Analytical and Clinical Evaluation of an Immunoassay for Estimating Immunogenicity of Infliximab and Etanercept in Indian Population

Canna Ghia¹, Shashank Akerkar², Shailaja Sabnis³, URK Rao⁴, Gautam Rambhad⁵

Abstract

Background/Objectives: Biologic anti-TNFs in India have improved the patient management. Significant proportions of patients lose response over time or do not respond. Possible explanations are suboptimal trough anti-TNFa concentrations or antibodies to anti-TNFs. The aim of this project was to set up and standardize an independent laboratory to test immunogenicity of anti-TNF biologics (infliximab and etanercept).

Methods: Three rheumatologists piloted this project approved by independent ethics committee and carried out in compliance with ICH/GCP guidelines. Pfizer supplied immunogenicity kits (Promonitor® - ELISA) to the independent laboratory (SRL labs). After informed consent, blood (5 mL) was collected before infusion of infliximab (n=8) or injection of etanercept (n=8).

Results: Mean age of 16 patients was 42.06 ± 12.89 years. While 4 patients tested negative for infliximab, one patient tested low positive and 3 patients were positive. Anti-infliximab antibody was detected in 1/8 patient (12.5%) and the blood level of infliximab was negligible.

Discussion: Anti-infliximab antibodies are found in 12%-44% of patients vis-à-vis anti-etanercept antibodies (0%-18%). Anti-etanercept antibodies are without apparent effect on effectiveness or adverse events. When anti-TNFa are used, therapeutic drug monitoring is of help for optimal clinical outcomes. It might be more cost effective to adjust anti-TNFa dosages according to serum drug concentrations. Clinicians should have access to immunogenicity testing facility in India. The results of the study were as per the observed percentages across the world.

Conclusion: This study met its objective of setting up and standardizing an independent laboratory for immunogenicity testing of anti-TNF biologics in India.

Editorial Viewpoint

• There are increasing number of indications for biologics and more affordability with availability of biosimilars.
• Biologics are known to loose responsiveness over time.
• This study was carried out to set up and standardized an independent laboratory to test immunogenicity of infliximab and etanercept.

Background

In the last decade, the use of biologics especially the anti-TNFs (infliximab and etanercept) in India have changed the way rheumatology was practiced. However, despite being overall effective, a significant proportion of anti-TNF treated patients lose response over time or do not respond. Possible explanations for this are less than the required trough anti-TNFa concentrations in a target range or antibodies that are being formed against these agents.¹ Low drug levels and immunogenicity are associated to a loss of clinical response in rheumatoid arthritis (RA) and spondyloarthropathy. Till recently, the immunogenicity of anti-TNF drugs was not perceived as a clinical issue and in the absence of an assay to measure the drug levels and anti-TNF antibodies, treatment failures due to anti-TNF alpha could be deciphered solely based on clinical outcome.² But now it is well known that there is a very strong association between low anti-TNF drug levels, positive anti-drug antibodies and the loss of clinical

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Received: 09.11.2015; Revised: 20.04.2016; Accepted: 26.04.2016
The prevalence of anti-infliximab antibodies in RA varies from 12% to 44% and seems to be inversely proportional to the level of serum infliximab and therapeutic response. In fact, patients with anti-infliximab antibodies discontinue the treatment earlier, because immunogenicity is one of the mechanisms behind the efficacy failure. A study done by Bendtzen K et al has shown that among 106 RA patients receiving infliximab, 13% were positive before the third infusion (6 weeks), 30% were positive at 3 months, and 44% were positive at 6 months accompanied by diminished trough levels of infliximab. Pascual-Salcedo D et al in their study concluded that the formation of anti-infliximab antibodies during treatment with infliximab was associated with a loss of clinical efficacy response, development of infusion reactions and discontinuation of treatment.

The use of etanercept has been associated with the development of anti-etanercept antibodies in 0% to 18% of patients, but without apparent effect on effectiveness or adverse events. Similarly, studies have shown the prevalence of anti-adalimumab antibodies from 1% to 87%. In a study done by Jung SM et al in 360 patients of RA and ankylosing spondylitis, the prevalence of immunogenicity was highest i.e. 17 (28.8%) with infliximab, 17 (10.4%) with adalimumab, and significantly lower 2 (1.4%) with etanercept.

Immunogenicity screening is required for drug development during clinical trials, because of efficacy and safety issues. Now the need for patient monitoring is recognized not only by the regulatory agencies and the industry, but also by the clinicians. The field of rheumatology desperately needs methods that provide clinically useful results, which are robust, sensitive enough and validated. Hence it is important that every clinician should have access to a standardized immunogenicity testing facility in a country like India, where this testing is not currently done.
Table 2: Assay cut-off points and interpretation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cut-point</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infliximab&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.035 μg/mL &lt; 0.035 μg/mL – Negative for Infliximab</td>
<td>0.035-1.5 μg/mL – Low positive for Infliximab</td>
</tr>
<tr>
<td></td>
<td>≥ 1.5 μg/mL – Positive for Infliximab</td>
<td></td>
</tr>
<tr>
<td>Anti-Infliximab antibodies&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 AU/mL &gt; 2 AU/mL – Positive for antibodies to Infliximab</td>
<td>2 AU/mL – Negative for antibodies to Infliximab</td>
</tr>
<tr>
<td>Etanercept&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.035 μg/mL ≤ 0.035 μg/mL – Negative for Etanercept</td>
<td>≥ 0.035 μg/mL – Positive for Etanercept</td>
</tr>
<tr>
<td>Anti-Etanercept antibodies&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142 AU/mL ≤ 142 AU/mL – Negative for antietanercept antibodies</td>
<td>≥ 142 AU/mL – Positive for antietanercept antibodies</td>
</tr>
</tbody>
</table>

