Autoimmune disease disorders have been noted in the medical literature since the mid-1960s. For example, direct connections between viral infections and autoimmune diseases were described specifically in hemolytic anemia. Today over 80 diseases are related to an immune system response to “self” which can damage organs, tissues and cells. Normally, the immune system functions to produce a response designed to protect against agents such as bacteria, parasites and cancerous cells. The response involves immune cells and or antibodies. Autoimmune disease will arise when the immune system attacks normal body constituents as if they were foreign. These autoantibodies, target their own cells, tissues and organs which in turn cause inflammation and tissue damage leading to autoimmune disorders.

Most autoimmune diseases are rare with the number of cases rising. Over 80% of those individuals with Autoimmune disease are women—systemic lupus erythematosus and Hashimoto Thyroiditis affecting 9-10 women for every man. Complicating the disease further, overlapping genetic traits increase susceptibility to many of the diseases. Patients may suffer from more than one disorder and the same family may face multiple autoimmune diseases. Autoimmune disease has no known cure however a variety of treatments specific to the disease are available.

Since autoimmune disorders cover a wide area, research approaches to developing therapeutic agents range from gene therapy to administration of antibodies, vaccines and cytokines. The future direction for the development of effective treatments may involve immunomodulation as opposed to immunosuppression.

The clinical picture in individuals with autoimmune diseases vary widely and given the generally poor knowledge and awareness of this area of illness, the need for better diagnostic support increases. Laboratory testing of autoimmune markers meets that need. In this edition of Somagen Quarterly we explore the Phadia EliA® test and show how highly specific autoimmune tests offer high clinical value.

The management of chronic inflammatory disease today is through the use of biotherapies known as anti-tumor necrosis factor (anti-TNFα) drugs (Infliximab, Adalimumab, and Etanercept). These biomarkers are capable of binding TNFα and blocking the action of the TNFα that is responsible for the inflammatory state. However patients that are treated with these therapeutics are not all responding well to the treatment and among those who are responders, the level of response is variable from one individual to another, but also for the same individual over time. Inside the pages of this edition of Somagen Quarterly we introduce a new supplier to Somagen Diagnostics, Theradiag who have developed a novel EIA test—Lisa Tracker which is used to measure the efficacy of these biotherapies. Through therapeutic drug monitoring, measurement of drug trough levels and anti-drug antibodies, clinicians are now able to better manage patients over their course of treatment.
EliA Testing

Autoimmune disease testing in the routine laboratory today is carried out by a variety of methodologies. While operational efficiency, assay sensitivity and specificity are the hallmarks of a good assay, the clinical value of the result is ultimately the main driver for good clinical decision making. For many tests quantitative results enable estimations on the severity and the prognosis of the disease. Quantitative results therefore have a higher clinical value than qualitative results which solely point out if the test result is ‘positive’ or ‘negative’.

EliA assays combine exceptional specificity with highest sensitivity and provide the guidance and safety clinicians need for their daily diagnostic decisions.

**Most important, the positive predictive values and likelihood ratios of EliA assays provide excellent values assuring high clinical usefulness in routine practice.**

"Phadia EliA® dsDNA assay provides the best specificity, positive predictive value and post-test probability in the study."  
Antico, Lupus 2010

"…. a positive result by EliA® CTD Screen had a higher likelihood ratio than a positive result by indirect immunofluorescence."  
Op de Beeck, Autoimmun Rev 2011

"…. IgA anti-tTG assay from Phadia had the highest likelihood ratio in this study."  
Vermeersch P, Clin Chim Acta 2010

"Importantly, positive predictive values of the automated tests (85.2%) in comparison to the CCP IgG 3.0 manual ELISA (72.5%) resulted in a better correlation with clinical RA."  

**EliA Autoimmune Assays provide test results with high clinical value, crucial for good clinical decision-making and the key for diagnostic success.**

These rapid serological assays (ANCA PR3s, MPOs and anti-GBM) should enable clinicians to make or dismiss a well-founded clinical diagnosis of AAC and anti-GBM disease and lead to rapid institution of appropriate therapy in case of a positive result and further diagnostic evaluation in case of a negative result respectively.”  

