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AESKU. SCIENCE

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Sjögren-Syndrome

New opportunities for early diagnosis

Lab automation: opportunity or threat?

AESKU.KIPP INSTITUTE: First Research Projects on the Starting Line

Challenge Lyme Disease

AESKU.AWARD 2006

Bridging the gap

Dr. Torsten Matthias, AESKU.DIAGNOSTICS

AESKU.DIAGNOSTICS' staff thinks innovation and straightforwardness are no contradiction but that the implementation of new developments into everyday laboratory work rather plays a significant role in innovation.
Therefore, also the third issue of AESKU.SCIENCE will report about new diagnostic options, which will make the diagnosis of autoimmune diseases not only safer and quicker, but in particular also more convenient.
AESKU.SCIENCE will focus now on Sjögren's syndrome to demonstrate, how complicated the diagnosis of an autoimmune disease can be, when no sensitive laboratory markers are available, and when the clinical picture is very unspecific at first sight. The new marker alpha-Fodrin allows identifying almost all patients, at least in the early stage of the disease.
Quality and profitability – another contradiction?
Medical laboratories all over the world need to fight economic pressure and to increase sample throughput at the same time, and to combine this even with improved quality of the results. Experienced lab experts know that automation is the only way to succeed in bridging the gap between these conflicting requirements.
Shorter output times, efficient sample flow, error reduction and more spare time for professional quality assurance and special diagnostic offers, all these positive achievements of automation clearly demonstrate that no area of laboratory diagnostics can escape this trend in the long term, including autoimmune diagnostics.
Therefore, AESKU.SCIENCE does not only discuss the technical features of new products optimally fitted for automation and their positive impact on the efficiency of laboratory diagnostics, but the current issue specifically focuses actual benefits which automation may have for the laboratory staff.

We wish you pleasant reading!

Editorial

Focus

News

Diagnosis of Sjögren's syndrome

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Establishing the diagnosis of Sjögren's syndrome has been difficult due to the lack of sensitive laboratory markers and also in the light of the unspecificity of complaints of dry eyes and mouth. Recently, antibodies against alpha-fodrin have been shown to be present in 93% of untreated patients with Sjögren's syndrome. Therefore, they can be used as a marker for screening of patients. When Sjögren's syndrome is suspected, antibodies against alpha-fodrin and SS-A should be measured and objective tests of saliva and tear production should be performed.

Sjögren's syndrome was named after Henrik Sjögren, who described the syndrome in 1933 as a combination of dry mouth and dry eyes in patients with rheumatoid arthritis (1). Sjögren's syndrome is a common autoimmune disease (prevalence approximately 1-2%) with lymphocytic infiltration of lacrimal and salivary glands, resulting in dry mouth and eyes, i.e. the sicca syndrome. Secondary Sjögren's syndrome is concomitant to other connective tissue diseases or to rheumatoid arthritis, while primary Sjögren's syndrome occurs alone. The differential diagnosis of Sjögren's syndrome and other causes of the sicca syndrome is often difficult. Up to 10% of the population suffer from the sicca syndrome as a cause of physiological ageing, infections (hepatitis C, HIV), sarcoidosis or iatrogenic causes (e.g. radiation or more than 200 drugs like tricyclic antidepressants or beta blokkers) (2,3). It is important to perform laboratory tests to differentiate between Sjögren's syndrome and other causes of the sicca syndrome, because - at least in its initial phase - Sjögren's syndrome can be treated by immune modulation; furthermore, extraglandular complications like arthritis and polyneuropathy or neoplasia develop during its course, which should also be treated as early as possible.

Clinical picture

The diagnosis of Sjögren's syndrome is mostly performed in patients complaining about actual or imaginary symptoms of reduced gland function like permanently burning eyes, parching thirst or glossodynia. Although such symptoms may result from Sjögren's syndrome, they are also complaints of depressive patients and therefore do (almost) not correlate with an objective reduction in tear and saliva production. Approximately 20-40% of the Central European population complain about actual or imaginary symptoms of dry (e.g. burning) eyes or dry mouth, respectively (2,3).

