Complications and optimisation of Mesalazine and anti-TNF-alpha therapy in inflammatory bowel disease
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Detection of infliximab levels and anti-infliximab antibodies: A comparison of three different assays

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Abstract

Background:
Formation of antibodies to infliximab (ATI) inversely correlates with functional drug levels and clinical outcome. Comparison of drug levels and anti-drug antibody monitoring is hampered by lack of standardization.

Aim:
To determine the correlation between three different assays for measuring infliximab and ATI.

Methods:
Serum samples and spiked controls (total 62) were evaluated in a blinded way in infliximab and ATI assays developed by Sanquin Amsterdam, Netherlands (A), Laboratory for Pharmaceutical Biology, KU Leuven, Belgium (B) and a commercially available kit from Biomedical Diagnostics (BMD), Paris, France (C) performed by the University Medical Center Groningen (UMCG), Netherlands.

Results:
All infliximab assays showed a linear quantitative correlation (Pearson r = 0.91 for A vs. B, 0.83 for A vs. C and 0.73 for B vs. C). Assay C detected infliximab in 11 samples (18%) not detected by A and B, including samples containing only ATI. All ATI assays showed a good linear correlation (Pearson r = 0.95 for A vs. B, 0.99 for A vs. C and 0.97 for B vs. C). Assay A detected ATI in five samples with low ATI that were not detected by assays B and C. Assay B did not detect ATI in three patient samples with low ATI according to assays A and C.

Conclusions:
There is a good correlation of infliximab and ATI measurements between these assays. Nevertheless, the BMD kit detected false positive infliximab levels in 18% of the samples.
Introduction

Antibodies against tumor necrosis factor alpha (anti-TNF-alpha) such as infliximab (IFX) and adalimumab (ADA) have proven to be effective in the treatment of the inflammatory bowel diseases i.e. Crohn’s disease (CD) and ulcerative colitis (UC) as well as in the treatment of rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and psoriasis. Both IFX and ADA are effective in inducing and maintaining remission of luminal and fistulizing CD and UC.1-2 These drugs are administered at fixed dose and intervals derived from dose finding studies for IFX3-4 and ADA.5-6 Observational studies showed that approximately 10 % of patients per year lose their response to these anti-TNF antibodies.7 One factor associated with loss of response is immunogenicity, whereby the production of antidrug antibodies is associated with infusion reactions and an accelerated antibody clearance resulting in lower anti-TNF antibody titers.8 Observational studies have demonstrated a relationship between IFX and ADA drug concentrations, the presence of antidrug antibodies and clinical outcome.9-12 In the case of loss of response with low drug titers without antibodies, increasing the dose or shortening of the dosing interval is effective.13 Whereas in cases of low drug titers due to anti-drug antibody formation, a switch to another anti-TNF is the preferable strategy.14 Therapeutic Drug and Immunogenicity Monitoring (TDIM) with early serial trough and antidrug antibody level measurements will probably optimize anti-TNF treatment.15-16 Therefore currently several studies are designed to dose IFX or ADA based on trough levels. Different assays are being used to measure drug and anti-drug antibody levels. The most commonly used assay types are the: enzyme-linked immunosorbent assay (ELISA) (i.e. used by laboratories in Spain, France, the Netherlands, Belgium and Israel but also provided as a kit by commercial companies), radio-immunoassay (RIA) (used by laboratories in Denmark and the Netherlands) and since recently a fluid phase mobility shift assay (provided by a commercial company in the United States). However, standardization of these assays to measure IFX or ADA trough levels and anti-IFX or anti-ADA antibodies is lacking. Several confounding factors such as drug interference and background can influence the measurement of biological drugs and antibodies to these drugs. This may result in poor specificity, sensitivity and reproducibility. In this study we determined the correlation between academically developed assays (Leuven and Amsterdam) that were used in several studies to detect IFX drug levels and anti-IFX antibodies17-19 and are routinely applied in patient diagnostics, and a commercially available assay (further referred to as BMD ELISA) used in the study of Pariente et al.20
**Materials and Methods**

We have set up a round robin experiment in which serum samples were sent around by three institutes and were analyzed in a blind manner by each institute. IFX and antibody levels to IFX were determined. The institutions participating in this interlaboratory test were: the Laboratory for Monoclonal Therapeutics, Sanquin Diagnostics (IFX and ATI assay A), Amsterdam (the Netherlands), the Laboratory for Pharmaceutical Biology, KU Leuven (IFX and ATI assay B), Leuven (Belgium) and the Department of Gastroenterology, UMCG (IFX and ATI assay C), Groningen (the Netherlands) who performed the BMD assay. All three institutions delivered serum samples derived either from the department of gastroenterology or from the department of rheumatology and Leuven and Sanquin provided quality control samples. No clinical details of patients were collected.

