CONFERENCE REPORT

ORGENTEC International Conference 2013

Pentahotel Wiesbaden

May 16 2013

On 16th of May 2013 more than 100 laboratory experts, clinicians and scientists from all over Europe joined for a top-class medical symposium about laboratory diagnostics of autoimmune and selected infectious diseases.

Diana Simon from the University of Pécs, Hungary, gave the first presentation. She talked about the significance of natural autoantibodies in cardiovascular disease. Natural autoantibodies bind to highly conserved epitopes of intracellular structures. They occur in low levels and with low affinity and contribute to regulation of the immune system.

Elevated levels of IgG anti-hsp60 antibodies are considered as risk factors for morbidity and mortality of coronary artery disease. Anti-CS antibodies of the IgM class presumably are protective in heart transplant patients; their role in cardiovascular disease has not been investigated before.

Natural autoantibodies against hsp60 and CS were present in peripheral blood and in lower amounts in pericardial fluid from patients with ischemic heart disease. In the risk groups, consisting of individuals with type 2 diabetes, smokers and adipose persons, significantly higher levels of anti-CS IgG occurred in adipose and diabetic individuals. In patients with diabetes and smokers with bypass grafting concentrations of anti-CS IgM where significantly reduced. These results are consistent with data from previous studies that found high amounts of anti-hsp60 antibodies related to coronary artery diseases.

The cardiovascular risk factors obesity, smoking, and diabetes, may be associated with a change in the ratio of anti-CS IgG and anti-CS IgM. Levels of anti-CS IgG increase and levels of anti-CS IgM decrease, suggesting a protective role for anti-IgM antibodies in cardiovascular disease.

With the next talk Ferdinand Vlaspolder from the Department of Microbiobiology, Medical Center Alkmaar, Netherlands, lead the audience from the immunology of the heart to infectious disease. “EBV-diagnostics – is it that easy?” was his topic.

He first gave a brief overview on Epstein-Barr virus transmission and clinical aspects of the disease. These range from silent, nonspecific infections in children with prolonged low-grade fever, lymphadenopathy, cough, rhinorrhea, and pharyngitis to the classical picture of infectious mononucleosis. EBV-associated tumors, e.g. Burkitt lymphoma or nasopharyngeal carcinoma may also occur when the disease progresses.

Infectious mononucleosis is long known and a whole bundle of tests have been developed to detect the infection in early and late stages of the disease. Ferdinand Vlaspolder reported on testing for anti-EBV antibodies on a high throughput automatic system, the Immulite 2000 Xpi (Siemens Healthcare Systems) versus Alegria®, the random access analyser by ORGENTEC that allows for individualised automated analysis of smaller numbers of samples.

They analysed 84 samples that had been difficult to determine with the routine Immulite method on Alegria® and checked discrepant samples with an immunoblot. IgG antibodies to the different virus antigens EBNA and VCA were tested for the detection of latent stage infections. VCA-IgM and ZEBRA-IgM (Alegria® only) served as markers for early infection.

Comparison of the results showed a very good correlation of the two automated systems with only a limited number of discrepancies. Analysing the discrepant samples by immunoblot indicated that Alegria® performed better than Immulite and proved to be a reliable system for EBV serology. Alegria® is especially suited for a lower number of samples and may be used for additional testing of samples with ambiguous results in routine high throughput testing. The ZEBRA assay on Alegria® can be a reliable and time-saving alternative to the respective immunoblot.

Back to autoimmunity: Mario von Depka Prondzinski from the Werlhof-Institute in Hannover, Germany, spoke about diagnostics and management of the anti-phospholipid syndrome.

The anti-phospholipid syndrome (APS) is an autoimmune disease that causes thrombosis, recurring miscarriage and intrauterine foetal death. These symptoms are not specific to the APS, they can also accompany other diseases, but only the blood of patients with APS contains specific autoantibodies against phospholipids like cardiolipin or phospholipid binding proteins like beta-2-glycoprotein I that can serve as markers for the laboratory diagnosis of the syndrome. Besides the detection of antibodies against cardiolipin or beta-2-glycoprotein I that can be detected by ELISA, blood coagulation tests for the so-called lupus anticoagulants (LA) also contribute to the diagnosis of the APS. In general, LA tests are more specific, whereas the cardiolipin and beta-2-glycoprotein I ELISAs are more sensitive. Treatment with anticoagulants may interfere with the LA-tests and with the ELISA assays. Tests for anti-cardiolipin always require beta-2-glycoprotein I as a cofactor.