Physicians should consider the occurrence of Sjögren's syndrome not only when the patients report subjective complaints, but also when objective consequences of dry glands occur like relapsing conjunctivitis, chronic sinusitis or potential extraglandular symptoms of Sjögren's syndrome like arthritis (in particular oligoarthritis) or polyneuropathy. Whenever one of these symptoms occurs, tear and saliva production should be measured, for example with Schirmer's test for tear production or the Saxon test for saliva production. Saliva production can also be assessed with scintigraphic methods, and inflammatory changes of the salivary glands including sialectasia can be detected by sialography of the parotid gland.

Laboratory diagnostics

Laboratory diagnostics should be performed to assess all patients with objective dryness of mouth and/or eyes and with potential manifestations of Sjögren's syndrome (arthritis, polyneuropathy). In the past, this mainly involved autoantibodies against SS-A (Ro) and SS-B (La), but also antinuclear antibodies and rheumatoid factors, while currently also tests for the detection of antibodies against alpha-fodrin are available. Also an invasive method can be performed, i.e. biopsy of the salivary gland. Their diagnostic values should be thoroughly considered:

Antibodies against SS-A (Ro)

Still today, many physicians consider antibodies against SS-A the best laboratory test for Sjögren's syndrome. These autoantibodies are detected in more than 90% of the patients, who were diagnosed by office-based physicians. However, the actual prevalence of antibodies in the Sjögren's syndrome is remarkably lower; in many studies it is only 30 – 40% (4), the specificity of antibodies against SS-A (Ro) may be even as low as 14%. Although antibodies against SS-A are a valuable laboratory marker of Sjögren's syndrome, the disease is not detected in many patients due to the lack of a positive autoantibody test.

Antibodies against SS-B (La) are more specific than antibodies against SS-A (Ro); however, they occur in approximately 20% of

Glossary

Schirmer's Test

Schirmer's test uses a 5 mm high and 35 mm wide filter paper strip inserted under the outer lid into the conjunctival sac to measure the production of tears. After 5 min, the distance is measured which the tears have run in the paper strip. The result is assessed pathological (i.e. positive Schirmer's test), if the distance is smaller than 5 mm.

Rose bengal test

Conjunctiva and cornea are stained with rose bengal - a dye for bacteriological and microscopic applications leading to a deep pink stain. The doctor compares the actual staining with a 1-9 score staining intensity sheet. The finding is assessed pathological from score 4. Accumulation of pigmentary defects equivalent to score 4 is also regarded pathological.

Biopsy of the lips

Specimens are removed from lips and microscopically diagnosed foci of inflammation.
Biopsies of the salivary gland are mostly taken from the lower lip. A supplemental lacrimal gland biopsy may be performed.

Saxon test

Saliva production is measured with multiple tests. The Saxon test can be performed most easily. The patients have to chew a gauze sponge for two minutes, and the sponge is weighed before and after chewing. The saliva volume collected in the sponge is determined by the weight difference.

Scintigraphy/sialography

To confirm an involvement of the large salivary glands, a scintigraphy of the salivary glands is performed including the demonstration of limited exocrine function and/or sialography with the demonstration of inflammatory changes of the parotid ducts. The imaging of frequently appearing parotitis increasingly involves ultrasound and now and then dynamic MRT.

the patients only. As antibodies against SS-B (La) occur only rarely when no antibodies against SS-A (Ro) are detected, their determination together with antibodies against SS-A (Ro) does not increase the sensitivity but only the specificity of the detection.

Antinuclear antibodies (ANA): earlier European classification criteria of Sjögren's syndrome considered antinuclear antibodies equal with antibodies against Ro and La as a laboratory criterion of Sjögren's syndrome. The sensitivity of these laboratory markers is excellent and above 80%; however - at least in low titers - antinuclear antibodies are detected in more than 25% of the population; therefore, their specificity for Sjögren's syndrome is only low.

Rheumatoid factors: In the past, also rheumatoid factors were used as a classification criterion of the Sjögren's syndrome. However, their sensitivity is considerably lower; they are detected in 30 – 40% of the patients only, and their specificity is low, too.

Salivary gland biopsy

A salivary gland biopsy is normally taken from glands of the lower lip. The risks involved in this intervention are low. However, a pathological biopsy is no reliable sign of the Sjögren's syndrome, because the diagnosis requires broad experience by both the performing physician and the evaluating pathologist. For example, a study was performed on the dual evaluation of biopsies. In half of the cases, the second diagnosis disagreed with the first (6). Biopsies only lead to clear results when the inflammation is severe, while the prevailing mild salivary gland inflammations are unspecific.

