Cut-off ranges of infliximab serum levels in Crohn's disease in the clinical practice

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Received: 12/08/2019 · **Accepted:** 23/01/2020

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ABSTRACT

Introduction: between 30 % and 40 % of patients treated with infliximab lose response during maintenance. Therapeutic drug monitoring could be used to optimize management in these situations. However, infliximab serum levels are not well defined. The aim of this study was to determine the cut-off range of infliximab serum levels in Crohn's disease patients in remission in the clinical practice.

Methods: an observational retrospective study was performed from 2016 to 2017. Patients were included with established Crohn's disease, who had been on a maintenance dose schedule of infliximab. Infliximab levels and antibodies to infliximab were measured at least twice in all patients, after induction and after six months of treatment. Clinical remission was defined as ≤ 4 using the Harvey-Bradshaw index. Cluster analysis was used to analyze the results.

Results: one hundred and five Crohn's disease patients were included in the study; 57.1 % were male with a mean age of 39 years (SD ± 12.9). The median (range) time of the disease was eleven years (7-15) and the median (range) time of follow-up was 32 months (22-38). Patients who achieved remission had infliximab serum levels between 4.26-8.26 ug/ml *versus* 0.06-1.43 ug/ml in patients who did not achieve remission after induction. Infliximab serum levels were 2.84-7.75 ug/ml and 0.05-2.69 ug/ml in patients who achieved remission *versus* those who did not achieve remission after six months of treatment. Overall, 4.26-8.26 ug/ml was found to be the best cut-off range for remission.

Conclusions: in our clinical practice, serum levels of infliximab in Crohn's disease patients should be higher than 4 ug/ml to achieve clinical remission.

Keywords: Infliximab levels. Infliximab antibodies. Therapeutic drug monitoring.

INTRODUCTION

Biological therapies introduced two decades ago, such as monoclonal antibodies targeting TNF, have revolutionized the treatment of inflammatory bowel diseases (IBD) (1). Infliximab (IFX) was the first drug approved by the European Medicines Agency (EMA) (2) for the treatment of moderate to severe Crohn's disease (CD). In pivotal clinical trials, IFX was effective for inducing and maintaining clinical remission (3,4).

Despite its efficacy, up to 20-40 % (5) of patients who were in clinical response will experience a secondary loss of response, with an annual risk of loss of response of 13-15 % patients/year (6). This loss of response could be due to the increased clearance of the drug in the presence of antibodies to IFX (ADA), which has been reported to be around 5-18 % (7,8). The empiric strategy recommended for the management of the secondary loss of response is intensifying the dose (increasing dosage or frequency) as well as optimizing the immunosuppressive drugs (9). In fact, the success of this strategy has been described in some studies (10,11). However, some studies have suggested that an optimization therapy based on IFX drug monitoring (TDM) is a more cost-effective strategy in patients with a loss of response (12,13).

In the post-hoc analysis of the ACCENT-1 trial, the best predictive response to IFX therapy was an IFX level of ≥ 3.5 ug/ml in week 14. Furthermore, in the Trough Concen-

Valdés Delgado T, Guerra Veloz MF, Castro Laria L, Maldonado Pérez B, Perea Amarillo R, Merino Bohórquez V, Sáez A, Caunedo Álvarez Á, Argüelles-Arias F. Cut-off ranges of infliximab serum levels in Crohn's disease in the clinical practice. Rev Esp Enferm Dig 2020;112(10):756-761

DOI: 10.17235/reed.2020.6539/2019

tration Adapted Infliximab Treatment (TAXIT) (14) study, higher sustained remission rates in CD patients with IFX levels around 3-7 ug/ml were reported. In the Yarur AJ et al. (15) study, IFX levels ≥ 10 ug/ml improved rates of fistula healing. However, the optimal IFX trough levels remain unclear. We have been measuring IFX levels and ADA for three years to optimize our biological therapy, using the ranges obtained in the TAXIT study. Nevertheless, we have noticed that the cut-off range both in our patients and in our clinical practice are not the same as in the previously mentioned study.

