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Alimentary Tract

Clinical relevance and inter-test reliability of anti-infliximab antibodies and infliximab trough levels in patients with inflammatory bowel disease

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ABSTRACT

Background: Treatment with infliximab is a common option for inflammatory bowel disease (IBD) patients. Therapeutic drug monitoring could improve treatment management.
Aims: To test inter-test reliability of two commercially available diagnostic kits for infliximab trough levels and infliximab antibodies, and their association with treatment outcomes.
Methods: 86 IBD outpatients on infliximab maintenance treatment were enrolled in a prospective cross-sectional study, 115 samples were available for inter-test reliability.
Results: Inter-test agreement was good both for trough levels (concordance correlation coefficient 0.78, weighted κ 0.60, Sperman's ρ 0.937) and for infliximab antibodies (weighted κ 0.79) measurement, when comparing Promonitor and ImmunDiagnostik kits.

According to manufacturers' cut-off values, trough levels were classified as undetectable (17%), low (21%) or in range (63%). The only significant associations were: mucosal healing ($p = 0.026$; OR 6.50), infliximab antibody status ($p = 0.0015$; OR 0.031) and adverse events ($p = 0.009$; OR 0.115). Higher trough levels were observed among patients on concomitant steroid/immunosuppressive therapy and among patients with dose-intensification. Infliximab antibodies were significantly associated to treatment-related adverse events ($p = 0.0003$, OR 30.42), and to lower trough levels, but not to other clinical variables.
Conclusion: The two tests performed equally well. Infliximab antibodies were associated to adverse events, while trough levels were not associated to treatment outcomes.

1. Introduction

Biotechnological drugs, mainly tumour necrosis factor (TNF) blockers, have become a major therapeutic resource [1–3] over the past 15 years for the treatment of inflammatory bowel disease (IBD), both Crohn's disease (CD) and ulcerative colitis (UC). Their effectiveness is well known among clinicians managing IBD, although exact mechanisms underlying primary lack of efficacy and of loss of response to TNF blockers are still poorly understood.

During recent years, reports of an association between anti-drug antibodies (ADAs) and adverse effects of treatments both in CD and in UC have surfaced [4–11]. Development of ADAs is usually considered to be associated to immunogenicity of monoclonal antibodies, and their occurrence is associated mainly with on-demand schedule of treatment (as compared to scheduled treatment) and with absence of treatment with co-immune modulators [8,10,12–17]. Measurement of drug trough levels (TLs) was shown to be associated to therapeutic efficacy [6,7,9,11,15,18–22], and more recently therapeutic algorithms integrating drug TLs in drug schedule and dosage were proposed [12,14,23].

Several diagnostic kits and technologies for ADAs and TLs monitoring have been introduced and marketed, although absolute reliability and exportability of different tests still requires independent validation.

Aim of this study was to evaluate correlations of antibodies to infliximab (anti-IFX Ab) and infliximab TLs (IFX-TLs) monitoring with clinical outcomes, as well as to explore inter-test reliability of two commercially available diagnostic kits.

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2. Patients and methods

IBD patients undergoing scheduled maintenance infliximab outpatient treatment in a tertiary centre were consecutively enrolled. Clinical characteristics, disease activity and concomitant treatments were recorded at the time of clinical observation. Blood samples were drawn at the time of infusions (trough); sera were separated and stored at −40 °C until evaluation, once or more times during the follow-up.

All patients were on 5 mg/kg infliximab treatment, and all samplings were after a minimum of 14 weeks after infliximab was started (no sampling was considered during induction regimen). Maintenance schedule, based on clinical characteristics requiring prior dose optimization (e.g. clinical-based optimization), was at variable intervals between 4 and 8 weeks.

2.1. Analytical setting

Two different enzyme linked immunosorbent assay (ELISA)-sandwich tests were used in order to determine IFX-TL and presence or absence of anti-IFX Ab: "Promonitor IFX Determination of Drug and Anti-Drug Antibodies Concentration" ("Promonitor", Menarini, Italy) and "TNF Blocker Monitoring/Antibodies against TNF Blocker" ("ImmunDiagnostik", ImmunDiagnostik, Germany). Out of 145 available samples, 115 were tested with both methods, and therefore available for inter-test reliability analyses.