Antibodies against alpha-fodrin as a marker of Sjögren's syndrome

Alpha-fodrin belongs to the spectrins, a family of ubiquitarily expressed cytoskeleton proteins. Alpha-fodrin is a 240 kDa protein forming a heterodimer together with beta-fodrin. The heterodimers are embedded into the plasma membrane and bind to actin, calmodulin and microtubules. Fodrines are involved in the formation of cell organelles and secretion processes. During apoptosis, e.g. occurring at chronic inflammations, the 240 kDa protein is cleaved by caspase 3 to smaller fragments, including 150 and 120 kDa fragments, which are neoantigens and induce the formation of autoantibodies.

Autoantibodies against alpha-fodrin were first described in 1997 in a mouse model of Sjögren's syndrome, i.e. the NFS/sld mouse, thymectomized on day 3 (7). Since this time, increasing evidence was found that the immune reaction against alpha-fodrin is involved in the pathogenesis of Sjögren's syndrome. The autoantibodies bind only to cleavage products of alpha-fodrin being formed by caspase 3 digestion, but not to the intact protein. The disease can be prevented in the Sjögren's syndrome mouse model by blokking caspase. Even the injection of alpha-fodrin and thus tolerance induction does not only prevent the late formation of antibodies against alpha-fodrin in the mouse model, but also the outbreak of the disease. On the other hand, the late injection of alpha-fodrin metabolites can induce Sjögren's syndrome in healthy mice (late injection does not lead to tolerance, but to an immune reaction against alpha-fodrin). Also the transfer of T-lymphocytes specific for alpha-fodrin induces Sjögren's syndrome in normal mice. Alpha-fodrin-specific T-cells were also detected in humans with Sjögren's syndrome. These studies demonstrate an essential role of T-cells directed against alpha-fodrin in the pathogenesis of Sjögren's syndrome. However, the role of the autoantibodies is still unclear. So far, they appear to be rather a diagnostic and activity marker than inducing factor of Sjögren's syndrome.

Sensitivity of the antibodies against alpha-fodrin in Sjögren's syndrome

The sensitivity of the antibodies against alpha-fodrin is assessed rather differently. This is due to

1. the quality of the diagnosis (sensitivity is very high when objective criteria are used like extraglandular complications of Sjögren's syndrome rather than subjective criteria like complaints about dryness),
2. the therapeutic treatment of the patients (antibodies against alpha-fodrin are activity markers of Sjögren's syndrome and normalize after immune-suppressive treatment) and
3. the stage of the disease (alpha-fodrin antibodies as activity markers cannot be longer detected in the late stage, when the glands are completely destroyed and inflammations do no longer occur).

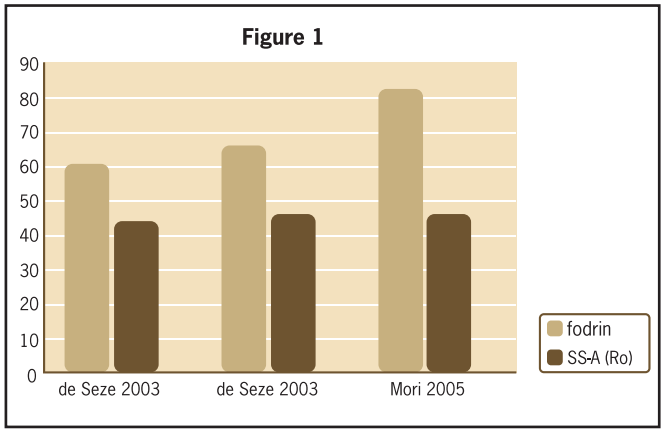

Figure 1: Prevalence (%) of antibodies against alpha-fodrin and SS-A in patients with Sjögren's syndrome und neurological manifestations in the studies of de Seze (14, 15) and Mori et al (16)

A prevalence of 97% of IgG antibodies against alpha-fodrin was reported in Japanese patients in the first study on the role of alpha-fodrin antibodies in Sjögren's syndrome; the antibodies were detected by immunoblots (7). The test was improved in more recent studies by the additional involvement of larger 150 kDa alpha-fodrin metabolites. This test detected a prevalence of even 98 % of IgG antibodies against alpha-fodrin (9) in patients, who were classified by the very stringent San Diego criteria of Sjögren's syndrome (8).