"In conclusion, newly developed EliA® methods for antiphospholipid antibody detection (Anti-cardiolipin and Anti-β2 Glycoprotein I) perform similarly to other ELISA assays and represent a useful tool for APS laboratory diagnosis in daily practice.”  

By offering specific markers (e.g., EliA dsDNA) which are appropriate for the follow-up of patients clinicians can benefit from support in patient management.

“Follow-up of SLE patients with nephritis showed good correlation between flare and dsDNA antibodies by EliA® dsDNA.”  
Poster Viander M. et al. 5th International Congress on Autoimmunity, Sorrento, Nov. 2006

“EliA® dsDNA detects anti-dsDNA antibodies in patients with renal involvement more frequently and with a significantly higher mean titer compared to patients without this complication.”  

EliA Menu

Thermo Fisher Scientific uses advanced techniques such as recombinant gene technology to develop the latest standardized, precise, reliable and easy-to-use diagnostic tests for clinical indications to include:

- Connective Tissue Disease – 20 markers for SLE (systemic lupus erythematosus), Scleroderma, Dermatomyositis/polymyositis, Sjögren’s syndrome, Mixed connective tissue disease
- Rheumatoid Arthritis – RF and CCP antibodies
- Antiphospholipid Syndrome – Cardiolipin and B2-Glycoprotein I antibodies
- Vasculitis and anti-GBM diseases – MPO, PR3 and GBM antibodies
- Coeliac and other gastrointestinal diseases – Tissue Transglutaminase, Deamidated Gliadin and Gliadin, Faecal Calprotectin, ASCA
- Thyroid Diseases – Thyroglobulin and Thyroid Peroxidase (TPO) antibodies
- Liver Diseases – LKM1 and M2 antibodies
- Other – Anti-IgA

What is EliA?

The EliA test is a fluorescence enzyme immunoassay (FEIA) and is designed as a sandwich immunoassay.

A well is coated with an antigen that is specifically recognized by target antibodies, representing markers for a particular autoimmune disease. If these specific antibodies are present in the patient’s blood sample, they will bind to the antigen. In the subsequent reaction step, an enzyme-conjugated secondary antibody binds to the target antibody, bound to the antigen. The enzyme transforms an added substrate into a fluorescent product. Comparing the fluorescence signal with that of calibrators of known concentrations enables the antibody concentration in the test sample to be determined.
Phadia 250 – operational efficiency

Phadia Laboratory Systems ensure productivity with operational efficiency with a broad autoimmunity menu covering clinically relevant autoimmunity markers for evaluation of the most common autoimmunity diseases, e.g., rheumatoid arthritis, coeliac disease and connective tissue diseases.

- Completely automated assays with 28-day calibration by antibody isotype
- All tests can be measured from one sample
- Onboard dilutions
- Throughput: 60 results/hour
- Connectible to Laboratory Automation Systems
- True walkaway instruments requiring minimal hands-on time
- Smart software applications for continuous on-line monitoring and secure data handling
- Random access, automatic reflex testing and stat capability
- Capacity and efficiency for low to high throughput laboratories
- Remote diagnostics through Phadia LabCommunity
- The largest quality assessment program in the world
- Excellent consistency over time and between countries, systems, laboratories and users

What is the benefit of using a highly specific autoimmunity test?