Therefore, the aim of this study was to find the cut-off range of IFX serum levels in CD patients in remission in the clinical practice.

MATERIALS AND METHODS

Study design

This was a retrospective observational study performed at the Hospital Virgen Macarena (Seville, Spain) from 2016 to 2017. The study was approved by the Research Ethics Committee of the Hospital Virgen Macarena and was performed following good clinical practice guidelines. All study participants or their legal guardians provided informed written consent prior to study enrollment.

Patients

The cohort included adult patients aged > 18 years with established CD who had been on a maintenance dose schedule of IFX. The serum trough levels of IFX and its antibodies were measured in all patients after induction (IFX level I) and after six months of treatment (IFX level II). These values were correlated with the clinical status of the patient. The Montreal classification status of all patients before enrollment was recorded.

Laboratory protocol

All the blood samples were collected according to standard operating outpatient procedures, before IFX infusion. All the tests were performed using an enzyme-linked immunosorbent assay (ELISA) with Progenika kits (PROMONITOR®).

Analysis

Clinical remission was defined using the Harvey-Bradshaw index (HBI ≤ 4). IFX levels were measured in all patients at two different times, after induction (IFX level I) and after six months of treatment (IFX level II). These values were correlated with the patient's clinical status by cluster analysis. Each IFX level was analyzed and compared with the patient's response. IFX was administered at a standard maintenance dose of 5 mg/kg every eight weeks. Changes in dose were administered by the clinicians in order to reach clinical remission.

Statistical analysis

Demographic and nominal results were reported as percentages and frequencies. Numerical results were reported as the average and standard deviation in cases of a normal distribution and as the median and interquartile range (IQR) in cases of a skewed distribution.

Cluster analysis was performed in two phases to find the groups with a predictable remission status. The cluster analysis used was called two-stage because the algorithm used by the SPSS statistical program to obtain the results is executed internally in two steps. The first of these consists in the formation of pre-conglomerates of the original cases and in the second step, the optimal number of conglomerates is determined according to the Bayesian Schwarz Criterion (BIC) or the Akaike Information Criterion (AIC). This type of analysis allows us to classify groups by qualitative and quantitative criteria. The referral prediction was based on IFX levels (level I and level II).

One of the main reasons for using cluster analysis is that since the IFX level is a quantitative variable, it allows ranges of values to be obtained according to the IQR in order to classify the patients in Remission as YES or NO. This also includes an area of uncertainty, where patients cannot be classified with a high reliability. Other tools such as logistic regression allow the percentage of patients correctly classified to be obtained, but it does not provide information about which patient groups are difficult with a good reliability according to IFX.

The quality of the results was based on the measure of the silhouette of cohesion and separation, referred to by Kaufman and Rousseeuw in 1990 (16). Confidence intervals (CIs) were calculated at 95 % and the value $\alpha = 0.05$ was adopted as the level of statistical significance. Statistical analysis was performed using SPSS 25 (IBM Corporation).

RESULTS

One hundred and five CD patients were included in the study; 57.1 % were male, with a mean age of 39 years (SD ± 12.9). The median (range) time of the disease was eleven years (7-15) and the median (range) time of follow-up was 32 months (22-38). Baseline demographics and phenotypic characteristics of the patients are shown in table 1; 265 IFX levels were measured during follow-up.

At the start of the study 61.9 % (65/105) of patients were in remission and 62.9 % (66/105) were in remission at the end of the study. Also, 61.9 % (65/105) were on the standard maintenance dose with IFX at the start of the study. During follow up, 24.7 % (26/105) of patients were given an increased dose and 7.6 % (8/105) were given a reduced dose. The median of IFX level I was 2.8 ug/ml (0.43-7.2) and IFX level II was 4 ug/ml (0.8-6.8 ug/ml) (Fig. 1). ADAs developed in 8.6 % (9/105) of patients, with a median IFX level of 0.01 ug/ml (0.01-2.2 ug/ml). After induction, five patients developed ADAs with a median value of 84.4 U/ml (24-196) and four after six months, with a median value of 5.7 U/ml (3.98-58).

