Investigating the link between disease activity and infliximab serum levels in rheumatoid arthritis patients

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Abstract

Objective

The aim of this study was to examine the extent to which infliximab (IFX) serum levels impact disease activity in rheumatoid arthritis (RA) patients.

Methods

In this cross sectional study, serum samples were taken prior to drug infusion from 60 RA patients who had been undergoing IFX therapy >12 months as a first line of biological treatment. Patient IFX levels were tested and then associated with clinical disease activity. Three DAS28 cut-off points, <2.6, <3.2 and <5.1 were used to determine whether detectable IFX levels were any predictor of clinical disease activity. Logistic regression analysis was run to check other possible factors associated with RA clinical outcomes such as MTX concomitant use, CRP and ESR.

Results

Sixteen (27%) out of the 60 patients tested negative; 28 (46%) presented subtherapeutic and 16 (27%) therapeutic IFX levels. Median IFX levels were higher in patients either in remission or showing low disease activity than in those with moderate and high disease activity (p=0.014). Significant association was found between IFX levels and clinical disease activity (p=0.001). Detectable levels of IFX shows better sensitivity and specificity to identify patients with DAS28<3.2 than to identify patients with DAS28<2.6 or DAS28<5.1. Conversely, the best DAS28 cut-off to identify detectable/undetectable IFX was 3.19, with AUC under ROC curve 0.804 (Sd.E 0.070), 76% specificity and 83% sensitivity (p<0.001). MTX use, CRP and ESR did not interfere with this association. Seven out of the 8 patients with anti-IFX antibodies presented DAS28>3.2 (p=0.005).

Conclusion

DAS28 and IFX serum levels were shown to have an inverse correlation. Undetectable IFX serum levels were associated to RA patients presenting DAS28>3.2 meaning that DAS28 <3.2 may be useful to clinicians to evaluate patient response to drug therapy.

Key words

rheumatoid arthritis, anti-TNF, infliximab, DAS28, disease activity, clinical response, biologics monitoring, enzyme-linked immunosorbent assay.

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Introduction

Disease progression in RA is known to be linked to many of the following indicators: rheumatoid factor (RF), anti-citrullinated peptide antibodies (ACPA), degree of disease activity as measured by the Disease Activity Score in 28 Joints (DAS28), and presence of acute phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values (1). Anchoring the treatment of RA is methotrexate (MTX) (2), a synthetic disease-modifying anti-rheumatic drug (sDMARD), effective as a standalone and combination therapy drug. Patients failing to respond to DMARDs with poor prognostic markers might be considered for a combination therapy of MTX plus a biological DMARD (bDMARD) (3, 4). This combination has resulted in improved physiological and clinical outcomes as well as decreased radiographic progression since the recent introduction of bDMARDs (5).

Infliximab (IFX, Remicade®, Centocor Ortho Biotech Inc.) is a chimeric monoclonal IgG1 antibody against tumour necrosis factor (TNF) that is approved for the treatment of rheumatoid arthritis (RA) (6) and other chronic autoinflammatory diseases (7-9) and has been proved to be highly effective in inducing and sustaining remission (10). Nevertheless, up to 40% of treated patients either fail to respond or lose response to it over time. Several factors are thought to influence negative response such as duration of treatment, baseline CRP values, use of concomitant immunosuppressive drugs and the formation of anti-drug antibodies (ADA) to IFX (11-15).

There is evidence which points to a link between disease activity and trough serum IFX levels and ADA, however, to date, the presence of ADA, low drug levels or both does not fully explain the lack or loss of response to treatment in all patients (16). The ELISA bridging assay is the most widely used technique to determine drug levels owing to cost and practicality. The problem, however, is that detection parameters have not been standardised meaning cut-off points are inconsistent between assays when the test result is positive (17).

There is a need to monitor drug levels in daily clinical practice so as to evaluate treatment efficacy and subsequent impact on disease activity (18). The primary aim of this study is to investigate whether there is a link between disease activity and IFX levels and the secondary aim is to evaluate whether RA-related outcome factors could influence the association between IFX levels and DAS28.

