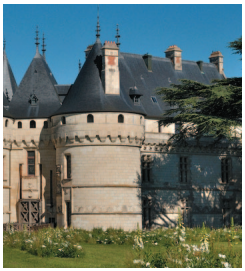
EASI™

European Autoimmunity
Standardisation Initiative



EASI Conference

ANA Testing – A Hot Issue

May 8th, 2010, 14:00 – 16:00 h (Hall B)
Ljubljana, Slovenia

European Autoimmunity Standardisation Initiative

Initiated and supported by Phadia

Dear EASI Friends,

We warmly welcome you to the 3rd EASI Conference, which will be held in coordination with the 7th International Congress on Autoimmunity in Ljubljana.

The EASI Committee, in line with this year's conference, would like to discuss the fascinating topic of the analysis of Antinuclear Antibodies as an aid in the diagnosis of Connective Tissue Diseases: ANA Testing – A Hot Issue.

Eight international speakers, noted for being at the top of their fields will gladly present their insights and experiences regarding ANA testing.
We would like to invite you all to share your opinions and practices with us and the other attendees of the EASI Conference.

We hope that these lectures and discussions will provide you with new information, which might help you to improve your daily laboratory or clinical routine.

The European Autoimmunity Standardisation Initiative (EASI) was founded with the intention of improving diagnostics in chronic rheumatic disorders by strengthening the collaboration between clinical and laboratory scientists responsible for autoimmune diagnostics at any given level of the health care systems in Europe.

Yehuda Shoenfeld
Congress President and Chairman of the EASI Network

EASI-ANA Testing – A Hot Issue

Supported by an Educational Grant from Phadia GmbH

Chairpersons:
Yehuda Shoenfeld · Israel
Michael Haass · Germany
Jan Damoiseaux · Maastricht University Medical Center, The Netherlands

Systemic Autoimmune Diseases: Clinical and Laboratory Challenges for the 21st Century ***Ricard Cervera*** · Hospital Clinic, Barcelona, Spain	14:00 – 14:15 h
Development of an Empirically Based Test Algorithm for Systemic Rheumatic Diseases: The Mayo Clinic Connective Tissue Disease Cascade ***Henry Homburger*** · Mayo Clinic College of Medicine, USA	14:15 – 14:30 h
Recommendations of the IUIS/WHO/AF/CDC Committee on the Standardization of Autoantibody Detection ***Pier Luigi Meroni*** · University of Milan, Italy-Ist G Pini - Ist Auxologico Italiano, Milan, Italy	14:30 – 14:40 h
Value-Added Reporting in Autoimmune Serology ***Xavier Bossuyt*** · University Hospitals Leuven, Leuven, Belgium	14:40 – 14:50 h
An Italian Multicentre Study for Application of a Diagnostic Algorithm in Autoantibody Testing for Autoimmune Rheumatic Disease ***Chiara Bonaguri*** · Parma Hospital, Parma, Italy	14:50 – 15:00 h
From ANA to ENA: Testing Algorithms in The Netherlands ***Jan Damoiseaux*** · Maastricht University Medical Center, Maastricht, The Netherlands	15:00 – 15:10 h
Portuguese Autoimmunity Laboratory Survey ***Carlos Dias*** · Hospital São João/Faculdade Medicina Universidade do Porto, Porto, Portugal	15:10 – 15:20 h
Evaluation of Autoimmundiagnostics in Austria Using the Dutch Autoimmune Questionnaire ***Manfred Herold*** · Innsbruck Medical University, Innsbruck, Austria	15:20 – 15:30 h
Detection and Clinical Relevance of the Fine Specificity of Antinucleolar Autoantibodies for the Diagnosis of Systemic Sclerosis ***Danilo Villalta*** · A.O.S. Maria degli Angeli, Pordenone, Italy ***Nicola Bizzaro*** · Ospedale, Tolmezzo, Italy	15:30 – 15:40 h
Diagnostic Value of Antibodies Against Ribosomal Protein P in SLE ***Torsten Witte*** · Medical School Hannover, Hannover, Germany	15:40 – 15:50 h

