Immunogenicity of biologicals

New diagnostic tests

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Blood and Beyond
Immunogenicity of biologicals

More and more proteins are used as therapeutic agents. Especially the use of therapeutic antibodies has been very successful. Well-known examples of therapeutic antibodies are TNFα-inhibitors like infliximab (Remicade®), adalimumab (Humira®) and etanercept (Enbrel®), which are applied for inflammatory diseases such as rheumatoid arthritis, Bechterew’s disease, Crohn’s disease, psoriasis etc. Rituximab (Mabthera®), directed against the CD20-antigen on B-cells and originally developed for treatment of lymphomas, has increasingly been applied for autoimmune diseases. Several other biologicals have been developed for cancer therapy, such as trastuzumab (Herceptin®) for breast cancer treatment.

Most therapeutic antibodies induce an unwanted immune response. The first generation of therapeutic antibodies was of murine origin (figure 1) and the immunogenicity of those antibodies has been studied extensively. Some 90% of patients treated with murine antibodies produced human anti-mouse antibodies (HAMA). Immunogenicity of therapeutic antibodies has been markedly reduced by replacing murine constant regions with human ones, resulting in chimeric antibodies such as infliximab and rituximab. About 50% of the patients treated with chimeric antibodies develop human anti-chimeric antibodies (HACA). Humanization of the variable regions further reduces immunogenicity. However, even fully human antibodies like adalimumab lead to the production of human anti-human antibodies (HAHA) in ca. 20% of treated patients. Antibody responses may also be influenced by patient-related factors such as genetic background, underlying disease, immunomodulating therapy and dosing schedule.

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<tr>
<th>Mouse</th>
<th>Chimeric</th>
<th>Humanized</th>
<th>Human</th>
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<td>(OKT3)</td>
<td>(infliximab)</td>
<td>(trastuzumab)</td>
<td>(adalimumab)</td>
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**Antibody response**

- HAMA
- HACA
- HAHA
- HAHA

**Immunogenicity**

*Figure 1. Engineered monoclonal antibodies and their immunogenicity in humans.*
Clinical consequences
The formation of antibodies against various anti-TNFα-antibodies in patients and the clinical relevance thereof has been demonstrated and published by the Sanquin research team (see key publications).
In several studies Sanquin and other research groups (ref. 6, 7) found a clear relation between levels of therapeutic antibody, HACA or HAHA production and efficacy of therapy.
For example:
• In RA patients treated with adalimumab, a clear correlation was found between antibody levels and therapy efficacy (ref. 1);
• Likewise, in RA patients treated with infliximab, HACA formation was associated with a reduced response to treatment (figure 2, ref. 2);
• In patients with Bechterew’s disease who were treated with infliximab, a correlation was found between absence of clinical response and the presence of anti-infliximab-antibodies, together with low infliximab trough levels (ref. 3);
• Besides a decrease in trough levels we found a relation between antibody formation and adverse effects such as infusion reactions (ref. 4) and serum sickness-like clinical reactions (ref. 5).

Current immunogenicity tests
Sanquin Diagnostic Services has developed tests to determine levels of biologicals and antibodies directed against biologicals. HAHA and HACA are quantified using validated antigen-binding tests (RIA), while levels of therapeutic antibodies are assessed using validated ELISAs, both on a routine basis. Serum samples are collected just prior to administration of the following drug dose, to prevent complex-formation of biological and antibody, which could lead to false-negative results.

Immunogenicity tests in development
Our standard assay format allows for quick development of new tests. Currently we are developing tests for abatacept, abciximab and omalizumab. To inform yourself on the most recent assays in development, consult our website regularly: www.biologicals.sanquin.nl. Please contact Sanquin to evaluate possibilities for development of tests in which you are interested: biologicals@sanquin.nl.

Personalized medicine
Determination of levels of biologicals and antibodies directed against these biologicals allows to optimize therapy for each patient. As each patient responds to treatment individually, next to the results of biological and antibody levels Sanquin also provides tailor made advice.

Test results and interpretation
• Good clinical response, adequate levels of biologicals and no antibody formation: continue treatment;
• Limited clinical effect, decreased drug level and a moderate antibody response: consider increasing the dosage or the dosing frequency;
• Absence of positive clinical effect, diminished drug level and a significant antibody production: consider switching to another anti-TNFα-blocking agent;
• No clinical improvement, sufficient serum drug levels and no antibody response: consider switching to a drug that intervenes in other immunological pathways.
Key publications


Sanquin Diagnostic Services

Sanquin Diagnostic Services performs blood testing in the field of immunology and transfusion medicine and advises medical professionals on the test results. Sanquin Diagnostic Services complies with the highest levels of quality with dedicated professionals and scientific support. Our immunogenicity assays are used for routine screening for both academic centres and hospitals in the Netherlands. We welcome medical professionals and researchers from outside the Netherlands to send us your samples.

Collaborations

Sanquin Diagnostic Services is supported by Sanquin Research, which is actively involved in research concerning assessment of immunogenicity of biologicals and assay development. Sanquin is a member of ARTHRON, the collaboration of academic and clinical centres in Amsterdam. These institutes, all with a profound knowledge of rheumatology, are involved in phase III trials for biologicals. They have a range of related biomaterials at their disposal.

Sanquin Diagnostic Services also cooperates with other knowledge institutes and companies involved in the development of biologicals on an international level.