In patients with suspected APS due to clinical symptoms and negative tests for anti-cardiolipin or anti-beta-2-glycoprotein I, testing for other phospholipid-antibodies is recommended. These antibodies are either directed against the proteins annexin V or prothrombin, or they bind to other phospholipids: phosphatidylserine, phospatidyl-choline, phosphatidyethanolamine or phosphatidic acid. These antibodies may be very helpful in problematic cases.

After a refreshing coffee break with many lively face-to-face conversations Angela Hall from the Imperial College Healthcare NHS Trust, London, UK spoke about two well-known biomarkers for the rheumatology lab.

In her presentation “Anti-CCP and rheumatoid factor: A multi-platform study” she presented data that may contribute to improvement of the performance of an immunology service laboratory. Taking actions for centralisation of immunology services and automation of centralised lab facilities also supports lab efficiency.

The study focused on two important immunology parameters rheumatoid factor (RF) and antibodies to cyclic citrullinated proteins (anti-CCP). They are both integral parts of the ACR/EULAR criteria for classification of rheumatoid arthritis (RA), and tests for these parameters are frequently requested.

In a multiparameter study they tested more than 100 samples for RF and/or anti-CCP by various methods: particle agglutination or turbidimetry (RF), ELISA and chemo-luminescence (anti-CCP). 78 samples in this data set were analysed for anti-CCP with the Abbot Architect and with ELISAs from Eurodiagnostica and ORGENTEC.

On average values measured with the ORGENTEC Anti-CCP ELISA were seven times higher than values determined with Architect and five times higher than the values of the Eurodiagnostica ELISA. Comparative analyses of the data indicated almost perfect agreement of positive and negative test results between the ORGENTEC Anti-CCP ELISA and the other two methods. Only a very small proportion of samples had discrepant results.

The evaluation of Anti-CCP testing in comparison with determination of RF resulted in a strategic adjustment of the service design: CCP will now be used as the first line screening test for RA. RF will serve as a confirmatory assay in clinically relevant groups after consulting the rheumatologist about the clinical facts.

Autoantibody testing may resemble a look into a crystal ball as Elio Tonutti from the Laboratory of Immunopathology and Allergy, Udine Hospital, Italy explained in his presentation about the predictive role of autoantibodies as markers for autoimmune disease.

Predictive tests are a challenge for both, the clinician and the laboratory specialist. Autoantibodies may precede the onset of the clinical phase of a disease for years, as has been demonstrated for various autoimmune disorders, like systemic lupus erythematosus, rheumatoid arthritis, primary biliary cirrhosis or celiac disease. What does a positive autoantibody finding in an apparently healthy individual mean? Are these antibodies really predictive for a disease or are they just an epiphenomenon? Is the test reliable or are the results false positives?

Evidence comes from a number of retrospective and prospective studies, which demonstrate, that numerous antibodies are detectable up to more than ten years before the onset of symptoms and the clinical diagnosis. Usually, their predictive value is high. It may be useful to look for multiple antibodies simultaneously because the risk for developing an autoimmune disease increases with the number of positive autoantibodies. Also in healthy individuals, transient titres of antibodies are detectable. Generally, these samples are only low positive and they are not errors of the laboratory.

In the near future new technological options like microarrays or proteomic profiling will offer the opportunity to determine large numbers of autoantibodies and other biomarkers simultaneously. The clinical interpretation of these complex tests increasingly depends on laboratory medicine. This is especially true for positive findings that were not required for clinical reasons but are included in the array.

Laboratory experts must be able to translate these data into relevant clinical information in order to transform the results of laboratory medicine into recommendations for the benefit of the patient.

At the end of an interesting and informative morning session, Karl Lackner from the University of Mainz, Germany, grasped the attention of the audience with the provoking question: “Is beta-2-Glycoprotein I really the key antigen in the pathophysiology of the antiphospholipid syndrome?”