A total of 62 samples were analyzed by all three institutes. Thirty six samples were clinical samples from patients containing different concentrations of IFX and ATI. The other 26 samples were calibrator samples in which a serum pool of healthy controls was spiked with known concentrations of IFX (n=10), ADA (n=1), antibodies to IFX (ATI) (n=10) or antibodies to ADA (ATA) (n=3) and two blank samples with only serum of healthy controls.

In Groningen the commercially available LISA-TRACKER Premium Infliximab kit (BMD Biomedical Diagnostics, Marne La Vallée, France) was used to measure IFX and antibodies to IFX. This kit is an ELISA and has a CE-label according to Directive 97/98/CE. Lower limit of quantification for IFX levels is 0.1 mg/l. The lower and upper limits of quantification for antibodies to IFX are 10 and 200 microgram/l. The kit was used conform to manufacturer instructions by a qualified person.

In Amsterdam, at Sanquin, an in house developed ELISA was used to measure IFX using the same procedures as described for ADA. Maxisorp ELISA plates were coated overnight with 2 μg/ml monoclonal anti-TNF-7 (Sanquin) in Phosphate buffered saline (PBS) at room temperature (RT). After five times washing with PBS/0.02% Tween (PT), plates were incubated for 1 hr at RT with recombinant TNFα (0.01 μg/ml) (Strathmann Biotech HmbH, Hannover, Germany) diluted in high performance ELISA buffer (HPE, Business Unit reagents, Sanquin). Next, the plates were washed and incubated for 1 hr with patient serum which was serially diluted in HPE. Subsequently, the plates were washed with PT and incubated for 1 h with biotinylated infliximab specific rabbit anti-idiotype antibody (0.25 μg/ml in HPE). After washing streptavidin-poly-HRP (Sanquin) (1/250000, in HPE) was added for 1 h at 37 °C. After washing the ELISA was developed with 100 μg/ml tetramethylbenzidine in 0.11 M sodium acetate (pH 5.5) containing 0.003% (v/v) H₂O₂. The reaction was stopped with 2 M H₂SO₄. Absorption at 450 nm was measured with an ELx808IU reader (BioTek Instruments Inc., USA)). Results were related to a titration curve of infliximab on each plate. The lowest level of quantification was 0.002 mg/l. To measure antibodies to IFX, an in house developed RIA was used. Briefly, one microliter of serum diluted in Freeze buffer was incubated with 1 mg protein A Sepharose (GE healthcare, Chalfont St. Giles, UK) in 800 ml of total volume. After overnight incubation, samples
Detecting IFX levels and anti-IFX antibodies

were washed and ¹²⁵I radioactive labeled infliximab F(ab’)2 fragments were added. After overnight incubation, unbound radiolabel was washed out and Sepharose-bound radioactivity was measured. Results of this test are commonly expressed by Sanquin as Arbitrary Units/ml, where 1 AE/ml equals approximately 10 microgram/l. The lower limit of quantification is 12 AE/ml.

