Pathology (■ xxxx) **xxx(xxx)**, xxx

CORRESPONDENCE

Subtyping anti-infliximab antibodies by the homogenous mobility shift assay (HMSA): potential utility in a pharmacokinetic case study

To the Editor,

Therapeutic drug monitoring (TDM) of anti-tumour necrosis factor inhibitors (TNFi) has an established role in the management of inflammatory bowel disease (IBD).[1] It may also be useful in the management of inflammatory joint disease, though the evidence is less clear.[2–4] Under-dosing and/or the presence of anti-drug antibodies (ADA) may be causative mechanisms for primary or secondary drug failure, particularly in the setting of a clinically ambiguous response. Differentiating between the two mechanisms has therapeutic implications. Here we describe the potential utility of the homogenous mobility shift assay (HMSA) in the detection of ADA and the added value it may provide to clinical decision-making.

Preparation of standard drug concentrations has allowed standardisation and potentially harmonisation of assays. Standardisation has also helped define therapeutic drug concentrations.[1,4,5] In contrast to drug concentrations there is no standardisation of assays for ADA detection which are reported to occur in up to 20−30% of patients.[6]

ADA can be of two types. Neutralising ADA (nADA) produce multimeric complexes when detected by HMSA and less often dimeric complexes (Fig. 1A). Non-neutralising/binding ADA (bADA), tend to be restricted to dimeric complexes (Fig. 1B).[7] nADA bind directly to the pharmacologically active site and interfere with the ability of the drug to bind to its target (TNF). bADA bind outside the active site and may not affect the drug-cytokine interaction but may increase drug clearance.[8]

We report a pharmacokinetic study of a patient using the HMSA method to detect and subtype ADA to infliximab. The patient gave written informed consent.

A 52-year-old male with steroid-responsive polyarticular psoriatic arthritis was found to be poorly controlled or intolerant to available disease modifying anti-rheumatic drugs (DMARDs) including adalimumab and etanercept, and none were subsequently continued. He typically required prednisone doses between 10 to 15 mg/day to maintain function and manual employment. Regular infusions of infliximab were commenced.

After an induction regimen, a therapeutic response was achieved, and maintenance therapy continued at infliximab 400 mg (5 mg/kg) IV every 8 weeks. He reduced prednisone to between 7 to 10 mg/day. By the fourth maintenance dose he experienced a 'wear-off' effect 4−6 weeks post-infusion, a pattern that persisted over several years of monitoring. During this wear-off period he increased prednisone up to 15 mg daily, until his next infusion when the dose was reduced again.

After 13 months, trough drug concentrations were measured by indirect enzyme-linked immunosorbent assay (ELISA) and found to be undetectable. Whilst ADA were not initially detected by competitive ligand binding ELISA (CLB-ELISA) retrospective analysis of samples using HMSA demonstrated weak dimeric ADA. Application to fund an increase in infusions to every 6 weeks was declined. We sought to understand the pharmacokinetics of infliximab and ADA to rationally maximise therapeutic benefit.

Serial weekly serum samples before and after two consecutive infusions over 95 days were obtained (Fig. 2). The study demonstrated accelerated serum infliximab drug clearance to undetectable concentrations by week four post-infusion. In contrast, ADA concentrations were initially low/undetectable post-infusion, then gradually increased to maximal concentrations by week four post-infusion before decreasing to a baseline low concentration prior to the next infusion. The patient's symptoms paralleled the infliximab and ADA kinetics with minimal symptoms during the period of high infliximab concentrations and absent/low ADA concentration followed by symptom development during the period of low drug concentration and high ADA concentrations.

Outside the study period dimeric complexes were consistently detected when measured by HMSA in the wear-off period (Fig. 1B). By CLB-ELISA no nADA were detected so by inference the dimers represented bADA only. With limited options available and on the presumption only bADA were present, the treatment regimen was maintained with reasonable control of symptoms. To date neither nADA by CLB-ELISA[9] nor multimers by HMSA have developed.

The HMSA method was developed by a group of researchers in San Diego[10] and has been used at Canterbury Health Laboratories, New Zealand, since 2018.