Clinical manifestations like thrombosis, abortion or thrombocytopenia and a signature autoantibody profile comprising anti-cardiolipin, anti-beta-2-glycoprotein I (beta-2GPI), and lupus anti-coagulans (LA) characterise the anti-phospholipid syndrome (APS). According to an international consensus statement, antibody titres must be high or intermediate on two occasions more than 12 weeks apart. Anti-cardiolipin antibodies should be measured with a standardised ELISA for beta-2-GPI dependent antibodies.

So far so good, but straightforward diagnosis of APS based on the consensus statements is not that simple, especially in patients with primary APS without any other autoimmune disease.

There is increasing evidence that phospholipid antibodies may belong to the natural antibody repertoire. Only limited data are available that explain the meaning of a positive anti-phospholipid titre without accompanying clinical symptoms.

Many patients express cofactor dependent and cofactor independent phospholipid antibodies simultaneously. Furthermore, patients may secrete phospholipid antibodies that depend on beta-2-GPI as a cofactor and antibodies that bind to beta-2-GPI only. Patients’ sera always contain beta-2-GPI and other cofactors. Therefore, it is not possible to perform an assay on serum samples in the absence of cofactors.

Phospholipid antibodies contribute to the APS by inducing a proinflammatory and procoagulant state. They activate signal transduction via toll-like receptors and interact with annexin on the cell surface. Dimerization of beta-2GPI by aPL activates other intracellular signalling cascades. Animal experiments demonstrate that antibodies against beta-2-GPI are not the only relevant pathogenic autoantibodies in the APS. Many open questions remain regarding APS pathogenesis.

Elena Tibenská from the Medirex Group in Bratislava, Slovakia, opened the afternoon session with the presentation of a study on HLA-DQ2/ HLA-DQ8 haplotypes and their correlation with serological markers in celiac disease (CD).

CD has a unique feature: a component from the environment, the protein gluten found in wheat, barley and rye, triggers the autoimmune reaction against the self-antigen tissue transglutaminase (tTG) in the gut endomysium. CD has a prevalence of up to 1 %. Children predominantly show gastrointestinal symptoms, e.g. recurrent diarrhoea, abdominal pain, failure to thrive or weight loss. In adults non-gastrointestinal manifestations like anaemia or vitamin B12-deficiency are common. CD is often associated with other autoimmune diseases of the thyroid or the liver and with type 1 diabetes.

Laboratory testing plays a key role in diagnosis of CD. Specific serological tests for anti-tTG and anti-Endomysium antibodies identify patients who need a biopsy of the small intestine mucosa. Antibodies against deaminated gliadin peptides (anti-DGP) support diagnosis in children under the age of two years.

CD is strongly associated with special HLA genotypes. Almost all patients with CD are HLA DQ2 and/or DQ8 positive. A positive anti-tTG-antibody test in an individual with HLA DQ2 and/or DQ8 is sufficient to confirm the diagnosis in symptomatic patients, according to the current guidelines of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN).

All relevant HLA alleles can be determined in parallel with a single DNA-microarray assay. At Medirex 462 patients had been tested for HLA-DQ2.2, HLA-DQ2.5, HLA-DQ2.2 + HLA-DQ2.5, HLA-DQ8 and HLA-DQ2 + HLA-DQ8. A high proportion (53%) of the test persons had either of the risk-haplotypes. Remarkably, 23 % of them were HLA-DQ8 positive. This is in contrast to other studies, which found DQ8 in about 10 % of the general population. This high frequency of HLA-DQ8 haplotypes thus may be a hint to a special genetic background of the Slovakian patient collective.

Reliability and quality of laboratory tests is always an issue, and the United Kingdom National External Quality Assessment Service (UK NEQUAS), the provider of external quality assessment schemes, regularly evaluates laboratory assays and the performing laboratories. Sheena Blackmore from the UK NEQAS Haematinics Scheme exemplified aspects of investigating laboratory performance under the topic "Testing for Pernicious Anaemia is not an exact science!" Pernicious Anaemia is a common autoimmune disorder in elderly people, which is characterised by the occurrence of autoantibodies against intrinsic factor or gastric parietal cells that are detectable in patients' sera by ELISA. Intrinsic factor has a sensitivity of 40-70 % and a specificity of 95-100 % for the detection of pernicious anaemia.