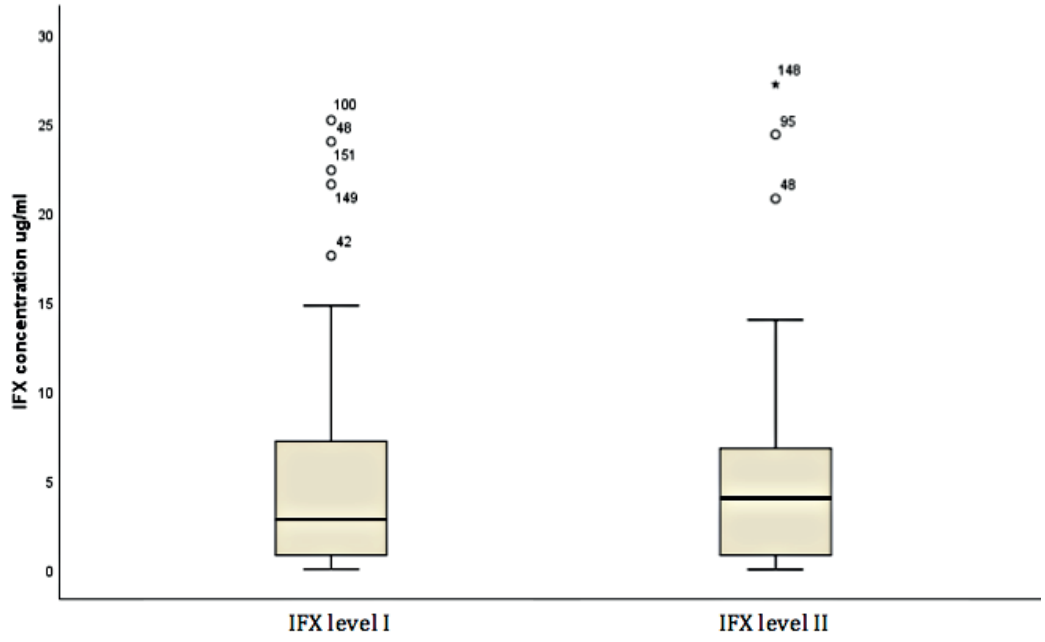
IFX interval concentration

IFX level I and remission status

The quality of the cluster was 0.72 (Fig. 2). Remission was achieved in patients who had IFX serum levels between 4.26-8.26 ug/ml.

Table 1. *Demographic characteristics of the patients*

Characteristics of CD patients (n = 105)		n (%)	CI (95 %)
Sex	Males	60 (57.1)	47.2; 67.1
	Females	45 (42.9)	32.9; 52.8
Age		37 (27-47)	
Age at diagnosis	A1 (< 17)	11 (10.5)	4.1; 16.8
	A2 (17-40)	80 (76.2)	67.6; 84.8
	A3 (> 40)	14 (13.3)	6.4; 20.3
Location at diagnosis	L1 (ileal)	29 (27.6)	18.6; 36.6
	L2 (colonic)	37 (35.2)	25.6; 44.9
	L3 (ileocolonic)	36 (34.3)	24.7; 43.8
	L3 + L4 (upper gastrointesinal tract)	3 (2.9)	0.6; 8.1
Disease behavior	B1 (nonstricturing, nonpenetrating)	56 (53.3)	43.3; 63.4
	B2 (stricturing)	23 (21.9)	13.5; 30.3
	B3 (penetrating)	26 (24.8)	16.0; 33.5
Perianal disease	Yes	51 (48.6)	38.5; 58.6
Naive IFX (IFX biosimilar)		42 (40)	30.1; 49.8
Switch IFX		54 (51.2)	41.4; 61.5
Infliximab original (Remicade®)		9 (8.6)	2.7; 14.4
Median age at diagnosis		26 (19-35)	
Median age at IFX initiation (IQR)		34 (24-43)	



