseDx) and by the comparative ELISA**

ELISA - enzyme-linked immunosorbent assay
FRET (ProciseDX) - fluorescence resonance energy transfer
TL IFX - trough infliximab levels
TL ADL - trough adalimumab levels

TL IFX		ELISA		
		LOW	NORMAL	HIGH
FRET (ProciseDX)	LOW	5	1	0
	NORMAL	1	7	0
	HIGH	0	0	10

LOW – trough infliximab levels up to 3 µg/mL
NORMAL – trough infliximab levels between 3.01 to 15 µg/mL
HIGH – trough infliximab levels over 15 µg/mL
Number of observed agreements: 22 (91.67 % of the observations)
Unweighted Kappa = 0.872
Standard error of Kappa = 0.085
95% confidence interval: from 0.705 to 1.00
*Weighted Kappa = **0.903***

TL ADL		ELISA		
		LOW	NORMAL	HIGH
FRET (ProciseDX)	LOW	5	0	0
	NORMAL	0	5	1
	HIGH	0	2	8

LOW – trough adalimumb levels up to 4.5 µg/mL
NORMAL – trough adalimumab levels between 4.51 to 10 µg/mL
HIGH – trough adalimumab levels over 10 µg/mL
Number of observed agreements: 18 (85.71 % of the observations)
Unweighted Kappa = 0.778
Standard error of Kappa = 0.120
95% confidence interval: from 0.542 to 1.00
*Weighted Kappa = **0.834***

Table 2 **Repeatability of infliximab and adalimumab trough level's FRET (ProciseDX) measurements in the capillary blood samples - analysis of 6 measurements in a single patient**

TL IFX - trough infliximab levels
TL ADL - trough adalimumab levels

	TL IFX, µg/mL	TL ADL, µg/mL
MEAN	17,65	14,85
MEDIAN	17,8	14,65
SD	1,47	2,94
MIN	15,8	11,6
MAX	19,6	18,7
1ST QUARTILE	16,2	11,9
3RD QUARTILE	18,7	17,6

Conclusion

Point-of-care detection of IFX and ADL trough levels by FRET is accurate, comparable to the reference method, analysis is sufficiently consistent. For clinical sites with a proactive approach to TDM, FRET may be a useful tool for decision-making regarding individualization of biologic therapy

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