R. Cervera
Autoimmune Diseases, Hospital Clinic, Barcelona, Spain

Systemic Autoimmune Diseases: Clinical and Laboratory Challenges for the 21st Century

Autoimmune diseases are conditions in which the immune system damages normal components of the individual. They may be classified, somewhat arbitrarily, into organ specific and systemic autoimmune diseases. Although the real prevalence of these diseases is not well known, some estimates indicate that more than 20% of the population suffer from one or another autoimmune disease. This figure could be even higher if it is confirmed the postulated hypothesis of an autoimmune mechanism for some more prevalent conditions, such as atheromatosis. Additionally, there is no doubt that "a little autoimmunity" is relatively common even in normal population. Therefore, it is not surprising that a significant number of Nobel Prizes have been awarded to scientists who deciphered some of the mysteries involved in the differentiation between self and non-self. Ten clinical and laboratory challenges for the 21st century will be briefly reviewed to emphasise the significance of autoimmune diseases in health care.

It is of note that our knowledge on the significance of autoimmune diseases in health care has greatly expanded in the recent years. However, the main challenge is still therapy. Let us hope that in the third millenium more revelations will be known to enable us to cure some, if not all, of the autoimmune diseases.

Presentation 2 – Abstract 1525

H.A. Homburger, MD
Professor (emeritus) Mayo Clinic College of Medicine, USA

Development of an Empirically Based Test Algorithm for Systemic Rheumatic Diseases: The Mayo Clinic Connective Tissue Disease Cascade

Algorithm testing is useful in situations of low disease prevalence where clinical signs and symptoms are compatible with multiple diagnoses. Test algorithms can improve overall diagnostic accuracy, speed evaluation of patients, eliminate unnecessary referrals to specialists, diminish the likelihood of inappropriate treatment and reduce overall costs. Screening tests are chosen to identify patients for further testing and follow up tests are used to avoid errors in diagnosis that may result because of poor test specificity. Comprehensive databases such as the College of American Pathologists Diagnostic Immunology Survey are helpful to identify those analytic methods that are suitable for use in a test algorithm. In the case of ANA, some analytic methods fail to detect all the clinically important auto antibodies.

At Mayo Clinic, we developed a test algorithm for systemic rheumatic diseases that uses the ANA test as a first order screen with a cut off for follow up testing for specific auto antibodies based on empirical data from our medical practice. Follow up tests are performed when the likelihood of finding a disease specific auto antibody is appreciable and patterns of test results are accompanied by appropriate computer coded interpretive comments. Quality assurance is achieved by real time tracking of auto antibody frequencies to identify biases that may develop because of changes in reagents. This presentation reviews the Mayo algorithm and test system, and shows how this algorithm can be implemented with different analytic methods for the screening ANA test.

P.L. Meroni
Internal medicine, Division of Rheumatology, University of Milan, Italy- Ist G Pini - Ist Auxologico Italiano, Milan, Italy

Recommendations of the LUIS/WHO/AF/CDC Committee on the Standardization of Autoantibody Detection

The impact of autoimmune diseases (AID) is growing from a clinical and from a laboratory point of view. Diagnostic assays are not only carried out in specialised laboratories but also in high throughput routine service laboratories. The number of the methods and their spreading increased the variability among the laboratories raising the problem of their reproducibility. On the other hand we do need a reliable test for an early diagnosis and a prompt treatment and for reducing the cost of repeated confirmatory tests or unnecessary further investigations.

New methodologies - in particular for antinuclear antibodies - have been applied to autoantibody detection in order to process larger volumes of samples more quickly and at less cost than the traditional ones. However, because of the lack of their standardization, inaccurancies in the results have been reported. A subcommittee of the American College of Rheumatology was set up in order to address this issue: the ANA Task Force. Specific recommendations have been suggested inaccuracies that represent a realistic preliminary step in the harmonization of diagnostic tests for autoimmune diseases.