Results of highly specific tests are more useful for clinicians due to the higher positive predictive value. For example Coeliac disease (CD) is characterized by a lifelong intolerance to gluten from wheat, barley or rye. The overall prevalence of CD in the general population is approximately 1%. However, there are many non-diagnosed patients because about two thirds of patients do not show the typical gastrointestinal symptoms and have so-called silent or latent CD. In contrast to other autoimmune diseases, coeliac disease has the advantage of having very sensitive and specific serological markers. A simple blood test can virtually rule out or confirm coeliac disease with almost 100% certainty. A positive screen test result usually leads to a confirmatory biopsy. Unnecessary biopsies should be avoided whenever possible, and particularly in children. High specificity of the screen tests for coeliac disease is therefore particularly important. Due to the prevalence of CD being only about 1% in a screening population, most suspected cases will prove not to have coeliac disease. Even a slight decrease in specificity will therefore dramatically increase unnecessary intestinal biopsies.

Quality Club – excellence through evaluation

Constant improvement is key to providing qualitative test results efficiently. Quality Club is an assessment program that monitors laboratory performance. Through a system of monthly reports, participating laboratories can evaluate their testing procedures to help maintain reliable, accurate test results. Members of Quality Club are part of the world’s largest testing community, one committed to monitoring and maintaining high-quality testing performance. With a membership of over 800 laboratories in more than 50 countries, Quality Club helps set the international standard for diagnostic testing.

As a member of Quality Club, you can:

- Assess laboratory performance against the highest quality standards.
- Note system errors and calibration problems.
- Maintain high accuracy and reliability in test results.
- Quality Club helps laboratories achieve superior testing performance and gain the confidence of healthcare professionals worldwide.

Somagen Diagnostics offers extensive instrument and autoimmune assay clinical expertise and support throughout Canada. Product and applications specialist’s help you get the knowledge and answers you need. To learn more about the Phadia 250 and EliA® please contact Somagen Diagnostics.
Inflammatory Disease Treatment

Patients treated for a variety of inflammatory diseases have seen considerable benefits from the use of genetically engineered antibodies that target inflammatory cytokines and cytokine receptors. Specific inhibition of the cytokine, tumor necrosis factor-α (TNFα), has brought about a revolution in the treatment of patients with several autoimmune diseases. These genetically engineered Anti-TNFα drugs account for over $40 billion spent to treat over 2 million patients.

Tumor Necrosis Factor - TNFα

Tumor necrosis factor alpha (TNFα) is a cytokine (cell signaling protein) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. The primary role of TNFα is in the regulation of immune cells. TNFα is produced primarily by monocytes and macrophages. It is found in synovial cells and macrophages in the tissues. It shares many properties with another cytokine - interleukin 1. It occurs in many inflammatory diseases, and also as a response to endotoxins from bacteria. TNFα promotes an inflammatory response, which, in turn, causes many of the clinical conditions associated with autoimmune disorders such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, psoriasis, and Crohn's disease.

Anti-TNFα Drugs

Anti-tumor necrosis factor (anti-TNFα) drugs are a class of drugs that have been used worldwide for over 10 years to treat inflammatory conditions such as rheumatoid arthritis, psoriatic arthritis, juvenile arthritis, Crohn's colitis, ankylosing spondylitis and psoriasis. They consist of biomarkers capable of binding TNFα and blocking the action of the TNFα that is responsible for the inflammatory state.

Three common biotherapeutic anti-TNFα drugs:

Remicade® – Infliximab

Infliximab neutralizes the biological activity of TNFα by binding with high affinity to the soluble and transmembrane forms of TNFα and inhibits binding of TNFα with its receptors. In rheumatoid arthritis, treatment with Infliximab reduced infiltration of inflammatory cells into inflamed areas of the joint as well as expression of molecules mediating cellular adhesion. In Crohn's disease, treatment with Infliximab reduced infiltration of inflammatory cells and TNFα production in inflamed areas of the intestine, and reduced the proportion of mononuclear cells from the lamina propria able to express TNFα and interferon.

Humira® – Adalimumab

Adalimumab binds specifically to TNF-alpha and blocks its interaction with the p55 and p75 cell surface TNF receptors. Adalimumab also lyases surface TNF expressing cells in vitro in the presence of complement. After treatment with Adalimumab, a decrease in levels of acute phase reactants of inflammation (Creactive protein [CRP] and erythrocyte sedimentation rate [ESR]) and serum cytokines (IL-6) was observed compared to baseline in patients with rheumatoid arthritis. A decrease in CRP levels was also observed in patients with Crohn's disease and ulcerative colitis.