In Leuven an in house developed direct ELISA was used to measure IFX based on a previously described method. Briefly, high binding 96-well plates (Costar, Sigma Aldrich, Germany) were coated overnight with TNF-alpha (Preprotech, UK) at 4°C. Plates were blocked with PBS/1% bovine serum albumin (BSA) (Sigma Aldrich, Germany) for 2 h at room temperature and samples were diluted in PBS/1% BSA and incubated for 2 h at 37°C. As detecting antibody horse radish peroxidase (HRP) linked monospecific rabbit polyclonal antibody (made in house) was used and plates were incubated at RT for 1 h. Plates were developed using 400 µg/ml o-Phenylenediamine (Acros Organics, Belgium) and 0.003% (v/v) H₂O₂ in 0.1 M sodium citrate 0.2 M di-sodium phosphate buffer pH 5. The reaction was stopped with 2 M H₃SO₄. Absorption at 490 nm was measured with an ELX808IU reader (Bio-tek Instruments Inc., USA). Results were related to a titration curve of infliximab on each plate. The cut off for an IFX positive sample was 0.3 mg/l. To measure antibodies to IFX, an in house developed bridging ELISA was used. Briefly, high binding 96-well plates (Costar, Sigma Aldrich, Germany) were coated for 72hr with IFX (Janssen Biologics, The Netherlands) at 4°C. Plates were blocked with PBS 1% BSA (Sigma Aldrich, Germany) for 2hr at room temperature and samples were diluted in PBS, 0.1%BSA and 0.002% (v/v) Tween 80 and incubated overnight at 4°C. As detecting antibody HRP linked infliximab (made in house) was used and plates were incubated at RT for 2 hr. Plates were developed using 400 µg/ml o-Phenylenediamine (Acros Organics, Belgium) and 0.003% (v/v) H₂O₂ in 0.1 M sodium citrate 0.2 M di-sodium phosphate buffer pH 5. The reaction was stopped with 4 M H₃SO₄. Absorption at 490 nm was measured with an ELX808IU reader (Bio-tek Instruments Inc., USA). Results were related to a titration curve of monospecific rabbit polyclonal antibody to IFX on in each plate. The cut off for an ATI positive sample was 1 mg/l equivalents.

Statistical analysis

To quantify the agreement between IFX levels from all three assays, the intraclass correlation coefficient (ICC) was calculated using the two-way mixed single measures test (absolute agreement) whereby a value of 1 represents complete agreement. Data are shown in a Bland-Altman plot in which the difference between two measurements is plotted on the Y axis, and the average of two measurements on the X axis. This plot allows comparing of two assay methods. An ideal agreement between two assay methods is represented by a flat line in the Bland-Altman plot. An ICC expresses agreement between results obtained by two different methods whereas a Pearson correlation only expresses the association between these results. To quantify the correlation between IFX levels and antibody to IFX levels from all three assays, the Pearson’s correlation coefficient was calculated whereby a value of 1 represents an ideal correlation between two methods. Correlation coefficients were compared using
a post-test following two linear regression models to explore whether the influence of the outliers significantly influenced the Pearson’s correlation coefficient. A p-value of <0.05 was considered statistically significant. The statistical software package SPSS version 17.0 (IBM, New York, USA) and GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, USA) were used for all analyses.

Results

A total of 62 samples were included to measure IFX and antibody to IFX (ATI) levels. We compared the IFX and ATI levels obtained with assays from three different institutions.

The correlation between IFX levels of the different assays was expressed by a Pearson $r$ correlation coefficient. The highest correlation was obtained when comparing IFX assay A versus B which gave a Pearson $r$ of 0.91 (P<0.0001) (figure 1A).

Four samples were above the higher limit of quantification (HLOQ) in IFX assay C and gave also a high signal in IFX assays A and B. These samples were left out of further calculations. Comparison of IFX assay A versus C gave a Pearson $r$ of 0.83 (P<0.0001) and IFX assay B versus C a Pearson $r$ of 0.73 (P<0.0001) (figure 1B, C).

Qualitatively, IFX assays B and C detected IFX in one healthy control sample spiked with ADA whereas in IFX assay A this sample was negative. Furthermore, IFX assay C detected IFX in 11 out of 62 samples (18%), not detected by IFX assays A and B. Five out of these 11 samples were calibrator samples, of which two samples only contained antibodies to IFX and three samples only contained antibodies to adalimumab. The remaining six samples were patient samples, all containing high ATI levels.

The agreement between IFX levels of the different assays was expressed by an intraclass correlation coefficient (ICC). Comparing IFX assay A with B gave the best agreement with an ICC = 0.91 (95%CI 0.86-0.95; P<0.0001). This is represented visually in figure 2A by a Bland-Altman plot where the average of the differences is close to zero, indicating that the two assay methods produce similar results. To a lesser extent, we saw an agreement between IFX assays A and C and IFX assays B and C: the ICC between IFX assays A and C was 0.73 (95%CI 0.58-0.83; P<0.0001) and 0.59 (95%CI 0.39-0.73; P<0.0001) between IFX assays B and C. The Bland-Altman plots of IFX assays A and C (figure 2B) and IFX assays B and C (figure 2C) show that as the average of two measurements increases, the difference increases.