The authors examined the sera of patients classified by various criteria with the Aesku.Diagnostics ELISA test. The results demonstrated more frequently IgA than IgG antibodies against alpha-fodrin. The analysis of sera of patients classified according to the San Diego criteria (8) confirmed the data of the Japanese authors:

IgA antibodies against alpha-fodrin were detected in 88%, IgG antibodies against alpha-fodrin in 64% and IgA and/or IgG antibodies against alpha-fodrin in 93% of the total population of 85 patients (10). In patients, who were classified according to the less stringent American/European consensus criteria (11), IgA antibodies against alpha-fodrin could be detected in 64% and IgG antibodies against alpha-fodrin in 50% of the patients.
However, other authors published lower prevalences of antibodies against alpha-fodrin in Sjögren's syndrome (12,13), which may be attributed to the therapy of the patients discussed above, the duration of the disease or the stringency of the diagnosis, respectively.

The stringency of the classification criteria of Sjögren's syndrome varies with respect to the specificity of the diagnosis. In particular the European criteria include subjective complaints of patients on dryness of mouth or eyes. However, these symptoms are in no way associated with the objective consequences of Sjögren's syndrome, i.e. reduced saliva and tear production.
Ideal studies on the sensitivity of laboratory markers of Sjögren's syndrome should include the screening process of the subjects with objective manifestations of Sjögren's syndrome. Three studies performed in this way investigated the prevalence of various markers in patients with Sjögren's syndrome, which were primarily characterized by polyneuropathy as a frequently occurring extraglandular complication. In all studies, the prevalence of the antibodies against alpha-fodrin was higher than that of antibodies against SS-A (Ro) (Fig. 1) (14-16). The superiority compared to antibodies against SS-A is even more remarkable, because these antibodies are a classification criterion of Sjögren's syndrome and therefore all comparative studies are characterized by a bias towards SS-A antibodies.

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Specificity

It requires great effort to perform studies on the specificity of antibodies against alpha-fodrin with respect to Sjögren's syndrome. From the formal point of view, the detection of IgA and IgG

	Dry mouth and eyes	Dry mouth only	Dry eyes only	Normal tear and saliva production
	n = 4	n = 4	n = 49	n = 111
SS-A (Ro)	0	0	1 (2 %)	0
IgA alpha-fodrin	3 (75 %)	0	4 (8 %)	2 (2 %)
IgG alpha-fodrin	2 (50 %)	0	1 (2 %)	2 (2 %)
IgA/IgG alpha-fodrin	3 (75 %)	0	5 (10 %)	4 (4 %)

Table 1: Prevalence of antibodies against SS-A, IgG and IgA antibodies against alpha-fodrin in 168 healthy volunteers characterized with respect to saliva and tear production.

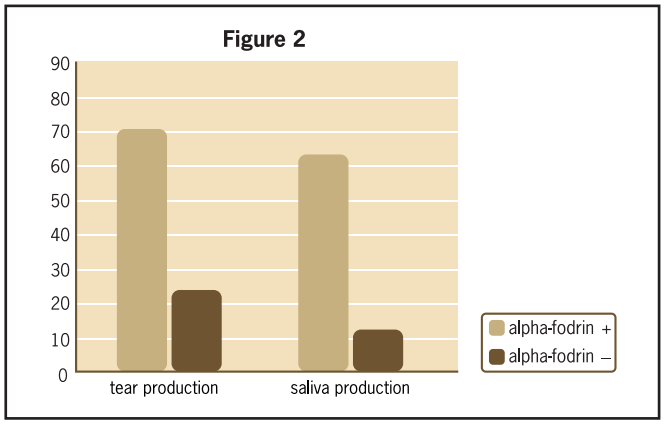

Figure 2: Probability (%) of the normalization of tear and saliva production during a three months hydroxychloroquine therapy in patients with or without alpha-fodrin antibodies

antibodies against alpha-fodrin increases in a number of chronic inflammatory diseases. For example, the prevalence of IgA antibodies against alpha-fodrin is approximately 10% in multiple sclerosis and rheumatoid arthritis patients and 2-3% in blood donors. The prevalence of IgG antibodies against alpha-fodrin is 10 - 20% in multiple sclerosis patients, 20% in rheumatoid arthritis and SLE patients and 2-3% in blood donors.