Materials and methods

Patients

For this cross-sectional observational study, a total of 60 patients diagnosed with RA under the American College of Rheumatology 1987 criteria (19) were recruited at the Department of Rheumatology of the Gregorio Marañón University General Hospital in Madrid and Marina Baixa Hospital in Villajoyosa (Spain) after signing the informed consent. This study was approved by the Medical Ethics Committees of both hospitals. Inclusion criteria were to be undergoing treatment with IFX, as a first line of biological therapy, and to have been receiving standard doses at 3mg/kg/8 weeks for at least 12 months. Disease activity was measured using the 28 joint count-disease activity score (DAS28), factoring in the erythrocyte sedimentation rate (ESR) and standard clinical variables for RA, *i.e.* swollen tender joints count (SJC/ TJC) and visual analog scales (VAS) for pain estimation. Disease activity status was classified as remission/non activity when DAS28<2.6; low activity, when ranging from 2.6 to 3.2; moderate when between 3.2 and 5.1, and high when >5.1.

Determination of IFX and ADA levels

A blood sample was taken from all 60 patients prior to infusion with IFX and centrifuged. Serum was frozen and stored at -80ºC. IFX serum levels were measured by an enzyme-linked immunosorbent assay (ELISA) Promonitor®-IFX kit (Progenika Biopharma, Spain) under blinded conditions, in strict adherence with the manufacturer's guidelines. The samples were sequentially diluted and a standard curve was constructed using a standard solution included in the kit. IFX serum levels of <0.053 μg/ml indicate negative; 0.053-

1.5 μg/ml, low positive and >1.5 μg/ml positive, which are interpreted and categorised as negative, subtherapeutic and therapeutic levels, respectively, mirroring the assay's data sheet.

Anti-IFX antibodies were detected by the ELISA assay Promonitor®-IFX kit (Progenika Biopharma, Spain) which tests as positive when >37 AU/ml, according with the manufacturer's guidelines. Previously-diluted samples were analysed in accordance with the standard curve included in the kit. Absorbances (OD) were analysed using the Analysis Software Solutions (MyAssays, Ltd 2009).

Statistical analysis

Quantitative variables are summarised as mean and standard deviation (SD) or median and interquartile ranges (IQR). Qualitative variables are summarised as frequencies and percentages.

The chi-squared test was used to evaluate the association between IFX levels catagorised as either negative, subtherapeutic or therapeutic or disease activity categorised according to DAS28 as remission, low activity, moderate activity and high activity. Categories were recoded if needed to avoid cells with expected frecuencies <5.1.Haberman residuals were used to detect cells with frequencies which significantly depart from independence hypothesis.

IFX levels were dichotomised as undetectable when <0.053 μg/ml (negative); or detectable when >0.053 μg/ml, which includes subtherapeutic and therapeutic levels. This was used to compute sensitivity (SE), specificity (SP), positive and negative predictive value (PPV and NPV) of detectable IFX levels to identify patients with DAS28 lower than 2.6 (remission); 3.2 (low) and 5.1 (moderate activity). Conversely a ROC curve was plotted to study the best DAS28 cut-off to identify patients with detectable/undetectable IFX levels.

The Mantel-Haenszel test was used to check association between dichotomised IFX levels and dichotomised DAS28 after controlling the effect of: i) MTX treatment (yes/no) ii) CRP (<0.5 mg/dl/>0.5 mg/dl) and iii) ESR (<14 mm/h />14 mm/h). To study factors associated to DAS28 moderate and high disease activity (≥3.2) a logistic regression model with a dichotomic DAS28 outcome lower or higher than 3.2 was run. First an univariate regression model was run using MTX treatment, dichotomised CRP and ESR and undetectable IFX levels as independent variables. Multivariate model using forced entry method and stepwise method was also run.

P-values <0.05 were considered significant. All analyses were performed using SPSS V.21.0 (SPSS, Chicago, Illinois, USA) and Prism version 5.03 Software (GraphPad Software© Rockfield, CA, USA).