Presentation 4 – Abstract 1391

X. Bossuyt
University Hospitals Leuven, Leuven, Belgium

Value-Added Reporting in Autoimmune Serology

In daily clinical practice, an important question clinicians are asking is "What is the probability of a patient having (or not having) disease X for a specific laboratory test result?" Likelihood ratios can help to solve that question. The likelihood ratio is the ratio of the probability (likelihood) of the specific test result in people who have the disease to the probability in people who do not have the disease.

In the presentation I will illustrate how test result specific likelihood ratios may add value and help with interpretation. The concepts will be illustrated for serological markers for celiac disease, small vessel vasculitis, inflammatory bowel disease and rheumatoid arthritis.

C. Bonaguri[1], A. Melegari[2], A. Ballabio[3], P. Terenziani[4], P.P. Dall'Aglio[5], A. Russo[1], L. Battistelli[1], R. Aloe[1], G. Lippi[1]

[1]Diagnostic Laboratory Department, Parma Hospital, Parma, [2]Diagnostic Laboratory Department, Modena Hospital, Modena, [3]Diagnostic Laboratory Department, Piacenza Hospital, Piacenza, [4]Diagnostic Laboratory Department, Reggio-Emilia Hospital, Reggio-Emilia, [5]Clinical Immunology Department, Parma Hospital, Parma, Italy

An Italian Multicentre Study for Application of a Diagnostic Algorithm in Autoantibody Testing for Autoimmune Rheumatic Disease

Aims

The presence of specific auto-antibodies in serum (i.e. ANA, anti-dsDNA, anti-ENA), is one of the major criteria for the diagnosis of Autoimmune Rheumatic Disease. As such, the request of these tests has grown exponentially in the laboratory practice. The aim of this study is to implement an universal guideline for reducing clinically inappropriate test requests of autoantibody testing in a broad geographic area (Parma, Modena, Piacenza, Reggio-Emilia).

Methods: This study, started in January 2008, is an observational research aimed to compare the number of tests (ANA, anti-dsDNA, anti-ENA) and the percentage of positive test results before and after implementation of the diagnostic algorithm.

Results

- A multidisciplinary team comprising clinical immunology and laboratory scientists was instituted, with the aim to collect and analyse diagnostic criteria, clinical needs, laboratory report formats, analytical procedures and number of tests performed.
- A common guideline for autoantibody testing, which poses ANA test at the first level, has been developed and implemented from January 2009.
- The results for the period January -June 2009 (12.738 tests) were compared to those of the same period in 2008 (13.067 tests). A significant reduction in the number of anti-dsDNA (26%) and anti-ENA (15%) was observed.

Conclusions

The development and implementation of diagnostic algorithms not only allowed a reduction in the number of second-level tests, but also increased their diagnostic specificity. The percentage of second-level tests positivity after implementation of the diagnostic protocol has also remarkably increased both for ENA (13% vs. 17%) and dsDNA (9% vs. 11%).

J. Damoiseaux[1], L. Bakker-Jonges[2], J.W. Cohen Tervaert[1], R. Derksen[3], D. Hamann[4], H. Hooijkaas[5], C. Kallenberg[6], I. Klasen[7], P. Limburg[6], R. Smeenk[4]

[1]Clinical and Experimental Immunology, Maastricht University Medical Center, Maastricht, [2]Laboratory of Clinical Immunology, Reinier de Graaf Gasthuis, Delft, [3]Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, [4]Sanquin Diagnostic Services, Amsterdam, [5]Immunology, Erasmus MC, University Medical Center, Rotterdam, [6]Rheumatology and Clinical Immunology, University Medical Center Groningen, Groningen, [7]Blood Transfusion and Transplantation Immunology, Nijmegen University Medical Centre, Nijmegen, The Netherlands

From ANA to ENA: Testing Algorithms in The Netherlands

Harmonization of testing algorithms is a goal of the European Autoantibody Standardization Initiative (EASI). The focus has been set on algorithms for anti-nuclear antibodies (ANA), anti-dsDNA antibodies, and antibodies to extractable nuclear antigens (ENA).