Enbrel® - Etanercept

Etanercept is a dimeric soluble form of the p75 TNF receptor that can bind TNF molecules. Etanercept inhibits binding of TNF-α and TNF-β (lymphotoxin alpha [LT-α]) to cell surface TNFRs, rendering TNF biologically inactive. Etanercept has been shown to affect several animal models of inflammation, including murine collagen-induced arthritis.

Loss of Response

While there are numerous benefits to patients receiving treatment with these drugs not all patients that are treated with these therapeutics are responding well to the treatment and among those who are responders, the level of response varies from one individual to another, but also for the same individual over time.

Several mechanisms can explain this variability:

• Different physiopathologies that lead to other pathways other than the TNFα pathways.
• Inter and intra-individual fluctuations of the effective serum concentration of the biopharmaceutical for a given dosage. These fluctuations arise from several elements that can be intricately linked for example,
• Neutralization of the active site of the biopharmaceutical, through anti-drug antibodies (ADAb) given the immunogenic characteristic of these molecules.
• An acceleration of the clearance of the biopharmaceutical, independent from the presence of ADAb.
• A high clearance of the biopharmaceutical, independent from the presence of ADAb.

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Immunogenicity and anti-drug antibodies

Biotherapies are immunogenic and can trigger the production of anti-drug antibodies (ADAb). The frequency of ADAb varies according to the molecule and depends on:

- The dose administered
- The treatment scheme
- The immunogenicity of each molecule, linked to their structure
- The individual pharmacokinetic variability

Theranostics

In order to determine a patient’s response to treatment with anti-TNFα drugs, a new in vitro diagnostic modality known as Theranostics has emerged with the objective to provide information with which to offer greater personalization of treatment. Theranostic testing is combined with a targeted biotherapy in order to predict or evaluate a patient’s response by measuring drug trough levels and ADAb’s. Besides screening for disease, its purpose is to enable clinicians to establish the treatment best suited for each disease, check the results of this treatment over time, modify this treatment and even change it should that be necessary due to side effects or lack of effectiveness.

Monitoring Patients Under Biotherapies

Theradiag, a global leader in the field of Theranostics has developed a novel biotherapy monitoring tool Lisa-Tracker, used to measure the individual or simultaneous dosage of prescribed drugs or anti-drug antibodies (ADAb). Lisa Tracker EIA Kits include a complete range of assays that offer standardized protocols, ready to use reagents, flexible formats, adaptable to all automated platforms and results in 3 hours.

Therapeutic Drug Monitoring

Diseases related to immune system disorders, such as rheumatoid arthritis, ankylosing spondylitis and inflammatory bowel disease, are chronic pathologies which affect more than 28 million people around the world. Biotherapies, frequently trigger immune responses from the patient, leading to treatment loss of response. There is a need for therapeutic drug monitoring to allow clinicians to assess patient responses in order to anticipate, optimize and change treatment.

Rational, Evidence Based Decisions for Patient Management - Predicting Outcomes

In a paper published in the American Journal of Gastroenterology entitled, Development of an algorithm incorporating pharmacokinetics of adalimumab in inflammatory bowel diseases1 a group headed by Dr. Xavier Roblin was looking at patients with Crohn’s disease or Ulcerative Colitis on Adalimumab (Humira) who received the standard dose 40mg sub cutaneous every other week and were not doing well.

Part one of the study

In this group of roughly 100 patients, Dr. Roblin’s team checked levels of Adalimumab and levels of antibody to Adalimumab just before they were to receive their next dose. Based on that profile they were able to place patients into three groups.

1. Patients with therapeutic levels of Adalimumab
2. Patients with low levels of Adalimumab with no antibodies to the drug
3. Patients with low levels of Adalimumab with antibodies to the drug

All patients underwent dose optimization which means if they were not doing well, on every other week, their dose was escalated to 40mg sub cutaneous “weekly”. If they still did not do well, ultimately they were switched to another anti-TNF drug Infliximab (Remicade).

