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Comparison of infliximab drug measurement across three commercially available ELISA kits

MONIQUE WEI MENG LEE[1], SUSAN CONNOR[2,3], WATSON NG[2] AND CATHERINE MEI-LING TOONG[1,3,4]

[1]Department of Immunology, Liverpool Hospital, Sydney, [2]Department of Gastroenterology and Hepatology, Liverpool Hospital, [3]University of NSW, and [4]Sydney South West Pathology Service, Liverpool Hospital, Sydney, NSW, Australia

Summary

The monitoring of infliximab drug levels aids in the management of several autoimmune diseases, notably inflammatory bowel disease. Several commercial kits are now available and approved by the Therapeutic Goods Administration (TGA) for the measurement of infliximab levels, but there have been limited verification or comparison studies to date.

Finding an assay that most accurately measures infliximab is essential for optimal drug titration and patient management. We performed this study to compare the performance of the Grifols Promonitor, Theradiag Lisatracker and R-Biopharm Ridascreen enzyme linked immunosorbent assay (ELISA) kits.

Preparations of serum containing known concentrations of infliximab were assayed using each kit, including in the presence of interference from anti-infliximab antibodies, autoantibodies and other biological agents.

The Lisatracker kit provided the most accurate determination of infliximab drug levels, however it yielded false positive results at low concentrations of infliximab. The average coefficients of variation (CVs) for the kits were 8% for Lisatracker, 5% for Ridascreen and 11% for Grifols. Infliximab measurements across all kits were affected by interference from antibodies to infliximab (ATI).

This study identified the Lisatracker kit as the most accurate in quantifying infliximab levels, although it was limited by false positive results at low concentrations of infliximab as well as interference from ATI. This has important implications for the monitoring and management of patients receiving infliximab therapy.

Key words: Infliximab; antibodies to infliximab; ATI; therapeutic drug monitoring.

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INTRODUCTION

Infliximab is a chimeric monoclonal antibody that neutralises the activity of tumour necrosis factor alpha (TNF-α) and binds with high affinity to its soluble and transmembrane forms. It is composed of human immunoglobulin constant domains and murine variable TNF-binding domains produced in genetically modified Chinese hamster ovarian cells. It is used in a number of autoimmune conditions notably inflammatory bowel disease (IBD), rheumatoid arthritis and spondyloarthritis.

It is known that 10–30% of IBD patients do not respond to TNF inhibitors, and up to 60% of initial responders later lose response.[1] One of the reasons for loss of response is immunogenicity, in which the development of antibodies to infliximab (ATI) occurs as a result of recognition of the drug as non-self. ATI are usually neutralising and block the binding site of infliximab to TNF-α.[1] They can lead to allergic reactions and their presence has been linked to subtherapeutic serum drug levels and clinical loss of response. By forming complexes with infliximab, ATI can significantly increase the clearance and reduce the bioavailability of infliximab.[2] Cohort studies and post-hoc analyses show that serum infliximab trough concentrations correlate with clinical response, remission and mucosal healing in IBD patients; low infliximab trough levels and presence of ATI are associated with worse clinical outcomes.[3,4]

Therapeutic drug monitoring (TDM) using a combination of trough infliximab concentration and ATI levels can guide therapy by allowing targeted dose intensification in patients who lose response due to low drug concentration, while avoiding dose escalation in patients who have developed immunogenicity. The cost efficiency of such a model has been verified both in a simulated model[5] and a randomised controlled study.[6] More recently, the TAXIT trial demonstrated that dose escalation in Crohn's disease patients with suboptimal drug concentrations (<3 μg/mL) results in better disease control, while dose reduction was successful in both Crohn's disease and ulcerative colitis patients with supra-optimal drug concentrations (>7 μg/mL), with resulting cost reduction and lower drug exposure.[7]

The Australian Therapeutic Goods Association (TGA) has approved three commercially developed Infliximab ELISA kits for use: Promonitor-IFX (Grifols, Spain), Lisatracker-Infliximab (Theradiag, France) and Ridascreen (R-Biopharm, Germany). Although some of these are already in research and clinical use, a comparative study of these kits has not yet been published. This study compares the performance of these three kits by measuring the infliximab levels of pre-prepared standards of known concentration. The possible interference to infliximab measurement from the

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presence of ATIs, as well as biological and pharmacological sources was also investigated.

MATERIALS AND METHODS

Preparation of standards

Infliximab (Remicade, Janssen-Cilag), adalimumab (Humira, AbbVie) and etanercept (Enbrel, Pfizer) were purchased from the Liverpool Hospital pharmacy. Infliximab was reconstituted with sterile water according to the manufacturer's instructions. Pharmacological interference samples were prepared using adalimumab and etanercept, purchased as pre-filled syringes at concentrations of 40 mg/0.8 mL and 25 mg/mL, respectively. Adalimumab and etanercept were diluted to achieve therapeutic concentrations of 20 μg/mL and 25 μg/mL, respectively.

Control serum was donated by two healthy males and one female volunteer, all without known medical conditions. Their full blood counts were within normal parameters and antinuclear antibody screens were negative. Physiological interference samples included samples from patients with positive rheumatoid factor ($n = 3$) and from patients with paraproteins (3 patients with IgM kappa paraprotein and 3 patients with IgG kappa paraprotein). Infliximab was added to each of the serum control and interference samples to achieve final infliximab concentrations of 0.2 μg/mL, 2 μg/mL, 3 μg/mL and 7 μg/mL.