Table I. Main demographic data of the patients included in the study.

Number of patients	n=60
Female gender n (%)	47 (78%)
Age/ years (mean, SD)	63 ± 12a
Concomitant DMARD use n (%)	49 (81.6%)
Concomitant MTX use n (%)	36 (60%)
IFX dose/ mg/kg (mean, SD)	3.5 ± 0.6
Duration of infliximab therapy/ years (mean, SD)	5.9 ± 1.24
DAS28 (mean, SD)	3.02 ± 1.25
RF positive n (%)	32 (53.3%)
Anti-CCP positive n (%)	45 (75%)
CRP/ mg/dl (mean, SD)	0.554 ± 0.59
ESR/ mm/h	15.8 ± 10.8
Infliximab concentration/ μg/ml (median, IQR)	0.65 (0.052-1.52)

SD: standard deviation; DMARD: disease-modifying anti-rheumatic drugs; IFX: infliximab; DAS28: Disease Activity Score (28 joints); RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide antibody; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IQR: interquartile ranges.

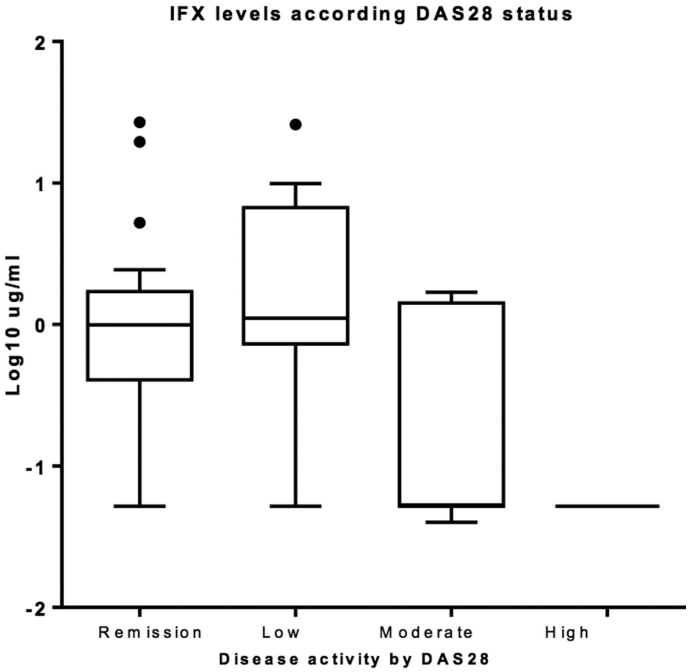

Fig. 1. IFX levels in patients with RA according to disease activity status (DAS28). Horizontal lines indicate median and IQR values.

Results

Demographic and clinical data

Demographic and clinical characteristics were obtained from the electronic medical database (MixeTB™ HGUGM and AIRE Marina Baixa) in those patients that met entry criteria (Table I). In brief, the majority of the 60 patients who entered the study were women (78%), with mean (SD) age of 63 (12) years. IFX was used as a first line of biological treatment due to inadequate response to previous conventional therapy. Mean (SD) treatment time with IFX was 5.9 (1.24) years. The most widely used disease modify-

ing anti-rheumatic drug (DMARD) was methotrexate (MTX) (60% of patients). Mean (SD) of serum C-reactive protein (CRP) concentration and erythrocyte sedimentation rate (ESR) was 0.365 mg/dl (0.59) and 15.8 mm/h (10.8), respectively. None of the studied patients presented chronic impairment of renal or liver function.

IFX levels and clinical disease activity

Sixteen out of the 60 patients (30%) presented negative IFX levels, 28 patients subtherapeutic IFX levels (43.3%; 0.73 μg/ml, IQR 0.44-1.09 μg/ml) and 16 therapeutic IFX levels (26.7%; 3.9 μg/ml, IQR 1.8-10.9 μg/ml). Spearman's correlation index of -0.316 (p=0.014) shows that there is a significant monotonically decreasing relationship between DAS28 and IFX levels, where DAS28 is reduced IFX level is increased. Boxplots in Fig. 1 show descriptive statistics of the logarithm of IFX levels in the four categories of DAS28 status; remission, low, moderate and high disease activity. Median log-IFX levels were higher in remission and low disease activity than in moderate and high disease activity.