The method uses size-exclusion high-performance liquid chromatography (SE-HPLC) to separate fluorochrome-labelled [AlexaFluor (AF); ThermoFisher, USA] drug and immune complexes by molecular size. Fluorescence intensity of eluents is continuously measured by a fluorescence detector and graphically recorded (y-axis) with time on the x-axis. Larger molecules (drug-ADA multimers) elute early, followed by smaller complexes (typically drug-ADA dimers) and finally unbound fluorescently-labelled drug. Internal standards producing additional peaks are used for quality control. Previous studies have shown the size of the drug-ADA immune complexes segregate bADA and nADA when compared to a competitive ELISA. A study comparing CLB-ELISA and HMSA confirmed that smaller, dimeric complexes are almost exclusively bADA, while HMSA multimers are exclusively associated with nADA activity.[7]

In brief the HMSA method is as follows. Serum samples are first treated with acetic acid to dissociate any serum drug-ADA complexes. This allows a level of drug-tolerance not observed in other assays such as CLB-ELISA or bridging ELISA. Fluorochrome-labelled infliximab is then added, and the acetic acid neutralised to allow immune complexes to reform by competitive binding of both labelled (added) and unlabelled drug (circulating). The assay is qualitative but reported on a semi-quantitative scale

Print ISSN 0031-3025/Online ISSN 1465-3931

Please cite this article as: Chow KL et al., Subtyping anti-infliximab antibodies by the homogenous mobility shift assay (HMSA): potential utility in a pharmacokinetic case study, Pathology, https://doi.org/10.1016/j.pathol.2022.08.013