To assess the interference to infliximab readings by the presence of ATI, a purified monoclonal IgG1 anti-infliximab antibody was purchased from AbD Serotec. This antibody specifically recognises free infliximab but not the infliximab/TNF-α complex and inhibits binding of infliximab to TNF-α, with a high affinity for infliximab ($k_d = 121$ pM) and concentration of 0.5 mg/mL (HCA233, AbD Serotec, USA). The ATI was added to each concentration of infliximab to achieve final ATI concentrations of 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL and 10 μg/mL. These concentrations were chosen to cover the range of ATI levels that may be detectable in patients receiving infliximab.[8] While *in vivo* ATI would be polyclonal, a high affinity monoclonal ATI was chosen for this study due to its ability to better characterise the nature of the antibody involved, particularly in determining the ability of neutralising antibodies to interfere with the results of the infliximab assay.

Determination of infliximab levels and anti-drug antibody levels

Commercial kits were obtained from Grifols (Promonitor-IFX, Ref. 506023000), Theradiag (Lisatracker Infliximab LT1002-48) and R-Biopharm (Ridascreen G09041). The samples were all run on an automated ELISA processor (Triturus, Grifols), programmed according to each of the kit manufacturer's instructions. Application specialists from each company were invited to verify the correct programming of the machine and supervise the analysis of the deidentified samples on the kits provided by that company.

The Promonitor-IFX is a capture ELISA with wells pre-coated with an anti-TNF-α human monoclonal antibody bound to human recombinant TNF-α, to ensure that the TNF-α structure is not disrupted and available to bind infliximab. The Lisatracker kit is a standard ELISA that uses TNF-α coated wells. The Ridascreen kit uses pre-coated TNF-α wells, to which the patient sample is applied and incubated. Following washing, a second incubation is performed with a specific monoclonal antibody against infliximab (MA-IFX6B7) conjugated with horseradish peroxidise. MA-IFX6B7 was isolated and characterised at KU Leuvin and only detects infliximab; other anti-TNF drugs such as adalimumab do not interfere with measurement.

Limited plates were supplied by each company and did not allow for multiple sample analyses of the one concentration. Seven samples were analysed at 3 μL/mL and 7 μL/mL to calculate the coefficient of variation (CV), chosen to reflect the therapeutic range.

Following analysis in the Liverpool Hospital laboratory, samples were stored for 1 month at −80°C. One blinded set of 49 samples aliquotted from the original samples was sent to the Melbourne laboratory of Grifols for parallel analysis, and another similar set was sent to the Paris laboratory of Theradiag. Unfortunately, the Ridascreen assay was approved by the TGA only after the study had been performed and there was insufficient sample to offer duplicate testing to R-Biopharm.

Ethics

The study was conducted in accordance with the principles of the declaration of Helsinki, with oversight by the local research ethics committee.

Statistical analysis

Analyses were performed using GraphPad Prism 6 (2014; GraphPad Software, USA). Coefficient of variation was calculated for replicates measured with each kit at infliximab concentrations of 3 μg/mL and 7 μg/mL. Data that were out of range were reported at the value of the limit of detection for graphing purposes.

RESULTS

Comparison between kits

Assays of the prepared infliximab samples at concentrations of 0 μg/mL, 0.2 μg/mL, 2 μg/mL, 3 μg/mL and 7 μg/mL were run on each kit according to the manufacturer's instructions. The three commercial kits compared well against one another, especially at lower concentrations of infliximab. Ridascreen and Grifols had poorer agreement when compared with other methods (Fig. 1).

The Lisatracker kit readings were the most accurate, although it produced a false low-level reading of 0.13 μg/mL for an infliximab concentration of 0 μg/mL. This was followed by Ridascreen then Grifols, both of which consistently produced readings lower than the known concentration of the prepared samples (Fig. 2). However, both the Ridascreen and Grifols kits produced accurate negative readings at an infliximab concentration of 0 μg/mL.

To calculate the coefficient of variation (CV), seven samples were analysed at concentrations of 3 μg/mL and 7 μg/mL. At a concentration of 3 μg/mL, the CV for Lisatracker was 9%, Ridascreen 5% and Grifols 14%. At a concentration of 7 μg/mL, the CV for Lisatracker was 8%, Ridascreen 5% and Grifols 8%. Figure 3 demonstrates a graphic representation and comparison of the CVs between each kit. The 49 samples sent away to the Grifols Melbourne and Theradiag Paris laboratories had significantly larger CVs compared to aliquots of the same samples analysed in our laboratory. At a concentration of 3 μg/mL, the CV for the Theradiag sendaway samples was 17%, and Grifols 24%. At a concentration of 7 μg/mL, the CV for the Theradiag sendaway samples was 15% and Grifols 15%.

Assessment of interferences to infliximab reading

The presence of high-affinity ATI demonstrated a clear linear dose-dependent interference to the infliximab assay for the Lisatracker kit; increasing concentrations of ATI corresponded to reduced levels of infliximab drug level readings (Fig. 4). The Grifols kit also showed interference to infliximab drug level readings in the presence of ATI; low concentrations of ATI resulted in higher infliximab drug level readings, while higher concentrations led to reduced readings (Fig. 4). With regard to the Ridascreen kit, little interference was observed in the presence of low concentrations of ATI, while higher concentrations resulted in reduced infliximab drug levels in a dose-dependent fashion (Fig. 4).

The presence of other biological agents also produced interference. The Lisatracker kit returned falsely high readings in the presence of etanercept and adalimumab. Both the Grifols and Ridascreen kits produced falsely low readings in the presence of etanercept, while adalimumab did not interfere significantly with these two kits. Supplied company