Based on those original three profiles, it was possible to predict how patients would do.

Patients in group two with low drug level and no antibodies did best during the phase of the trial when the dose was doubled (every other week to weekly). Whereas patients in the third group who already had antibodies to the drug did not do well with dose optimization nor did the first group who had therapeautic levels of the drug.

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Part two of the study

In part two of the study the patients switched to Infliximab. Those who had low levels of drug with antibody did well when they were switched. In spite of having therapeutic levels of drug, those in the first group remained symptomatic and did not do well. It did not matter if they increased the dose of Adalimumab or switched to Infliximab; 90% of these patients did not do well. Based on this pharmacokinetic profile, it was possible to organize patients into three groups in order to help predict how to treat them.

In his practice, Dr. Ed Loftus a gastroenterologist at Mayo Clinic in Rochester Minnesota looks at patients to determine the proper course of treatment using a similar approach. With his patients that are not doing well on Adalimumab he measures the drug trough level and antibodies. If there is a low level of drug with no antibodies, he increases the dose of Adalimumab; those with a low level of drug with antibodies, he switches these patients are switched to another anti-TNF drug. If they have a therapeutic level of Adalimumab and yet they continue to not do well, then he looks to switch to another drug out of class with a different mechanism of action, such as an anti-integrin antibody.

Dr Loftus believes that this approach represents the future of Inflammatory Bowel Disease—we will see this more and more with biologic drugs where clinicians are able to measure drug trough levels, quantify the amount of antibody to drug and then make rational evidence based decisions on how to manage these patients.

Proactive Therapeutic Drug Monitoring Has Long Term Benefits

In an article entitled, Proactive Therapeutic Concentration Monitoring of Infliximab May Improve Outcomes for Patients with Inflammatory Bowel Disease: Results from a Pilot Observational Study published in the journal, Inflammatory Bowel Diseases, Dr. Byron Vaughn Assistant Professor of Medicine at the University of Minnesota, examined at the role of proactive Infliximab drug monitoring in patients that are started on Infliximab. Infliximab is an anti-TNF biologic which has been used for 15 – 16 years to treat Crohn’s disease and more recently Ulcerative Colitis. This drug is very effective however it is a biologic and is a protein. Patients’ immune systems can develop antibodies to this big protein molecule and some patients while they initially respond well can lose response over time.

This paper outlined a proactive approach which looked at 48 patients to measure levels of Infliximab and antibodies to Infliximab regardless of how they were doing. Levels were measured even if doing well clinically. Another control group of 78 patients was part of the study where no monitoring or a more reactive approach was carried out.

Where drug levels were detectable but less than 5, patients would have their dose increased by a relatively small amount, 50 – 100 mg per dose. If drug level were undetectable, small adjustments would be made to the dose – not doubling as would be done typically. If two levels measured were higher than the therapeutic range (over 10) the team would cut back the dose. In 48 patients where proactive monitoring was conducted, adjustments needed to be made on most of these patients. Initially in approximately ¼ of patients, the dose was increased, whereas in 10% the dose was reduced. Over time the majority of patients had adjustments made. It turned out that this resulted in patients remaining on Infliximab significantly longer. Staying on drug was a proxy for doing well—had patients not done well they would have been switched to another drug. Over several years, 10% proactive group stopped Infliximab vs 31% in standard treatment. A proactive approach meant less patients needed to discontinue Infliximab. Using a Kaplan-Meier framework a 5 year probability of staying on the drug was 86% if using a proactive approach vs 50% remaining on the drug if using the reactive or no approach.

The study conclusions stated: In this pilot observational study, proactive therapeutic concentration monitoring (TCM) of Infliximab frequently identified patients with low or undetectable trough concentrations and resulted in a greater probability of remaining on Infliximab.

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