Categorical IFX levels and DAS28 status

Significant association was found (p=0.001) between IFX levels (negative, subtherapeutic and therapeutic) and DAS28 status using a chi-squared test. Thirteen out of 16 patients with negative IFX levels (81.3%) showed moderate or high disease activity; the other 3 IFX-negative patients (18.8%) were either in remission or presented low disease activity. Conversely, 4 out of 16 patients with therapeutic IFX levels (25.0%) presented moderate disease activity whereas the other 12 patients with therapeutic IFX levels (75.0%) were either in remission or showed low disease activity (Table II). Haberman residuals demonstrated that the proportion of patients with negative IFX levels showing moderate and high disease activity was significantly higher than expected (p<0.05), whereas the proportion of patients with negative IFX levels showing remission and low activity was lower than expected (p<0.05).

Table II. Association between IFX categorical levels and DAS28 status.

DAS28 status	IFX levels							
	Negative		Low positive		Positive		Total	
	n	%	n	%	n	%	n	%
Remission	2	12.5%	14	50.0%	8	50.0%	24	40.0%
Low	1	6.3%	6	21.4%	4	25.0%	11	18.3%
Moderate	9	56.3%	8	28.6%	4	25.0%	21	35.0%
High	4	25.0%	0	0.0%	0	0.0%	4	6.7%
Total	16	100%	28	100%	16	100%	60	100%

χ^2-test: for analytical purpose remission and low activity were grouped in one single category (remission/low activity) and moderate and high activity were grouped in one single category (moderate/high activity). χ^2: 0.001; df. 2; p=0.001.

Table III. Sensitivity, specificity, positive and negative predictive values of detectable IFX levels (>0.053 μg/ml) to predict disease activity with DAS28.

DAS28 cut-off	Disease activity	Detectable IFX levels			
		SE	SP	PPV	NPV
DAS<2.6	Remission	48%	89%	0.91	0.42
DAS<3.2	Low	76%	78%	0.89	0.58
DAS<5.1	Moderate	100%	22%	0.75	1.00

Detectable IFX levels and DAS28 cut-off

Levels of IFX were dichotomised as undetectable or detectable (≤0.053 μg/ml or >0.053 μg/ml, respectively) in accordance with the assay's instructions. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to detect three different DAS28 cut-offs: remission (DAS28 <2.6), low (DAS28 <3.2) and moderate disease activity (DAS28 <5.1), all of them considered independently (Table III). Although specificity of detectable IFX levels to detect DAS28<2.6 was high (89%), the sensitivity was low (48%). We found that sensitivity of detectable IFX levels to detect DAS28<5.1 was perfect, but the specificity was very low (22%). The sensitivity and specificity of detectable IFX levels to detect DAS28<3.2 were 76% and 78%, respectively. Conversely, ROC analysis using undetectable and detectable IFX levels as classification variables was run to establish the optimal DAS28 cut-off points to classify IFX levels. Optimal DAS28 cut-off was found to be 3.19, with AUC 0.804 (Sd.E. 0.070), p<0.001 with 83% sensitivity and 76% specificity. (Fig. 2) These findings indicate that detectable IFX levels show greater sensitivity and specificity in terms of detecting low disease activity (DAS28 <3.2) than other DAS28 cut-offs.

IFX levels and factors associated to RA outcome

The association between undetectable IFX levels and DAS28>3.2 was not significant when the Mantel-Haenszel test was used to control the effect of MTX treatment (yes/no), CRP (<0.5 mg/dl/>0.5 mg/dl) and ESR (<14 mm/h />14 mm/h). Mantel-Haenszel χ^2 were 12.113 (p=0.001); 10.862 (p=0.001); and 12.113 (p=0.001), respectively. The univariate logistic regression model only found significant association between undetectable IFX levels and moderate and high DAS28 disease activity (p<0.001) as well as multivariate models with forced entry of variables (Table IV). When stepwise method were run only the IFX levels was retained in the final model. The odds ratio for undetectable IFX levels was 11.200 (CI95%: 2.996-41.871), which indicates that probability of DAS28>3.2 is much greater in patients with undetectable IFX levels.