The correlation between antibody to IFX levels of the different assays was also expressed by a Pearson $r$ correlation coefficient (figure 3). All three ATI assays showed a good correlation: comparing ATI assay A versus B gave a Pearson $r$ of 0.95 (P<0.0001); ATI assay A versus C gave a Pearson $r$ of 0.99 (P<0.0001) and ATI assay B versus C gave a Pearson $r$ of 0.97 (P<0.0001). Six samples were above the higher limit of quantification (HLOQ) in ATI assay C and gave also a high signal in ATI assays A and B. These samples were excluded from the calculation of the Pearson correlation.

Qualitative analysis showed that ATI assay A detected ATI in 5 samples with low ATI
Figure 1. Correlation of IFX levels (mg/L) between (a) Amsterdam (IFX assay A) and Leuven (IFX assay B), (b) Amsterdam (IFX assay A) and BMD (IFX assay C) and (c) Leuven (IFX assay B) and BMD (IFX assay C).

Figure 2. Bland-Altman plot of IFX levels to compare two assay methods: the difference between two measurements (mg/L) is plotted on the Y-axis and the average of the two measurements (mg/L) on the X-axis. Dotted lines represent the 95% limits of agreement. A) Amsterdam (IFX assay A) versus Leuven (IFX assay B), B) Amsterdam (IFX assay A) versus BMD (IFX assay C) and C) Leuven (IFX assay B) versus BMD (IFX assay C).
that was not detected by ATI assays B and C. ATI Assay B did not detect ATI in 3 patient samples with low ATI according to ATI assays A and C.

The agreement between antibody to IFX levels obtained with the three different assays could not be calculated since the assays report different outcome measures (i.e. Arbitrary Units/ml, microgram/l equivalents, microgram/l). This is due to the fact that until now no human ATI standard is available which is necessary to calibrate each assay. This is not the case for the determination of IFX levels since the drug is available at a known concentration, provided by the manufacturer and is equally used in all three assays.
Discussion

A round robin test was performed in which clinical samples and quality control samples were sent around from three institutions and were analyzed for IFX levels and ATI independently. Three institutions participated in this interlaboratory test: the Laboratory for Pharmaceutical Biology, KU Leuven, Leuven (Belgium) and Sanquin Research, Amsterdam (the Netherlands) because their in house developed assays are widely used and the department of Gastroenterology, UMCG, Groningen (the Netherlands) that tested the commercially available BMD ELISA. All three institutions delivered patient samples and Leuven and Sanquin provided quality control samples.

We found a good correlation and agreement in IFX levels measured with all three assays. Nevertheless, the BMD ELISA detected IFX in 11 samples (18%) not detected by the ELISA from Amsterdam or Leuven. These false positive samples were six clinical samples with high ATI levels and five quality control samples which only contained serum from healthy controls that was spiked with either ATI (two samples) or antibodies to ADA (three samples). The reason why the BMD ELISA detects false positive IFX levels could be because of non-specific binding or the use of a non-specific secondary antibody (anti-human IgG). It has to be noted that in contrast to the assays from Amsterdam and Leuven that were carried out in a research laboratory, the BMD assay was performed in a clinical pharmacology laboratory. However, the BMD assay was carried out by a qualified person conform to manufacturer instructions in an adequate setting.

We found a good correlation in ATI levels measured with all three assays. We did not perform specific tests to investigate the drug interference of IFX when detecting ATI but this was optimized by each institute. This is of pivotal importance because when IFX is present in a serum sample, this will form complexes together with ATI, thereby impeding the detection of ATI (= drug interference). Therefore, the detection and quantification of ATI is not always possible when IFX is present and those samples should be considered ‘inconclusive’ for ATI. The result of the RIA from Amsterdam for ATI is considered inconclusive when the IFX level is above 1 mg/l compared to 0.3 mg/l for the bridging ELISA from Leuven. In the manufacturer’s manual of the BMD kit, no interference of IFX is mentioned when measuring ATI and no information is given when a serum sample should be considered ‘inconclusive’.

The sensitivity of the three assays to detect ATI was comparable, with a slight advantage for the RIA in detecting low ATI concentrations. As the RIA is less sensitive to drug interference than the ELISA, it is able to detect low ATI concentrations in the presence of drug. Thus, antibody development may be detected a bit earlier. However, in vivo these low level ATI may only influence IFX to a limited extent.

We did not correlate the results of the tests with the clinical image of the patient (for the clinical serum samples). The main goal of this comparative test was to evaluate the ability of the tests to detect typical, clinical meaningful levels of drug and anti-drug antibodies without false positives or negatives. Comparing assays is critical for understanding and interpreting data of different clinical studies.

