Monitoring immunogenicity of protein-based TNF antagonists

A recent review by Hendy *et al*¹ discusses the use of anti-tumour necrosis factor (TNF) drug and anti-drug antibody (ADAb) measurements to achieve a personalised approach to treating patients with inflammatory bowel disease with loss of response (LOR) to protein-based TNF-antagonist therapy. While highly appreciated and filling an important gap in the current management of patients with LOR, the review leaves out important pharmacimmunological information related to drug immunogenicity, particularly the clinical relevance of technologies used to measure ADAb.²

It should be noted that blood levels of infused or injected anti-TNF drugs vary considerably in

the periods between repeated drug administrations ([figure 1](#)). Assessing circulating drug levels is therefore highly dependent upon the time from drug administration to blood sampling. If ADAb develops, these variations are even more complex, and assessments of drug and ADAb become dependent on time of blood sampling and on the kinetics of binding between drug and ADAb. The latter governs formation of immune complexes containing drug and ADAb, the size and stability of these complexes, their ability to bind and activate other serum components such as complement and, subsequently, elimination of the immune complexes from the circulation. In this scenario, the test characteristics of a given assay will markedly affect the reported levels of both drug and ADAb. While a test designed to assess drug levels in serum should be able to detect unbound drug, is it also capable of detecting one or multiple drug molecules in complex with one or several ADAb? Similarly, a test for ADAb should be able to detect free antibodies, but can it 'see' ADAb bound to drug molecules?

As pointed out by Hendy *et al*,¹ the conventional approach to test for ADAb is to test serum samples collected at the end of a therapeutic cycle (trough levels, see [figure 1](#)). Even though this provides a surrogate measurement of ADAb kinetics, trough-level measurements are far from optimal when judging the influence of ADAb on therapeutic efficacy.² The major reason for recommending trough-level measurements is that the most commonly used assays for antibodies to TNF antagonists are drug-sensitive, that is, they cannot reveal the presence of ADAb in a sample containing excess of drug.³ This includes ELISA for quantification of antibody against TNF antagonists. The technology is the most widely available assay and also the most drug-sensitive assay currently used. ELISA is unable to detect ADAb at times close to drug administration, as the comparatively huge

Figure 1 Kinetics models for anti-tumour necrosis factor drug and anti-drug antibody (ADAb) in serum samples of an immunised patient. The figure represents a patient treated with intravenous infusions of infliximab. In cases of subcutaneously administered drugs, the rise in drug levels is expected to be less steep and the peak levels more blurred. Note that the appearance of ADAb usually is not detectable until weeks/months after ADAb start being produced (after the 2 infusion), and that all or sizable amounts of ADAb are absorbed out or masked in ADAb—drug immune complexes after every drug infusion. This will affect test results for ADAb even in trough samples.

recommended dosages of biological TNF inhibitors result in circulating drug levels that supersede the levels of circulating ADAb days or even weeks after drug delivery (figure 1).

More importantly, however, one should realise that the read-out from a drug-sensitive test, including ELISA, may be affected weeks after infusion or injection of the drug, especially in the early stages of immunisation where ADAb levels are low. This means that ELISA may report false-low or false-negative ADAb findings even in trough serum samples. In recognition of this problem, some investigators who use ELISA for detection of ADAb report the antibody status as ‘inconclusive’ if drug is detectable in a serum sample without measurable ADAb.⁴ The inability of ELISA to accurately detect ADAb is relevant also in cases with subcutaneously injected TNF antagonists.

A shortcoming of all binding assays is that they do not reveal whether or not antibodies against biological TNF antagonists are neutralising or merely drug-binding but non-neutralising.² This is important because routine binding assays do not inform about the underlying binding kinetics; that is, affinity of binding and association/dissociation kinetics that decide whether or not an observed binding *ex vivo* between drug and

ADAb is having an effect *in vivo* that has clinical significance. An example of therapeutic relevance would be that the binding counteracts the ability of an anti-TNF drug to compete with high-affinity cellular TNF receptors in a manner that prevents TNF-induced cell signalling *in vivo*. Of consequence for both therapeutic outcome and safety would be if the binding generates immune complexes with/without activation of complement *in vivo*, as this might enhance drug clearance and/or cause side effects.

A rational approach to the problems facing anti-TNF- α biotherapies would be to discover underlying pharmacokinetic, pharmacodynamic and immunogenicity issues with clinically relevant technologies so that continued therapy can be tailored according to individual needs. One approach to accomplish this is to use therapeutic algorithms as discussed in the review by Hendy *et al.*¹ However, the use of this algorithm, first proposed by us⁵ and later supported by others,^{1 6–10} is seriously compromised if the underlying data are unreliable in the clinical setting.

Considering the complex scenario outlined in figure 1, and the shortcomings of ELISA as a clinically relevant tool for ADAb detection, we find that there are serious limitations to the use of ELISA for

assessments of immunogenicity of TNF antagonists and, therefore, that the test should be used with great caution as a tool for therapeutic guidance.¹ It is also of concern that the uncertainty surrounding the clinical consequences of drug immunogenicity will persist if the *in vivo* relevance of technologies used for therapeutic monitoring continues to be ignored.

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¹In contrast to Hendy *et al.*, we have no knowledge of laboratories that provide ELISAs that ‘give reliable ADAb levels in the presence of drug’.

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