For determination of IFX-TLs, in Promonitor method the plate is covered with immobilized TNFα through a monoclonal antibody. After incubating the samples and the standard, the amount of freely circulating drug is determined by binding to a second specific monoclonal antibody conjugated to enzyme. The drug concentration is determined through colorimetric reaction. The signal obtained is proportional to the serum drug level. A ten-point calibration curve allows the measurement of the drug in six different dilutions for each sample (1:10–1:10,240). The level of serum IFX was classified according to manufacturer's cut-off values into: within range for concentrations >1.5 μg/ml, low range between 0.053 and 1.5 μg/ml, and absent/undetectable for concentrations <0.053 μg/ml.

In ImmunDiagnostik method, TNFα is coated directly into the microtiter plate. A six-point calibration curve allows the measurement of the drug in each sample diluted 1:200. The level of serum IFX is considered within optimal range for concentrations >5 μg/ml, low between 0.8 and 5 μg/ml, and absent/undetectable <0.8 μg/ml.

For both kits, in the presence of anti-IFX Ab, IFX-TLs are likely to be absent, due to analytical interaction.

For anti-IFX Ab determination, with the Promonitor method the plate is coated with the drug; after incubating the samples and the control, detection is conducted using the previously biotin-labelled drug. The antibody concentration is determined through colorimetric reaction and the signal obtained is proportional to the amount of anti-drug antibodies in the patient. A ten-point calibration curve from a positive control allows the measurement of anti-IFX Ab. With the ImmunDiagnostik method, in a first incubation step, anti-IFX Ab from the sample are bound to the IFX coated on the plate. In a further incubation step, peroxidase-labelled antibody is added. After incubating with the substrate (tetramethylbenzidine), an acidic stop solution is then added and the intensity of the colour is directly proportional to the amount of bound anti-IFX Ab from the sample. The threshold value for anti-IFX Ab with Promonitor kit, expressed in arbitrary units, is 37 AU/ml, while with ImmunDiagnostik it is positive when its optical density is twice the optical density of negative control.

2.2. Statistical analysis

Descriptive statistics were used before univariate analysis. Inter-test reliability was tested by means of Bland–Altman analysis and with Passing and Bablok regression. The Bland–Altman plot [24,25], or difference plot, is a graphical method to compare two measurements techniques. In this graphical method, the differences between the two techniques are plotted against the averages of the two techniques. The plot is useful to reveal a relationship between the differences and the averages, to look for any systematic biases and to identify possible outliers. The Bland and Altman plot may also be used to assess the repeatability of a technique by comparing repeated measurements using one single method on a series of subjects. The Passing & Bablok [26] method allows methods comparison, it is a linear regression procedure with no special assumptions regarding the distribution of the samples and the measurement errors. The result does not depend on the assignment of the methods (or instruments) to X and Y. The slope and intercept are calculated with their 95% confidence interval, which are used to determine whether there is only a chance difference between intercept and 1 and between slope and 0.

Chi square or Fisher exact test were used, as appropriate, to test association of categorical variables. Relevance of difference of IFX-TLs in different clinically meaningful subgroups of patients was tested by means of Wilcoxon or Kruskal–Wallis test, as appropriate. The Kruskal–Wallis test (H-test) is an extension of the Wilcoxon test and is used to test the hypothesis that a number of unpaired samples originate from the same population. If the null-hypothesis, being the hypothesis that the samples originate from the same population, is rejected ($p < 0.05$), then the conclusion is that there is a statistically significant difference between at least two of the subgroups.

Analyses were carried out with MedCalc (ver 14.8.1, Mariakerke, Belgium) statistical software. For every analysis, p value less than 0.05 was considered significant.

2.3. Sample size

According to the hypothesis that mean IFX-TLs be subtherapeutic in patients with active disease and normal/high in patients in remission, leading to a difference of (at least) 5 μg/ml in mean TLs (referring to ImmunDiagnostik), and accepting a standard deviation in both groups equal to median difference, the sample size (with alpha error and beta error both set at 0.05) was calculated at 27 patients per group. As multiple comparison were expected, and since the magnitude and symmetricity of enrolment in different subgroups could not be anticipated, we aimed to a much larger sample size of roughly 100 samples.

3. Results

Overall 86 patients with at least one sampling were evaluated, leading to 145 samples. Patients' characteristics are reported in Table 1.

3.1. Inter-test reliability analysis

Inter-test reliability was evaluated in 115 samples, which were read in duplicate with Promonitor and ImmunDiagnostik tests; the remaining single readings were available with Promonitor kit only.

Distribution of IFX-TLs rejected normal distribution both with Promonitor and ImmunDiagnostik tests ($p < 0.0001$ for both variables). Anti-IFX Ab distribution and titres rejected normal distribution.