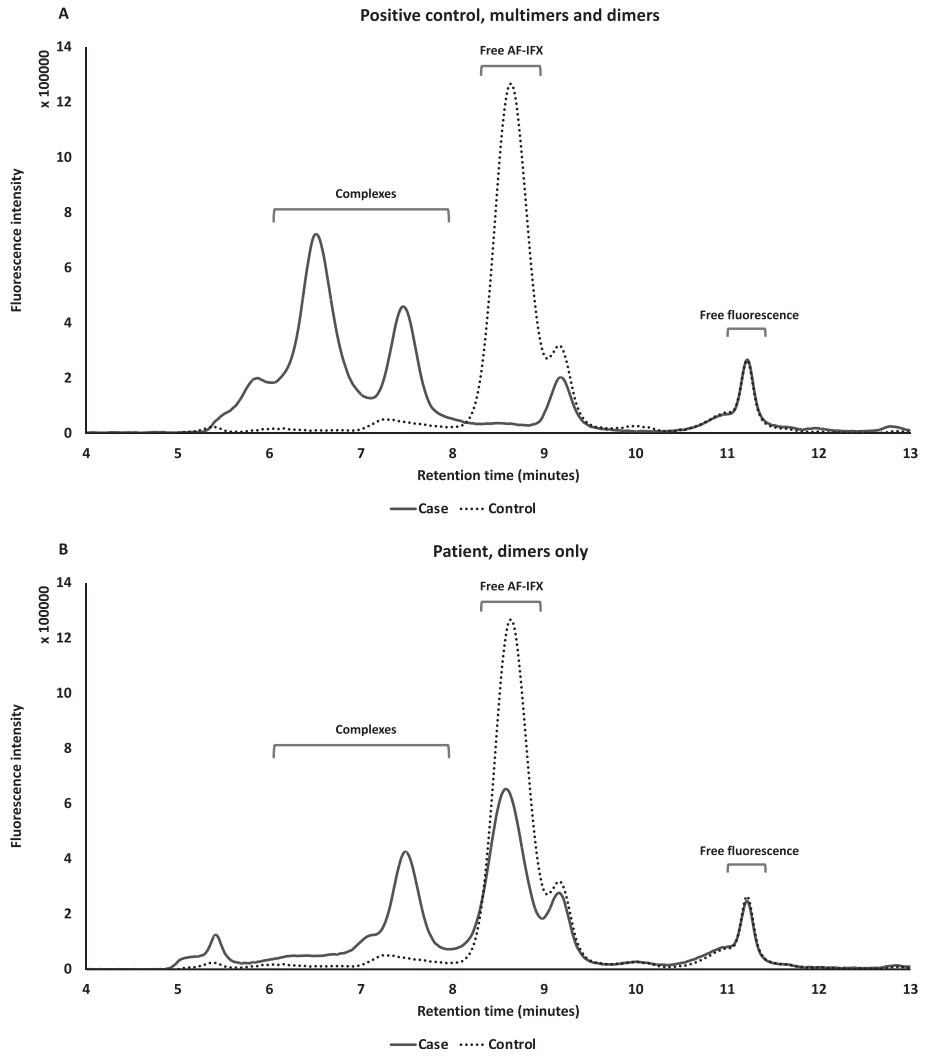

Fig. 1 HMSA chromatograms from anti-infliximab antibody detection of a known positive control sample (A) and the psoriatic arthritis case patient (B). The fluorochrome-labelled drug and drug-anti-drug antibody complexes are separated by size exclusion using high performance liquid chromatography (column BioSep 5 μm SEC-s3000 290 Å; Phenomenex, USA). Fluorescence intensity of each eluent is continuously recorded on the 'y' axis with time on the 'x' axis. Large protein complexes elute early followed by subsequent eluents containing successively smaller proteins or protein complexes. Calibration by the instrument manufacturer using protein standards allows an estimate of molecular weight at each peak. (A) The solid line represents the HMSA profile of an illustrative sample containing both multimeric and dimeric infliximab-ADA complexes. These proteins are represented by the area under the curve (AUC) of the fluorescent peaks between 5.5 and 9.0 minutes. The dotted line represents the negative control sample with only fluorochrome-labelled infliximab present. The AUC between ~5.5 and 7.0 minutes represents multimeric complexes (molecular weight greater than ~600 kD) whereas the AUC between ~7.0 and 8.0 minutes represents dimeric complexes (molecular weight ~300 kD). The AUC between ~8.0 and 9.0 minutes represents fluorochrome-labelled infliximab (molecular weight ~150 kD). Note that in this example the AUC of the multimeric and dimeric peaks contains 95% of the total fluorescence of the negative control sample's fluorochrome-labelled infliximab peak. The other peaks and shoulders in the profile are not directly relevant to interpretation but do act as internal controls. (B) Only dimeric complexes are evident in this profile with 45% of the AUC of the fluorochrome-labelled infliximab peak from the negative control sample 'shifted' into the AUC of dimeric peak. No multimeric complexes have been detected. AF-IFX, AlexaFluor-infliximab.

by calculating the ratio of labelled drug to labelled drug-ADA complexes i.e. the 'mobility shift' of labelled drug into drug-ADA complexes. The assay is performed in a 'homogeneous' liquid phase minimising conformational distortion of proteins that may occur in solid phase assays. Run-to-run variability is reduced by reporting ADA in arbitrary units (AU) as a standardised ratio using the negative control included in each assay run. One arbitrary unit (AU) means there is perfect alignment of the sample curve over the control curve indicating undetectable ADA. A discriminating cut-off value of 2 AU has been obtained after assaying multiple infliximab-free serum samples including those containing rheumatoid factor activity.

The detection of ADA is most reliable when drug concentrations are low, so pragmatically only samples with drug concentrations <2 μg/mL are tested for ADA unless a specific request is made to test at higher drug concentrations.[11] Each sample is spiked with 5 μg/mL of AF-infliximab so that in most cases the concentration of labelled drug exceeds the concentration of circulating infliximab. When the circulating infliximab concentration exceeds 5 μg/mL a competitive advantage occurs between circulating drug and the labelled drug, diminishing analytical sensitivity for ADA detection. Interestingly in the case described, ADA dimers were detected at a drug concentration of 27 μg/mL (i.e., 5.4-fold higher than labelled drug). The fact that dimers were detected even at this high circulating drug concentration suggests bADA bind to more heterogeneous sites outside the drug binding domain.

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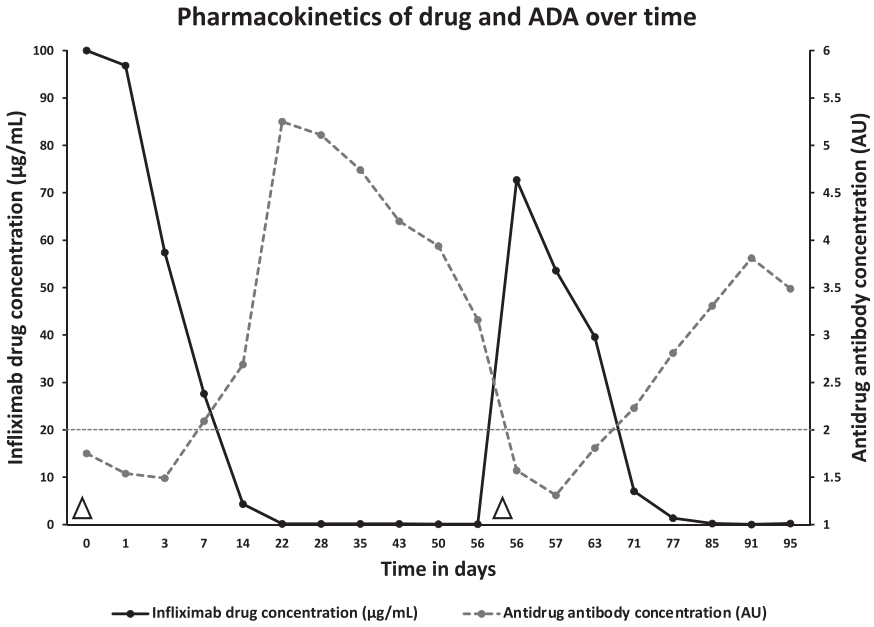

Fig. 2 Pharmacokinetic study over 95 days of the patient on infliximab with two separate infliximab infusions at day 0 and day 56. Serum drug concentrations show a progressive decline in serum infliximab concentration on days 0−22 and days 57−72. Reciprocally ADA are detectable (defined as >2 AU, horizontal dotted line) by day 7, reaching a maximum by day 22 and declining until after the next infusion. This pattern is repeated in the second study between days 57 and 95. For a derivation of ADA arbitrary units refer to narrative. Δ, infliximab infusions.