IFX level I: after induction

IFX level II: after six months

Fig. 1. *IFX concentrations.*

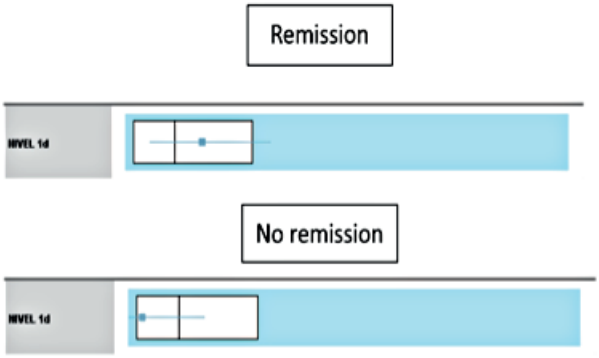


Fig. 2. *Cluster analysis. IFX levels in time I.*

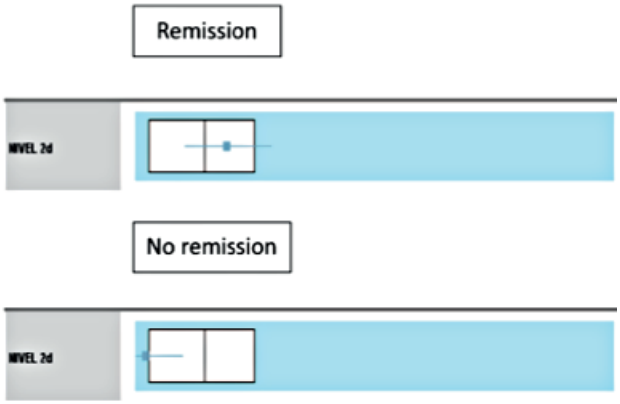


Fig. 3. *Cluster analysis. IFX levels in time II.*

Patients who did not achieve remission had levels between 0.06-1.43 ug/ml. The uncertainty zone was between 1.43-4.26 ug/ml. The uncertainty zone means that there is a range of IFX values where the patient is not classified with a good reliability. Thus, providing information for the correct classification with a high success rate for the rest of IFX values.

IFX level II and remission status

The quality of the cluster was 0.78 (Fig. 3). Remission was achieved in patients who had IFX serum levels between 2.84-7.75 ug/ml. Patients who did not achieve remission had levels between 0.05-2.69 ug/ml. The uncertainty zone was between 2.63-2.84 ug/ml. The most restrictive interval of IFX concentration related to remission was 4.26-8.26 ug/ml (Table 2).

Multivariable analysis

Age, sex, perianal disease and time under IFX therapy did not appear to have any influence on IFX serum levels in the multivariable analysis.

DISCUSSION

It has been reported that up to 20-40 % (5) of patients who were in clinical response will experience a secondary loss of response. An annual risk for loss of response could occur in around 13-15 % patients/year (6). Therapeutic drug monitoring might help us to prevent this loss of response (12,13). However, the optimal IFX trough levels remain unclear.

Table 2. *Intervals of infliximab concentration (ug/ml)*

Crohn's disease			
IFX level I	**IFX level II**	**Intervals**	**Clinical response**
0.06-1.43	0.05-2.69	0.06-1.43	No remission
1.43-4.26	2.69-2.84	1.43-4.26	Uncertainty zone
4.26-8.26	*2.84-7.75*	*4.26-8.26*	*Remission*

The aim of our study was to obtain evidence of IFX levels in our patients in remission and compare them with data recently published. In our clinical practice, it was demonstrated with a high level of certainty that the best range for predicting remission was 4.26-8.26 ug/ml. After this analysis, we have implemented a proactive attitude to TDM in our hospital, intensifying IFX treatment in those cases in which levels are under this range.