IFX-ADA concentration and DAS28 status

Eight patients (13.3%) showed positive IFX-ADA and 52 patients (86.7%)

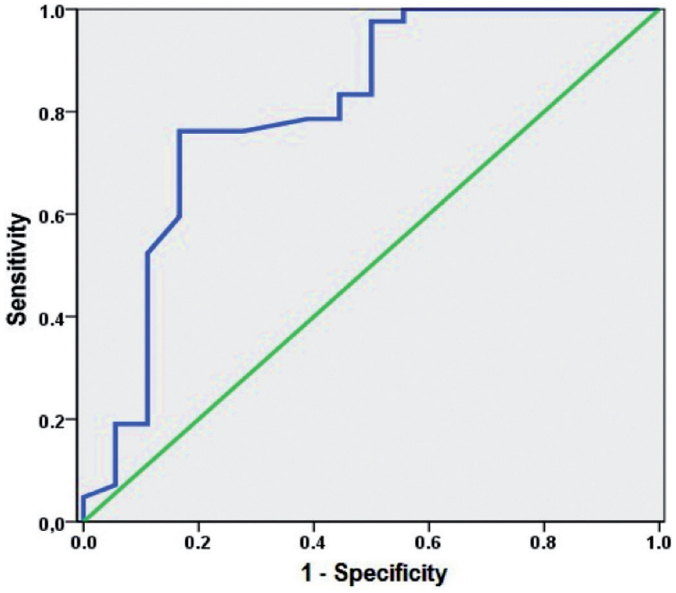

Fig. 2. ROC curves analysis using disease activity (DAS28) in RA patients to predict IFX undetectable or detectable.

negative IFX-ADA. A significant association between ADA positive and undetectable IFX levels was found. Eight out of 18 patients with undetectable IFX levels (44.4%) *versus* 0 out of 42 patients with detectable IFX levels showed positive ADA (Fisher exact test; $p<0.001$). Regarding DAS28 status, one out of 36 patients (2.8%) with a DAS28<3.2 showed positive ADA, whereas 7 out of 24 patients (29.2%) with DAS28>3.2 showed ADA positive ($p=0.005$). When ADA was positive, DAS28≥3.2 was predicted with 29% SE, 97% SP, with an 88% and 67% of PPV and NPV, respectively.The presence of positive ADA was significantly associated to undetectable IFX levels and to DAS28<3.2.

Discussion

The study aimed to correlate IFX levels and disease activity in RA patients. We tested a standard kit assay and considered three possible clinical situations using the RA DAS28 gold standard. Furthermore we evaluated whether other RA-related outcome factors influenced IFX levels and DAS28.

It is well known that there is an association between IFX levels and disease activity and our study has confirmed these results. Highest rates of test sensitivity and specificity were evident when IFX was present in patients presenting low disease activity. Conversely, when evaluating patients in remission, IFX level determination resulted in lower sensitivity. We think that a DAS28≤3.2 serves as a useful benchmark when determining serum IFX levels to evaluate patient response to therapy.

Besides the DAS28-IFX relationship, we also looked at other disease markers which might have influenced results. It is reported that the concomitant use of MTX with IFX favour a more sustained remission due to a synergistic effect (20-22). Our results showed the use of MTX had no effect on the DAS28-IFX levels relationship. Of note, seven out of eight ADA positive patients were undergoing MTX concomitant treatment, which is interesting as MTX can influence the rate of ADA formation (23, 24). It is widely accepted that CRP levels are a disease activity marker in RA patients and that, unlike ESR, they have been associated to anti-TNF therapy response (25) *i.e.* high IFX levels correlate with low CRP levels (26, 27). Our particular study did not reveal any evidence that CRP and ESR levels had any impact on either IFX levels or treatment response which suggests that IFX levels are more sovereign in terms of influencing RA disease activity than the aforementioned two factors.