Table 3: Drug and antibody concentrations cut-offs for infliximab and etanercept

<table>
<thead>
<tr>
<th>Drug level</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infliximab</td>
<td>Negative for Infliximab</td>
</tr>
<tr>
<td></td>
<td>Low positive for Infliximab</td>
</tr>
<tr>
<td></td>
<td>Positive for Infliximab</td>
</tr>
<tr>
<td>Anti-Infliximab antibodies</td>
<td>Positive for antibodies to Infliximab</td>
</tr>
<tr>
<td>Etanercept</td>
<td>All patients were positive for Etanercept</td>
</tr>
<tr>
<td>Anti-Etanercept antibodies</td>
<td>Antietanercept antibodies were not observed even in a single patient</td>
</tr>
</tbody>
</table>

Results

A total of 16 patients enrolled into the study (Mean age 42.06 ± 12.89 years). Of these 16 patients, 12 were males and 4 females. Eight patients each were on treatment with infliximab and etanercept respectively. The dosage of infliximab used was 3 mg/kg at recommended time intervals whereas etanercept used was either 25 mg once weekly (n=2), 25 mg twice weekly (n=2) and 50 mg once weekly (n=4) as per the rheumatologist standard of practice. All these patients had high disease activity and were on concomitant therapy with methotrexate (15 mg once a week).

Assay cut-point is defined as the level of assay above which a sample is defined to be positive for the presence of the particular biologic and below which a sample is defined to be negative for the presence of biologic. A systematic and statistical evaluation of assay responses for a subset of samples that are judged to be representative of drug-naïve target patient/subject population helps establish a valid assay cut-point. The cut-point of the assay can be considered valid for any type of rheumatology patient treated with these biologics.

Promonitor™ assay cut-points<sup>a</sup> are provided in Table 2.

This study analyzed the serum samples of biologic treated patients for serum drug levels and antibody formation. Drug concentration as well as antibody concentration cut-offs for infliximab and etanercept are listed in Table 3.

It was seen that all 8 patients had positive blood levels of Etanercept (Table 3). Antietanercept antibodies were not observed even in a single patient. While 4 patients tested negative for infliximab, one patient tested low positive and 3 patients were positive. Anti-infliximab antibody was detected in 1 patient (12.5%) and the blood level of this biologic was negligible (Table 3). The said patient was on infliximab since 1 year wherein the biologic was given once in 3 months.

Response failure can be associated with low trough levels of anti-TNFα biologicals consequent to development of antibodies<sup>1</sup> Though this patient on infliximab developed antibodies, the study was not designed to test this hypothesis. Further the sample size was far too low to comment on this aspect.

Discussion

Therapeutic drug monitoring is the optimal way to achieve effective treatment when biologics with inter-individual variability such as anti-TNFα are used. These biologics work best over a small blood concentration range. Below this range, serum anti-TNFα drug concentrations are probably too low to have a therapeutic effect. And above this range, to prevent adverse reactions, reduction in dose or extension of interval in between doses might be possible without the loss of clinical response. Taking into account the high cost of these agents, it might be more cost effective to adjust anti-TNFα dosages according to serum drug concentrations.<sup>1,12,13</sup>

The differences in assays...
and diverse patient populations studied make a direct comparison difficult. However, the results of this study are on the similar lines as those reported from previous studies, which have suggested that antibodies against infliximab are found in 12 to 44% of patients receiving this biologic and seems to be inversely proportional to the level of serum infliximab and therapeutic response. Hence early monitoring may help optimize dosing regimens for individual patients, reduce side effects, and obviate prolonged use of inadequate infliximab therapy. In a few cases, anti-infliximab antibodies will be positive before the worsening of clinical symptoms, therefore one can anticipate clinical failure before hand and bring about a change in anti-TNF therapy much before.

In a country like India, where biologics are used in extreme cases because of their cost, it becomes more than important, that they should be used judiciously. Hence drug immunogenicity should be considered patients receiving biological therapies. This study met its basic objective of setting up and standardizing an independent laboratory to test the immunogenicity of anti-TNF biologics (infliximab and etanercept).

The increasing number of biologic treated patients, in combination with this new assay, presents a unique opportunity to study the anti-antibody immune response in India. A proactive research partnership between the rheumatology fraternity and pharmaceutical organization resulted in a setting up of an independent laboratory for testing the immunogenicity of anti-TNF biologics (infliximab and etanercept) available in the country.

If the use of this monitoring tool is propagated to monitor the patient’s status and complement clinical assessment, management of patients especially in India; where anti-TNFs are used in late stages of the disease can be improved. One can also save costs because in a significant number of cases, antibody generation will precede clinical worsening of symptoms. As these tests demonstrate clinical utility they can be used to speed up the decision making process. It is expected that with the setting of this laboratory, as increased number of clinicians become aware, they will make use of this tool to better the management of patients treated with biologics. If monitoring is not routinely possible or challenging, antidrug antibodies should be tested as soon as a worsening of the clinical improvement is detected. This is the only way to take advantage of immunogenicity assay. It will also be noteworthy to see if every patient of biologic requires immunogenicity testing at some point in his treatment.

References