To find out the current practice the Dutch EASI team has designed a questionnaire consisting of 57 items in the categories: organization (n=4), ANA testing (n=14), anti-dsDNA antibody testing (n=8), anti-ENA antibody testing (n=15), and the testing algorithm (n=16). This questionnaire was sent to all participants (n=81) of the quality assessment program for these antibodies. Participants from abroad (n=2) and from diagnostic companies (n=2) were excluded. Sixty-six diagnostic laboratories responded (86%). ANA, anti-dsDNA antibodies, and anti-ENA antibodies are performed in 58, 63, and 62 laboratories, respectively. Major findings are:

- 11 laboratories do not test ANA by IIF,
- 14 laboratories screen ANA with a 1:40 dilution without titration,
- 16 laboratories report anti-dsDNA antibody as qualitative results,
- 8 laboratories only report positive anti-ENA antibody specificities,
- 19 and 14 laboratories have no algorithm for ANA in relation to anti-dsDNA antibodies or anti-ENA antibodies, respectively.

Results were reported in a meeting organized by the national foundation for quality assessment in clinical laboratories (SKML). Although the results may not be translated into strict guidelines for autoantibody testing, they will enable recommendations on how to test for and report results. Communication of these recommendations will increase the awareness of the relation between these antibody categories and, eventually, may result in further harmonization of the testing algorithms.

C. Dias[1], J. Ramos[1], M. Carvalho[2], M.J. Sousa[3], EASI Portugal
[1]Hospital São João/Faculdade Medicina Universidade do Porto, [2]Hospital Santo António, Porto, [3]Hospital Amadora Sintra, Sintra, Portugal

Portuguese Autoimmunity Laboratory Survey

On behalf of the Portuguese European Autoimmunity Standardization Initiative (EASI) we performed an approach to the Portuguese clinical labs that could be using autoimmunity assays. Our goal was to map the country status on the autoimmunity lab diagnostic in terms of antinuclear (ANA), anti double-stranded DNA (anti-DNA) and extractable nuclear antigens (anti-ENA) antibodies.

We used a strict Portuguese translation of the Dutch EASI survey, in agreement with the EASI meeting held on the 9th Dresden Symposium on Autoantibodies. This survey includes a total of 57 multiple-choice test questions, from lab classification, ANA, anti-DNA, anti-ENA testing and diagnostic algorithms and other clinical issues. A total of 160 mailings have been posted with a deadline for the survey submission on the 31st of December 2009.

Our preliminary results reveal that 61% of the ANA results are based on immune-fluorescent assays, with a wide range of cutoffs (4% 1/40, 40% 1/80, 56% 1/160, with one lab reporting an age related cutoff). The tittering of the IF assay is dealt with a wide range of options, from no titer (4%), up to an unlimited tittering (4%). Also, the reporting of the ANA results has a wide range of options.
On the anti-DNA and ENA assays a wide range of variations in assay performance and reporting have been revealed.

The lack of standardization in anti-nuclear assays results in significant variation of performance and reporting options. The Portuguese EASI branch has targeted this issue as main goal, so that guidelines are issued in 2010.

M. Herold, EASI Austria
Department of Internal Medicine 1, Innsbruck Medical University, Innsbruck, Austria

Evaluation of Autoimmundiagnostics in Austria Using the Dutch Autoimmune Questionnaire

Introduction

There is no stardardized procedure for detecting autoantibodies. IIF on Hep-2 cells is still the method of choice but not possible in all laboratories. Beside technical equipments like an immunoflurescence microscope experience in viewing the slides is necessary. Immunoassays are easy to handle but results often differ from IIF. This study was performed to evaluate the laboratory methods used in Austria to ANA.