The usefulness of establishing anti-TNF drug levels for clinical purposes has been proven beyond reasonable doubt, in our view. Nevertheless, translating accurate cut-off points into every day clinical practice is proving to be more fraught, not least due to assay discrepancies in terms of sensitivity, specificity, lower limit detection and diverse cut-off points (28). We consider that the qualitative results (detectable/undetectable) might be a better approach to be correlated with disease activity. We hope our results mean we can move closer to creating an internationally-accepted scale. In particular, we found that values greater than 0.053 μg/ml, including subtherapeutic and therapeutic levels, were predictors of low disease activity; unlike other authors who described an optimal cut-off level greater than 1μg/ml (26, 29, 30). We surmise that optimal design for IFX level determination strongly depend on the biological context of the assay, disease and patients.

Evidence collected by Vicent *et al*. (31), in a review published in 2013, shows that results vary not only in terms of the used assay, but also in definition of cut-off values and therapeutic range of drug levels and how results should be interpreted by the clinician and this is why we believe that categorical results

Table IV. Univariate and multivariate regression logistic models with DAS28 (< 3.2 or ≥ 3.2) as dependent variable.

	Univariate regression models			Multivariate regression model (forced entry method)		
Independent variable	OR	CI95%	*p*	OR	CI95%	*p*
MTX treatment (yes / no)	1.600	0.547-4.681	0.391	1.131	0.321-3.978	0.848
CRP (≤0.5 mg/dl / <0.5 mg/dl)	2.273	0.780-6.620	0.132	1.204	0.301-4.814	0.793
ESR (≤14 mm/h / >14 mm/h)	1.250	0.444-3.521	0.673	1.111	0.305-4.044	0.873
IFX (≤0.053 μg/ml / <0.053 μg/ml)	11.200	2.996-41.871	<0.001	10.378	2.626-41.006	0.001

and establishment of cut-off therapeutic drug ranges could be more helpful in terms of potential use in clinical practice. To date there is no consensus regarding optimal therapeutic IFX levels with studies published differing on cut-off values for their respective clinical practice. However, there is a very clear need to standardise and to perform comparative studies between laboratory assays if universal clinical guidelines are to be drafted.

Undetectable IFX levels was associated with DAS28>3.2, whether IFX levels are categorised as if categorises the DAS28. Our findings suggest that in patients with DAS28>3.2, IFX levels should be monitored to determine drug bioavailability because the probability to have undetectable IFX levels is 11.2 folds higher than in those patients with DAS28<3.2. On the one hand, if a patient presents a DAS28>3.2 and detectable IFX levels, a different biological therapy might be considered. On the other hand, if a patient has a DAS28>3.2 and undetectable IFX levels, there is a greater likelihood the patient could have developed ADA, so we should consider screening for those. For instance, in our study seven out of sixty patients with positive ADA simultaneously presented moderate and high disease activity, therefore therapeutic alternatives would need to be considered in light of DAS28 assessment (32).

There are some limitations to be mentioned, *i.e.* the number of patients studied and the cross-sectional nature of our study. Longitudinal studies will be needed to determine optimal cut-off points taking into consideration biological variability between patients. We considered the ADA positive sample size too small to be associated with IFX levels and disease activity. Other studies using similar techniques of mirroring IFX levels with disease activity in RA patients described higher median IFX levels in patients with low disease activity than in patients with high disease activity. Therefore the nature of this study is not unprecedented and it is worth noting other research areas which have tried to establish a link between IFX levels and disease outcomes, for instance, in inflammatory bowel disease (IBD) (33, 34).

Conclusion

This study reinforces the importance of accurate IFX levels determination, particularly in RA patients with moderate to high disease activity under the DAS28 scale. A DAS28 score <3.2 could be considered pivotal in the evaluation of patient response to IFX. Our results show no correlation between MTX concomitant use, CRP and ESR levels and clinical response to IFX treatment.

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