It is the problem of specificity studies that the participants may actually have non-diagnosed secondary Sjögren's syndrome. As the majority of patients with objective dryness of mouth and eyes do not report subjective complaints, mostly no corresponding diagnosis has been performed. Therefore, the authors have initiated studies to establish a potential association of antibodies against alpha-fodrin with secondary Sjögren's syndrome. It was demonstrated that 1/6 of the patients with primary-progressive MS suffer from Sjögren's syndrome. This patient group had antibodies against alpha-fodrin more often than against Ro or La (14). At least in primary progressive MS, the supposed unspecificity of the alpha-fodrin antibodies was instead improved sensitivity with respect to the previously undiagnosed Sjögren's syndrome.

To establish the specificity of the detection of antibodies against alpha-fodrin in a normal subject population, the authors performed a so-called beer-for-blood study (17). For this study, volunteers were recruited at a summer fair of the Medizinische Hochschule Hannover with the slogan "400 ml beer for 4 ml blood". The subjects were all characterized with respect to saliva and tear production and the presence of autoantibodies against Ro, La and alpha-fodrin. In all 168 subjects, who were rewarded with free beer following their participation, only antibodies against alpha-fodrin but not antibodies against Ro and La were associated with objective dryness of eyes and mouth. Prevalences of IgA and IgG antibodies against alpha-fodrin in subjects with normal tear and saliva production were 2% each, thus resulting in specificities of 98% (Table 1).

Association of antibodies against alpha-fodrin with the disease activity of Sjögren's syndrome

In patients, who underwent salivary gland biopsies, the concentrations of IgA and IgG antibodies against alpha-fodrin correlated with the degree of lymphocyte infiltration of the salivary glands. Antibodies against alpha-fodrin also correlated with the severity of eye dryness (18). Antibodies were also measured in the course of patients, who were treated with antimalarials or glucocorticosteroids. Autoantibody concentrations normalized within 3 months. It was also demonstrated that the prevalence of autoantibodies correlates with the stage of the disease and is highest in the early phase (19). It is assumed that the early stage offers the only opportunity of re-normalizing tear and saliva production with an immune-modulatory therapy. For example, we noted during the follow-up of patients with Sjögren's syndrome, who were treated with hydroxychloroquine, that tear production normalized in 7/10 of the patients with but only in 2/8 of the patients without autoantibodies against alpha-fodrin ($p = 0.07$). Saliva production normalized in 5/8 of the patients with but in only 1/7 of the patients without autoantibodies against alpha-fodrin ($p = 0.08$) (Fig. 2). Currently larger studies are being designed to demonstrate, whether antibodies against alpha-fodrin indicate an active and treatable stage of Sjögren's syndrome.

Recommended sequence of diagnosis

Sjögren's syndrome should less be suspected when subjective dryness of mouth and eyes is reported, but rather, when typical symptoms appear. These include, as discussed above, relapsing conjunctivitis without indication of allergic causes, accumulated infections of the respiratory tract, polyneuropathy (in particular sensory axonal polyneuropathy) and other neurological complications like primary-progressive multiple sclerosis.

The examination should be followed by the parallel investigation for autoantibodies against alpha-fodrin, Ro (SS-A) and L (SS-B); also the occurrence of a sicca syndrome should be evaluated with simple tests, like Schirmer's test for the measurement of tear production and the Saxon test for the measurement of saliva production.

Should objective dryness of mouth or eyes be established and antibodies against alpha-fodrin or Ro, respectively, be detected, the occurrence of Sjögren's syndrome is highly probable. An additional biopsy of the salivary gland is indicated in case of doubt, in particular, when the diagnosis of Sjögren's syndrome is intended to result in therapeutic measures. This includes for example the decision for a year-long immuno-suppressive therapy at polyneuropathy related to Sjögren's syndrome.