Recently, Pariente and co-workers published a retrospective study in which they
investigated a cohort of IBD patients losing response to IFX. The correlation between clinical response, IFX trough levels (IFX serum level just before next infusion) and ATI was investigated. They concluded that in IBD patients who lose response to IFX, clinical improvement upon intensification of IFX therapy, was irrespective of IFX trough level or presence of antibodies to IFX. This study was performed with the commercially available BMD ELISA. Our results – showing that the BMD ELISA cannot distinguish between IFX and ATI in certain clinical samples and might show false positive results of IFX – shed another light on this study. When measuring false positive IFX levels in serum samples of patients with high levels on ATI, this will not correlate with the clinical outcome.

The results of Pariente et al. are also in stark contrast to previously published papers proving a correlation between IFX trough levels and clinical outcome in Crohn’s disease and ulcerative colitis patients. Furthermore ATI have been linked to a higher incidence of infusion reactions, adverse events and an increased risk for loss of response. Recently it has been shown that in patients with good IFX trough levels, the presence of ATI is associated with a higher CRP during treatment. However, also after discontinuation of anti-TNF, the measurement of anti-drug antibodies could be of clinical use. Ben-Horin et al. showed promising results in a small cohort of IBD patients that the decline of anti-drug antibodies could have an impact on the outcome of re-induction of the anti-TNF. Due to this increasing evidence for the use of therapeutic drug and immunogenicity monitoring in the management of chronic inflammatory diseases, there is a high need for standardized assays that can accurately detect IFX and ATI in sera.

**Conclusions**

There is a good correlation of IFX and antibody to IFX level measurements between the assays developed by Sanquin Research (Amsterdam), the Laboratory for Pharmaceutical Biology (Leuven) and the commercially available kit from BMD. Nevertheless, the BMD kit detected false positive IFX levels in 18% of the samples, including samples only containing antibodies to IFX and antibodies to ADA. Finally, the authors agree that existing assays to measure biological drugs and anti-biological drug antibodies need to be standardized.
References

16. van de Casteele N. 1159 Results on the Optimisation Phase of the Prospective Controlled Trough Level Adapted Infliximab Treatment (TAXIT) Trial. 5 2012;142:211-212.


Letter: detection of infliximab levels and anti-infliximab antibodies – comparison of three different assays; author’s reply

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In response to Parussini’s comments on our article\textsuperscript{1,2}: we regret that Theradiag Innovation (bmd) cannot support the results and formally contests the conclusion of our article. In their letter it is stated that the published results are not consistent and they suspect an issue occurring during the pre-analytical process of the samples. In order to select the best test for clinical practice the UMCG performed the tests with the LISA tracker and took part in a round robin experiment together with Sanquin Diagnostics and the Laboratory for Pharmaceutical Biology as mentioned in the paper. The test was performed according the manufacturer’s protocol by a technician of the UMCG, who was instructed by the manufacturer. Apart from the eight presumed false positive samples all the other results were consistent with the other assays, therefore a bad compliance of the product protocol is highly unlikely. In order to improve their test and not to dispute the results, Theradiag received eight samples (seven clinical samples, one QC sample) from our study stated as false positive Infliximab (IFX) levels. Three out of eight samples that were retested at Theradiag were still false positive. These tests were not performed in a blinded way in contrast to the set-up of the initial round robin experiment. Furthermore, there appear to be some inaccuracies in the graph depicted in the letter of Parussini as UMCG found levels up to 2.2 mg/L (not 5 mg/L as Theradiag erroneously mentioned) (Table 1). The LISA tracker uses non-specific anti-human IgG as a detecting antibody, which is known to cause false positive results due to aspecific binding of serum proteins leading to false positive results. Referring to the 22 extra samples recently measured by Theradiag and the Laboratory for Pharmaceutical Biology, the infliximab results obtained by Theradiag were now consistently lower and one sample was now even false negative. This underestimation of infliximab results is peculiar. The LISA tracker is used in several publications and journals, but they never performed a study to determine the correlation between different assays for measuring IFX levels.\textsuperscript{3}

The authors’ declarations of personal and financial interests are unchanged from those in the original article.\textsuperscript{2}

Table 1. Comparison of the IFX levels in eight out of 11 samples interpreted as false positive in the present study. Theradiag still shows a false positive result in three out of eight samples. The UMCG did not find levels up to 5 mg/L IFX such as Theradiag suggests in their graph.

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References


