

Standardization of Autoimmune Diagnostics in Germany

Activities of the German Group in the European Autoimmune Standardization Initiative

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ABSTRACT: The German Regional Group of EASI was established during the annual Meeting of the German Society of Immunology in Kiel in September 2005. Since this initial informative meeting, an active core group of about a dozen rheumatologists, immunologists, and laboratory specialists has been generating starter projects. In general, these projects do focus on clinically associated diagnostic questions, and do integrate a variety of specialists with profound knowledge in several related subjects. The aims of the German EASI group are to contribute to the definition of standards and to improve patient care. Therefore, the group is establishing guidelines for the diagnosis of autoimmune diseases,

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to standardize and improve their quality, combining the experience of clinical and laboratory specialists. The diagnostic activities focus currently on systemic lupus erythematosus (SLE) and on rheumatoid arthritis. These activities include laboratory investigations and diagnosis through clinical manifestations. Standardized diagnostics cannot be based solely on vague symptoms and positive laboratory tests. In laboratory diagnostics, standardization and implementation of objective methods for the detection of autoantibodies has been identified as a central challenge. Here, immune fluorescence techniques and the evaluation of RibP are used as first parameters that could improve SLE diagnostics. Furthermore, guidelines and proposals from scientific medical organizations, and in particular from other national EASI groups will be adapted to the German health system. A cornerstone of implementation is the identification and logistic preparation of existing serum banks, the definition of gaps that should be bridged, and, particularly, the definition and collection of adequate control groups. Through these measures, the German EASI group will provide a standardized diagnostic model of autoimmune disorders throughout Europe starting in the field of rheumatology. Diagnostics may become more rational, efficient, faster, and cost-efficient. Patients with autoimmune rheumatic disorders will profit from receiving an earlier and more accurate diagnosis, which again will allow earlier therapeutic intervention and lead to a better long-term clinical outcome.

KEYWORDS: autoimmunity; standardization; guidelines; laboratory diagnostics

INTRODUCTION

Despite an excellent health system in Germany and high standards in medical care and research, patients suffering from autoimmune diseases are not always diagnosed in the best way. This may cause delayed onset of adequate therapy. For the patients, this may be related to unjustified pain, poorer prognosis, secondary complications, and an important loss of quality of life.

Reasons for that may be at least in part:

- Insufficient connection between research and medical care
- Separation of scientific activities between laboratory and clinic
- Delayed transfer of scientific knowledge into clinical practice
- Presence of guidelines with controversial recommendations
- Missing data about relevance of international publications for German population
- Release of diagnostic tests without adequate validation in autoimmune cohorts
- Discrepancy between peer-viewed diagnostic guidelines and actual routine situation

- Financial restrictions and inadequate health policy regarding optimal autoantibody determination
- Slight knowledge in immunodiagnostics by resident doctors

EASI aims to address and overcome these issues. The German Regional Group has been established during the September 2005 annual Meeting of the German Society of Immunology in Kiel.

THE GERMAN EASI GROUP

The active core group of the German EASI is formed by rheumatologists, immunologists, and laboratory specialists that are engaged in the field of autoimmune diseases and cover the whole field from research and laboratory diagnostics to clinical care, treatment, and clinical studies. Our strategy aims to include active members that represent all parts of German health system, including doctors in an own practice, clinicians, laboratory specialists, and academic institutions.

CURRENT PROJECTS

According to the aims of EASI projects focus on clinically associated diagnostic questions. Thereby, we integrate a variety of specialists with profound knowledge in several related subjects. So far, the following first projects are under investigation:

- Algorithms in autoimmune diagnostics
- Evaluation of guidelines
- Status and availability of serum banks

ALGORITHMS IN AUTOIMMUNE DIAGNOSTICS

A major challenge in standardizing autoimmune diagnostics is the diversity of methodological aspects. Several questions should be addressed, for example, what procedures of laboratory diagnostics can be proposed as golden standards or which tests give specific information for a certain clinical situation?

Starting in the field of systemic lupus erythematosus (SLE) diagnostic we have analyzed the diagnostics situation of the indirect immunofluorescence (IIF) on HEp-2 cell, a widespread and useful tool as a first-line test system. Furthermore, we wanted to get a clearer picture of the value of rib P antibodies for SLE diagnostics evaluating rib P antibody tests within clinically well-defined patient cohorts.

HEp-2 Analysis

The determination of non-organ-specific autoantibodies based on indirect immunofluorescence using HEp-2 cells, a permanently growing epithelial cancer cell line, represents the first and necessary step in most accepted protocols for autoantibody screening in the diagnostics of chronic rheumatic diseases. Nevertheless, unexpected controversial procedures could be identified by interviewing high-quality labs in this field. Such differences are not only caused by HEp-2 cell subtypes or preparations,¹ but represent technical equipment, experiences, and customs of every laboratory staff.^{2,3} We are analyzing these protocols and plan to propose a consensus guideline, not only to harmonize diagnostics, but also to generate reproducible rules that can be used in automatization activities.^{4,5}

RibP-Antibodies in SLE Diagnostics

Autoantibodies directed to RibP have been discussed to provide further information in diagnostics of SLE.⁶ To verify the diagnostic benefit by detecting this marker, the sensitivity and specificity of antibodies against RibP were evaluated using seven different commercially available enzyme-linked immunosorbent assay (ELISA) kits. Sera from 115 patients with SLE were collected in outpatient departments; the majority of the patients was in remission. The sensitivity of antibodies against RibP varied from 3% to 14%, the specificity, assessed in 118 controls, was 90% in one test, 96% in a second, and 100% in the remaining five tests. Currently, the sensitivity of RibP antibodies is being evaluated in active SLE. To explain these findings, clinical data of all patients are revisited at the moment.