Conclusion

So far, Sjögren's syndrome was often overlooked, because no sensitive laboratory marker was available. Now, antibodies against alpha-fodrin allow to identify at least all patients in the early stage of the disease. The new laboratory test can be used for screening in addition to antibodies against Ro and La, when Sjögren's syndrome is suspected. Sjögren's syndrome should be suspected, whenever objective dryness of mouth and eyes occurs, but also when infections of the upper respiratory tract occur more frequently than normal or when polyneuropathy is detected. In future, early diagnosis of such patients should be possible, thus allowing the timely initiation of an immune-suppressive to ameliorate the symptoms of the disease.

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Green light for the new AESKU homepage

AESKU.DIAGNOSTICS' new homepage will provide product information on more than 100 various ELISA tests covering a broad spectrum of autoimmune diseases - figures, data and facts about novel test systems, immuno-analyzers, immunofluorescence tests, parameters in the serological diagnosis of infectious diseases - manuals and practically oriented advice and tips - background information on many clinical pictures - the most current information on research and development: not only the company AESKU.DIAGNOSTICS has developed with incredible speed since its establishment only seven years ago, but also the expanding product range increases the demand for information from day to day.

More and more, the internet becomes the most important source of information, because it provides most current knowledge tailored to individual needs.

Of course, this does only apply to well-organized websites. The information explosion - in particular in the internet - requires a clear structure to allow quick access and orientation. In particular in the internet, rapid service is important for the users. This means direct access to all issues, because daily lab work does not leave much time to search information within complicated structures.

AESKU.DIAGNOSTICS is currently building an expressway to users, research partners and the interested public to provide them with the diversity of information - the new AESKU homepage.

The website will present the company itself and will give a well-structured overview of the complete product diversity and detailed information on its multitude of applications, together with scientific and practical support; articles, news and working instructions will be downloadable.

The new homepage will be available from 1 January 2007. All curious individuals are invited to see an exclusive preview at the MEDICA. We are sure you will like it!

AESKU.SCIENCE - IMPRESSUM

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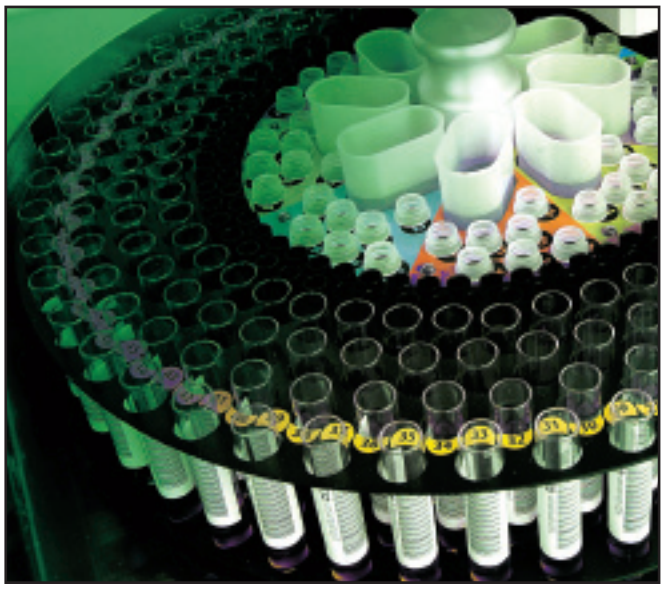
Lab automation: opportunity or threat?



Shorter patient stays in medical institutions, increasing awareness of patient safety and often dramatic staff shortage are three of the major reasons why labs need to tighten up their processes by an increased level of automation, wrote Robert A. Browning of the Baptist Hospital of East Tennessee Laboratory, Knoxville, Tennessee, U.S.A (1).

In addition, hospital labs will undergo an actual shift in paradigms, said Dr. Johannes Aufenanger of the Klinikum Ingolstadt in Germany in his lecture on the occasion of the Annual meeting of the Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (DGKL) in the beginning of October 2006 in Mannheim. Today, clinical labs are considered cost centers in the worst case or profit centers in the best case, but they will have to change to become true service centers in the future. This requirement essentially involves optimum workflow from pre-analytics to the transfer of the test results.

Is lab automation the ideal approach?

Not only in the U.S., but also in many other countries worldwide with well-developed health systems the economic success of hospitals significantly depends on shortest possible patient stays. However, the introduction of DRGs in Germany and other countries sets new standards, also in Europe.