In contrast, nADA binding is more restricted. Only the active site on one Fab arm is available in a one-to-one ratio with each nADA binding site.[12] At the same time the other infliximab Fab active site is available to act as an abutment for another nADA, allowing bridging of drug at a relatively low concentration to form multimers of circa 600−700 kD. Why bADA exclusively develop in one patient while nADA +/− bADA in another is not known.

Other than increased joint symptoms during the 'wear-off' period the patient has not experienced features that might suggest immune complex disease such as renal involvement or skin rashes. However, the bADA appear to be associated with increased drug clearance. Theoretically, the resulting impaired therapeutic response could be improved by either increasing the drug dose or potentially decreasing the interval between infusions. Unfortunately, funding was not made available to allow testing of these potential therapeutic options.

Despite the HMSA's ability to subtype ADA, it is important to remember that such binary classification of nADA vs bADA does not necessarily reflect activity *in vivo*. Functional neutralisation *in vivo* depends on relative concentrations, subclass and affinities of ADA, cellular density of TNF receptors, and variability in partitioning of drug and ADA within body compartments.[8]

Nevertheless, this case demonstrates the potential clinical utility of subtyping ADA to infliximab in a patient with psoriatic arthritis with secondary loss-of-effect using the HMSA method. Subtyping ADA may allow a more nuanced approach to apparent drug failure as, depending on sample timing, it can indicate the underlying cause of low drug levels better than measuring drug levels or detecting ADA alone. This is especially important when there are limited therapeutic options available. Further research is required to determine whether such discrimination has value in a wider group of patients.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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1. Mitrev N, Casteele NV, Seow CH, *et al*. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther* 2017; 46: 1037−53.
2. Syversen SW, Goll GL, Jorgensen KK, *et al*. Effect of therapeutic drug monitoring vs standard therapy during infliximab induction on disease remission in patients with chronic immune-mediated inflammatory diseases: a randomized clinical trial. *JAMA* 2021; 325: 1744−54.
3. Stamp LK, Barclay M. Therapeutic drug monitoring in rheumatic diseases: utile or futile? *Rheumatology (Oxford)* 2014; 53: 988−97.
4. Chen DY, Chen YM, Tsai WC, *et al*. Significant association of antidrug antibody levels with serum drug trough levels and therapeutic response of adalimumab and etanercept treatment. *Ann Rheum Dis* 2015; 74: e16.
5. Sanmarti R, Inciarte-Mundo J, Estrada-Alarcon P, *et al*. Towards optimal cut-off trough levels of adalimumab and etanercept for a good therapeutic response in rheumatoid arthritis. Results of the IMMUNOREMAR study. *Ann Rheum Dis* 2015; 74: e42.
6. Quistrebert J, Hassler S, Bachelet D, *et al*. Incidence and risk factors for adalimumab and infliximab anti-drug antibodies in rheumatoid arthritis: a European retrospective multicohort analysis. *Semin Arthritis Rheum* 2019; 48: 967−75.
7. Hock BD, McKenzie JL, Goddard L, Smith SM, McEntyre CJ, Keating PE. Discrimination of anti-drug antibodies with neutralizing capacity in infliximab- and adalimumab-treated patients: comparison of the homogenous mobility shift assay and the affinity capture and elution assay. *Ther Drug Monit* 2018; 40: 705−15.
8. Atiqi S, Hooijberg F, Loeff FC, Rispens T, Wolbink GJ. Immunogenicity of TNF-inhibitors. *Front Immunol* 2020; 11: 312.

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9. Hock BD, Stamp LK, Hayman MW, Keating PE, Helms ET, Barclay ML. Development of an ELISA-based competitive binding assay for the analysis of drug concentration and antidrug antibody levels in patients receiving adalimumab or infliximab. *Ther Drug Monit* 2016; 38: 32−41.
10. Wang SL, Ohrmund L, Hauenstein S, *et al*. Development and validation of a homogenous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. *J Immunol Methods* 2012; 382: 177−88.
11. Barclay ML, Karim S, Helms ETJ, *et al*. Infliximab and adalimumab concentrations and anti-drug antibodies in inflammatory bowel disease control using New Zealand assays. *Intern Med J* 2019; 49: 513−8.
12. van Schie KA, Kruithof S, Ooijevaar-de Heer P, *et al*. Restricted immune activation and internalization of anti-idiotype complexes between drug and antidrug antibodies. *Ann Rheum Dis* 2018; 77: 1471−9.

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