For monitoring laboratory performance, single positive or negative samples from the haematinics scheme are distributed to the participating laboratories. Participants then report a positive or negative finding for intrinsic factor antibody. Each sample sent out has a designated response (DR). Performance of the lab is scored according to the degree of concordance of the test results with the sample DR.

Unexpected or unexplained positive results for intrinsic factor may be related to the method used in the lab, to a certain lot number and to features of the sample material. If the reasons for discrepant test results are method or lot related close cooperation with the participating lab and the test manufacturer may contribute to settle these issues. Equivocal samples may be retested with an independent reference test and NEQAS further investigates the sample in question with regard to clinical and drug history of the donor or presence of heterophilic antibodies. If present, information on antigen source and epitope composition is also taken into account. Although the observed differences may be isolated incidents they point out that care should be taken when interpreting test results as, in spite of good laboratory performance, 'false positive' results may occur.

“Are ACPA positive and negative rheumatoid arthritis the same disease?” Zoltan Szekanecz from the University of Debrecen, Hungary, addressed this question in his talk.

Antibodies against citrullinated protein and peptide antibodies (ACPA) are a hallmark of rheumatoid arthritis (RA). They are an essential diagnostic tool for this disease and they often occur years before symptoms become evident. ACPA production is the result of a complex interaction of the genetic repertoire of the patient and environmental triggers, e.g. smoking or periodontitis. However, some RA patients never produce detectable levels of ACPA. Evidence is emerging from various studies, that these patients share genetic risk factors.

ACPA bind to various proteins, like fillagrin, fibrinogen, vimentin or alpha-enolase from oral bacteria and. ACPA tests are integral parts of the ACR criteria for classification of RA.

Genes and environmental factors contribute to ACPA production and to RA development in ACPA negative RA patients. A risk of about 30 % for developing ACPA positive RA is associated to the HLA-DRB1*04 haplotype. A number of other gene loci are also related to ACPA positive RA, e.g. PTPN22, IL23 and IL23R. HLADRB1*13 confers the risk of developing ACPA negative RA. Genome wide analysis reveals major differences between ACPA positive and ACPA negative RA with the HLA strongly dominating in the ACPA positive disease. Smoking is the most important environmental trigger for ACPA positive RA, but smoking does not contribute to ACPA negative disease. Besides smoking, excessive caffeine consumption and oral contraceptives increase the risk of developing ACPA positive RA, while alcohol consumption lowers the risk. In contrast, obesity slightly enhances the risk for ACPA negative RA. ACPA negative RA is less progressive and less destructive compared to ACPA positive disease. ACPA positive patients respond better to treatment with MTX or biological. Taken together, the differences between ACPA positive and ACPA negative RA support the hypothesis, that both are distinct disease subsets with different pathogenesis.

At first sight, the last presentation of the day seemed to be a nice distracting intermezzo. Candace Zaiden reported on her life and career as a professional opera singer. From the very beginning on the high school stage, she took the audience to the inspiring Tanglewood Music Festival in Berkshires, Massachusetts; she described her studies at Boston University and her first professional steps in Ohio and California.

Germany is a country with many opera theatres and a great tradition in classical music, this was the reason for her to move first to Koblenz and then to the Nationaltheater Mannheim. Here she was engaged to the ensemble of the opera chorus and had the possibility to perform solo parts.

However, an enemy from the inside always endangered this apparently easy storybook career. At the age of twelve, Candace Zaiden had been diagnosed with Lupus erythematosus. In defiance of the disease, she took the chance to start into a life as an artist and made her dreams for the future come true. After 20 years of a successful singing career, the disease now has taken its toll. The physical stress of acting on stage became harder to bear; drug effects damaged the voice and forced her into early retirement. Sharing Candace Zaiden's story reminds us what we are working for and makes aware, that also at the bench we are always dedicated to the good of the patient.