EVALUATION OF GUIDELINES

Our aim is to review and evaluate guidelines, overviews, recommendations, and proposals from several authors, scientific medical organizations, and from other national EASI groups for the diagnosis of autoimmune diseases.⁷⁻⁹ Differential diagnostics of patients suffering from SLE has been chosen for first investigation and checked for actuality and how these guidelines can be adapted to the German specific situation.

Especially in this field, a huge variety of similar but not identical guidelines have been published, including criteria and recommendations by scientific medical organizations.^{10,11} We would like to develop a guideline that covers the relevant medical aspects and prove to be a realistic and feasible guideline on the basis of the German reimbursement system. In addition to a highly sophisticated guideline for the specialist, a definitive need for a guideline for

the family doctors is also requested, because they see the patients when showing early symptoms of the disease.

STATUS AND AVAILABILITY OF SERUM BANKS

Most activities involving laboratory investigations require well-defined patient groups in serum banks. We have established a register of existing serum banks in Germany. We focused on the existing capacities, the logistic preparation of samples, and defined gaps that should be bridged. This revealed an acute need for a well-structured bank fulfilling a multitude of requirements, not only for patients' samples but also concerning definition and collection of adequate control groups.

The goal of our activities is to establish a serum and data bank based on standardized and company-independent approaches regarding serum sampling and storage as well as ascertainment of the necessary clinical and histological data of disease and adequate control groups for the following activities:

1. Comparison between different methods (including novel multiparametric assays) for the determination of each diagnostic important autoantibody entity
2. Comparison of assays of different companies
3. Evaluation of novel or potential novel diagnostic relevant autoantibodies
4. Evaluation of novel assays
5. Establishment of round robin tests

These results of those investigations should help in defining guidelines for an optimized and cost-effective autoantibody (or autoantibody profile) analysis for the appropriate autoimmune disease.

CONCLUSION

Activities of EASI groups will contribute to standardized diagnostic models of autoimmune disorders throughout Europe starting in the field of rheumatology. So, diagnostics may become more rational, efficient, and cost-efficient. Clinicians treating patients and the patients suffering from autoimmune rheumatic disorders will profit from these activities.

APPENDIX

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REFERENCES

1. HAHM, D. & U. ANDERER. 2006. Establishment of HEp-2 cell preparation for automated analysis of ANA fluorescence pattern. *Cytometry A* **69**: 178–181.
2. SHACKNEY, S., D.R. EMLET, A. POLLICE, *et al.* 2006. Guidelines for improving the reproducibility of quantitative multiparameter immunofluorescence measurements by laser scanning cytometry on fixed cell suspensions from human solid tumors. *Cytometry B* **70**: 10–19.
3. HIEMANN, R., N. HILGER, U. SACK & M. WEIGERT. 2006. Objective quality evaluation of fluorescence images to optimize automatic image acquisition. *Cytometry A* **69**: 182–184.
4. SACK, U., S. KNOECHNER, H. WARSCHKAU, *et al.* 2003. Computer-assisted classification of HEp-2 immunofluorescence patterns in autoimmune diagnostics. *Autoimmun. Rev.* **2**: 298–304.
5. WIJK, A. 2004. Guidelines and approaches to autoantibody testing. *In* From Animal Models to Human Genetics: Research on the Induction and Pathogenicity of Autoantibodies. K. Conrad, M.P. Bachmann, E.K.L. Chan, M.J. Fritzler, R.L. Humbel, U. Sack, Y. SHOENFELD, Eds.: 441–446. Pabst Science Publishers. Lengerich, Berlin, Riga, Rom, Wien, Zagreb.
6. ARNETT, F.C. & M. REICHLIN. 1995. Lupus hepatitis: an under-recognized disease feature associated with autoantibodies to ribosomal P. *Am. J. Med.* **99**: 465–472.
7. STATENS SERUM INSTITUT. 2005. Autoimmun. Serologi. DANAK Reg. Nr. **397**.
8. GEBECAUER, L. & J. LUPAC. 2006. Standard operating procedure (SOP) evaluation of autoantibody fluorescence patterns on HEp-2 cells. DYNEX. Praha.
9. CONRAD, K., W. SCHÖBLER & F. HIEPE. 2006. Autoantikörper bei Systemischen Autoimmunerkrankungen. *In* Immundiagnostische Bibliothek. K. Conrad & U. Sack, Eds.: 1–251. Pabst Science Publishers. Lengerich, Berlin, Riga, Rom, Wien, Zagreb.
10. <http://www.dgkl.de/autoimmundiagnostik/lupus.html>.
11. HOCHBERG, M.C. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **40**: 1725.