Methods

A questionnaire recently used in the Netherlands (Jan Damoiseaux, Netherlands, 2009) and translated into English was used. The questionnaire with 57 questions regarding autoimmune diagnostics was sent to all laboratories joining the Austrian quality control for laboratory diagnostics. 39 completed questionnaires were returned. 37 laboratories use Hep-2 cells, two laboratories ELISAs.

Results

For ANA screening, a serum dilution between 1/40 and 1/160 (median 1/80) is used and positive samples are titrated up to dilutions between 1/2560 and 1/20480 (median 1/5120). All laboratories describe common patterns. In most laboratories ANA slides are evaluated by two coworkers independent of each other. Special algorithms for ANA as well as for ENA diagnostics are usually used.
Conclusion: To optimize laboratory procedures in autoantibody testing and and to improve the communication between laboratories and clinicians is necessary to know what is the usual diagnostic procedure and how are results reported in each country. A comparison on an international level should help to harmonize the algorithms for a cost effective and rational autoantibody testing. We therefore used the same questionnaire as the Netherlands some months ago hoping that other countries will follow.

Danilo Villalta[1], Nicola Bizzaro[2]
[1]Allergy and Clinical Immunology- DML, A.O. "S Maria degli Angeli", Pordenone, Italy
[2]Clinical Pathology, Civic Hospital, Tolmezzo, Italy

Detection and Clinical Relevance of the Fine Specificity of Antinucleolar Autoantibodies for the Diagnosis of Systemic Sclerosis

Autoantibodies against nucleolar antigens (ANoA) are characteristically found in systemic sclerosis (SSc) whose clinical features, organ involvement, natural history and survival are correlated with SSc-associated serum autoantibodies.

The predominant ANoA are anti-U3 RNP (fibrillarin-AFA), anti-PM-Scl, and anti-Th/To antibodies, that are detected in 10-15% of SSc patients. Moreover, anti-RNA polymerase III (anti-RNAP III) autoantibodies, which however are not exclusively associated to the nucleolar pattern, are found in 5-23% of SSc. AFA-positive patients seem to have more frequent skeletal muscle involvement and pulmonary arterial hypertension (PAH); anti-Th/To positivity has been associated with poor survival due to pulmonary fibrosis and PAH; anti-PM-Scl antibody presence has been historically associated with polymiositis/scleroderma overlap syndrome, and anti-RNAP positivity with a higher frequency of scleroderma renal crisis. Unfortunately, until recently, the only specific method for their identification was the radioimmunoprecipitation (IP) assay, a cumbersome technique not suitable for routine use. Recently, ELISA methods for the detection of anti-RNAP III and anti-PM-Scl have been developed, which are highly accurate and may represent a valid substitute to IP in a clinical setting. Development of an ELISA method for AFA detection is currently in progress and the preliminary results are satisfactory.

T. Witte
Medizinische Hochschule Hannover, Hannover, Germany

Diagnostic Value of Antibodies Against Ribosomal Protein P in SLE

Aims

To study the diagnostic value of antibodies against ribosomal protein P (RibP) for SLE in a "real-life situation".

Methods

All the 105 patients that were referred to our outpatients´ clinics for the first time for diagnostic evaluation of SLE in 2009 and had at least one clinical ACR classification criterion of SLE were included into the study. Antibodies against RibP were measured using an automated test system (EliA RibP, Phadia, Freiburg, Germany), antibodies against dsDNA by Farr assay. The diagnosis was established by the physician without knowing the results of the RibP antibodies.

Results

Antibodies against RibP were present in 5/25 patients with SLE, but in none of the 80 non-SLE patients (with other CTDs, vasculitis, sarcoidosis, undifferentiated arthritis). All of the 5 patients with antibodies against RibP had active disease (SLEDAI score at least 7). 1 of the 5 SLE patients with antibodies against RibP did not have antibodies against dsDNA.

Conclusion

Antibodies against ribosomal protein P are specific for SLE and are helpful in the evaluation of patients with active disease, in particular in the absence of antibodies against dsDNA.

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