Labs are urged to provide their results as quickly as possible to ensure early treatment and thus fastest possible recovery and dismissal. At the same time, this economic pressure makes highest demands to the reliability of the results. Test repeats or event treatment odysseys due to misdiagnoses cause considerable unnecessary costs.

Of course, reliable lab results are tremendously important for treatment quality and thus patient safety.

According to information given by the Institute of Medicine (IOM), 1 million patients per year are injured by malpractice in the U.S., almost 100,000 die (2). The IOM stresses that the major source of malpractice is previous diagnostic error.

Surely, clinical anamnesis still plays the most important role in diagnosis. However, depending on clinical picture or situation, up to 80% of the data used by physicians to meet vital decisions about the further treatment may originate from lab analyses. Rapid response and reliable results are required; labs are requested to perform the right test for any patient at the right time to prevent malpractice whenever possible.

Staff shortage in labs is due to intentional reduction of employees or stagnating staff size despite steadily increasing sample volumes. A study published in 2003 states that alone in the U.S. 8,000 jobs remain vacant each year (3).

In addition, new blood is missing in many states. The average lab employee in the U.S. today is significantly older than forty.

Experienced lab experts all over the world agree that automation is the solution to this problem. Only the increased implementation of specific, well-coordinated automation solutions will master the discrepancy between quality and profitability.
Automation

- provides lab results quicker, a deciding factor in the face of decreasing hospital stays;
- makes sample flow in routine diagnostics more efficient and provides for extra time for special diagnoses or professional stat diagnostics;
- reduces error-prone manual work;

The human factor counts!

It is easy to outline the positive effects of automation on the efficiency of lab diagnostics, but are there also benefits for the lab staff?
The most important arguments in the favor of automation from

the employee's view are easier work and a safe working environment. Automation frees day-to-day work from monotonous activities like sample processing and frees resources for important tasks which should be the actual work of qualified employees: interpretation of results and quality assurance.

Nevertheless, many individuals are skeptical about the implementation of automation. Most of them consider automation "assembly-line work", which may lead to the need for less qualified staff and in the end even the loss of the own workplace.

Many hospitals intending to re-structure the workflow in their labs fight potential negative feelings, worries and opposition by parallel so-called "change management" involving the employees in the change process.

The reason is obvious: acceptance and motivation of the lab staff are deciding for efficient exploitation of the option made available by automation and for long-term success in quality and profitability. It is therefore important to integrate all individuals into the - sometimes considerable - change process related to the implementation of automation solutions.

This approach is obviously successful. When re-structuring was intended, the Malmö University Hospital in Sweden involved its personnel already in the early steps of planning and decision finding (4). Of course, it was clearly outlined in the beginning, which changes would be unavoidable, on the other hand, it was also explained how patients and staff would profit from the intended changes. This early integration of the employees of a lab responsible for two houses with 920 beds in total, i.e. approximately 4.2 million tests per year, achieved that automation was not perceived as a threat but as the positive option, which automation will be under ideal circumstances - an opportunity to improve the quality of one's own work, even under stress.

Also the University Hospital Mannheim, Germany, actively integrated its employees into the implementation of automation in the Institute for Clinical Chemistry, a re-structuring process which stretched over several years.(5) It was possible to create an open and transparent atmosphere in a workshop involving all affected individuals. Project manager Dr. Dieter Hannak said that the workshop was well accepted, in particular by the employees, because they were given opportunity to express their thoughts and feelings, to become integrated, to discuss and - most important - to get answers to their questions.

Only for large labs?

At first glance it seems that automation will demonstrate its positive effects particularly in high-volume labs with high sample throughput and a large range of diagnostic parameters. However, also smaller-scale labs can benefit from automation.

An impressing example is discussed by Browning in his report outlined above (1): the Baptist Hospital of East Tennessee is a smaller municipal institution with less than 350 beds. Nevertheless, the implementation of automation within the last years did not only optimize workflow but also reduce the incidence of malpractice.

The hospital was even able to fight staff shortage. Its offer combining safe working environment and well-structured processes leaving room for qualified work allowed to attract new committed employees.

Lab automation: opportunity instead of threat!