Over the last few years, most published studies have explored the available evidence of the relationship between IFX trough levels and remission (17,18). Vande Casteele N et al. (19) reported that an IFX concentration of

> 2.79 µg/ml (AUC = 0.681; 95 % CI: 0.632-0.731) and ADA concentration of < 3.15 U/ml (AUC = 0.632; 95 % CI: 0.589-0.676) were associated with remission. In the multivariable analysis, they showed that the drug concentration and ADA were independent predictors of remission. Some years later, the same authors reported that patients reaching drug concentrations between 3-7 ug/ml were more likely to maintain remission (14). In our study, using cluster analysis, it was determined with a high level of certainty that IFX levels between 0.06-1.43 ug/ml were associated with no response. However, an important area of uncertainty between 1.43-4.26 ug/ml was found that should be further discussed. Perhaps, patients with mild CD could be in remission with this range. Unfortunately, no answer to this was found in this study. Some authors suggest that higher levels are likely needed compared to the levels required for clinical remission in order to neutralize systemic inflammation and achieve deep remission with mucosal healing (20-22). Furthermore, Roblin et al. (23) showed in a prospective study that the trough levels of IFX differ according to the therapeutic outcomes expected in patients under IFX maintenance therapy. Patients who were in clinical remission with normal CRP and fecal calprotectine had higher IFX-levels (5.9 *versus* 2.1 ug/ml, p < 0.001) in comparison to those who were in clinical remission without strictly normal biomarkers. In our study, the therapeutic outcome was only clinical remission, which could be considered as a study limitation.

The American Gastroenterological Association (AGA) presented its guidelines on TDM in IBD in 2017. They suggested that trough concentrations of ≥ 5 ug/ml should be achieved in patients who are experiencing secondary loss of response in order to obtain remission (24). These levels could be considered as similar to ours.

There are some predictors of loss of response of anti-TNF therapies such as patient factors (gender, smoking, weight), disease characteristics (type, location, severity) and drug factors (pharmacokinetic, pharmacodynamics, immunogenicity) (25,26). Immunogenicity, the propensity for patients to develop anti-drug antibodies against the monoclonal agents, can result in increased drug clearance that directly leads to reduced trough levels. This can lead to loss of response, infusion reactions and the need for dose intensification or the need to switch to another therapy (27). Antibodies against IFX can be found in 8-60 % of patients (8,20) and may form soon after the first infusion. In our study, 8.6 % of patients developed ADAs with a median IFX level of 0.01 ug/ml. Our patients had a median ADA level of 84.40 U/ml and 5.55 U/ml at the first and second tests, respectively. It is known that low IFX levels (< 1 µg/ml) and the presence of detectable antibodies are associated with worse clinical outcomes (8). Steenholdt et al. (28) concluded that IFX < 0.5 µg/ml associated to anti-IFX antibodies ≥ 10 U/ml yielded the highest overall accuracy to determine loss of response.

Our study is inherently limited by the retrospective nature of the design. The data were recorded using the clinical electronic records of patients and this could result in a loss of information. Furthermore, another important limitation of our study is that remission was based only on clinical symptoms and biomarkers were not used in this study. Therefore, IFX levels and biomarkers of remission such as PCR, fecal calprotectine or mucosal heading were not correlated. On the other hand, we consider that this study is very useful in our clinical practice, because we have implemented a proactive attitude to TDM based on our results.

In conclusion, this study provides valuable data on the levels of IFX, which should be reached in patients with CD in our clinical practice. Likewise, it has been possible to establish the cut-off points of IFX for which clinical remission is expected for patients with CD (4.26-7.75 µg/ml). These data closely resemble those reported by the reference studies in this field of work and therefore, could be used in our clinical practice to optimize treatment with IFX.

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