In summary, the implementation of practically oriented automation solutions in the lab means:

- for treating physicians and nursing staff: clearer, more reliable results together with shorter, more uniform turnaround times;
- for lab employees: not only a better organized and safer working environment, but under optimum conditions also room- and time for qualified work;
- for patients: more safety and improved quality of treatment due to reduced possibilities of error;
- for medicine in general: a new awareness of the importance of laboratory diagnostics as a centralized service center, because labs play a major role in improving efficiency and the quality of diagnosis and treatment at the same time.

The diversity of positive effects of automation clearly demonstrates that on the long run all areas of lab diagnostics will have to follow this trend to profit from its benefits; this applies of course also to autoimmune diagnostics.

(bp)

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(3) Coordinating Council on Clinical Laboratory Workforce. Medical laboratory organizations take action offer solutions to address serious laboratory staffing shortage. February 7, 2003.

(4) ECL, October 2005, Volume 23, No. 5, S. 12

(5) Hannak, D.; Neues Laborkonzept für das Klinikum Mannheim, Management & Krankenhaus 10/2006, S. 32

The story of hare and tortoise

Do you know the story of hare and tortoise? In this tale, the smart and persistent tortoise wins a race against the much faster hare, due to its proper timing and continuity.

The everyday situation in the lab is just the same - a race against increasing sample volumes and rising costs.

Despite this daily pressure, high quality, validity and reliability of the results are needed for the benefit of patients and physicians. This is also true from the economic point of view, because repeated tests or incorrect diagnoses may cause considerable extra costs.

This makes both situations actually comparable. Like in the tale of hare and tortoise, the apparently slower but more intelligent solution will "make the race", when both profitability and high quality are required - also in the laboratory environment.

At first glance, speed and quality may appear contradictory. Both at the same time can be only achieved by test systems, which will optimally adapt to a varying working environment.

The new *AESKULISA*® format - a further step towards easier automation

Within the last years, daily laboratory routine has dramatically changed due to the introduction of fully automated ELISA analyzers. The need for reliable and safe but cost-effective diagnostic tests is still increasing.

Therefore, AESKU.DIAGNOSTICS has designed its *AESKULISA*® product range strictly for automation requirements. The deciding factor is the unique feature of the product range, which has been designed according to the principle "unique but equal": standardized protocols, standard curves and cut-off values provide maximum standardization within the product range.

Therefore, the tests are ready for automation, thus allowing high sample throughput - a remarkable benefit in the face of an increasing demand for profitability, not only in large, but also in smaller laboratories.

Ready-to-use reagents reduce processing and working time for the user. Increased safety is provided by clear color-coding of all reagents, buffers and vials. In addition all calibrators are color-coded depending on concentration to prevent mix-up of various concentrations.

From 2007, the new 30-30-30 test format will be available. It is characterized by standardized incubation conditions, pronouncedly extended temperature tolerance and even less effort. AESKU.DIAGNOSTICS even goes one step further - all *AESKULISA*® assays will be adapted to the requirements of all common lab analyzers and less ideal working environments.

The objective is rather to adapt the *AESKULISA*® product range to the real lab environment than to request changes in day-to-day lab routine!

Three major improvements were implemented to make daily laboratory routine as easy and efficient as possible:

New incubation scheme 30- 30-30 min

The new and homogeneous incubation scheme of 30-30-30 min perfectly meets the requirements of any sort of automated system as it allows a more efficient and time-saving scheduling of various tests.
The result is a far better utilization of the capacity of the instrument - more tests can be processed in a given time.
Like the tortoise in the tale of hare and tortoise, the user wins much time, although the test system seems to be slower at the first glance.

The new test format of the *AESKULISA®* product range sets new standards in automation.
The new standardized incubation schedule of the *AESKULISA®* product range allows the more efficient combination of multiple test runs.

Extended temperature range 20°C to 32°C

The temperature range of the tests has been extended to 32°C which allows more stable and reproducible results on automated systems as the additional heating of the instrument itself can easily be compensated.

Special vials for calibrators and controls

The new vials for calibrators and controls have been developed exclusively for AESKU.DIAGNOSTICS - a special plastic material guarantees minimal absorption of proteins and maximum stability of the control material.

The size and shape of the new vials have been designed especially to better fit directly into most automated systems.
It is no longer necessary to decant calibrators and controls, the vials can be used directly out of the kit.

(cl)

The Story

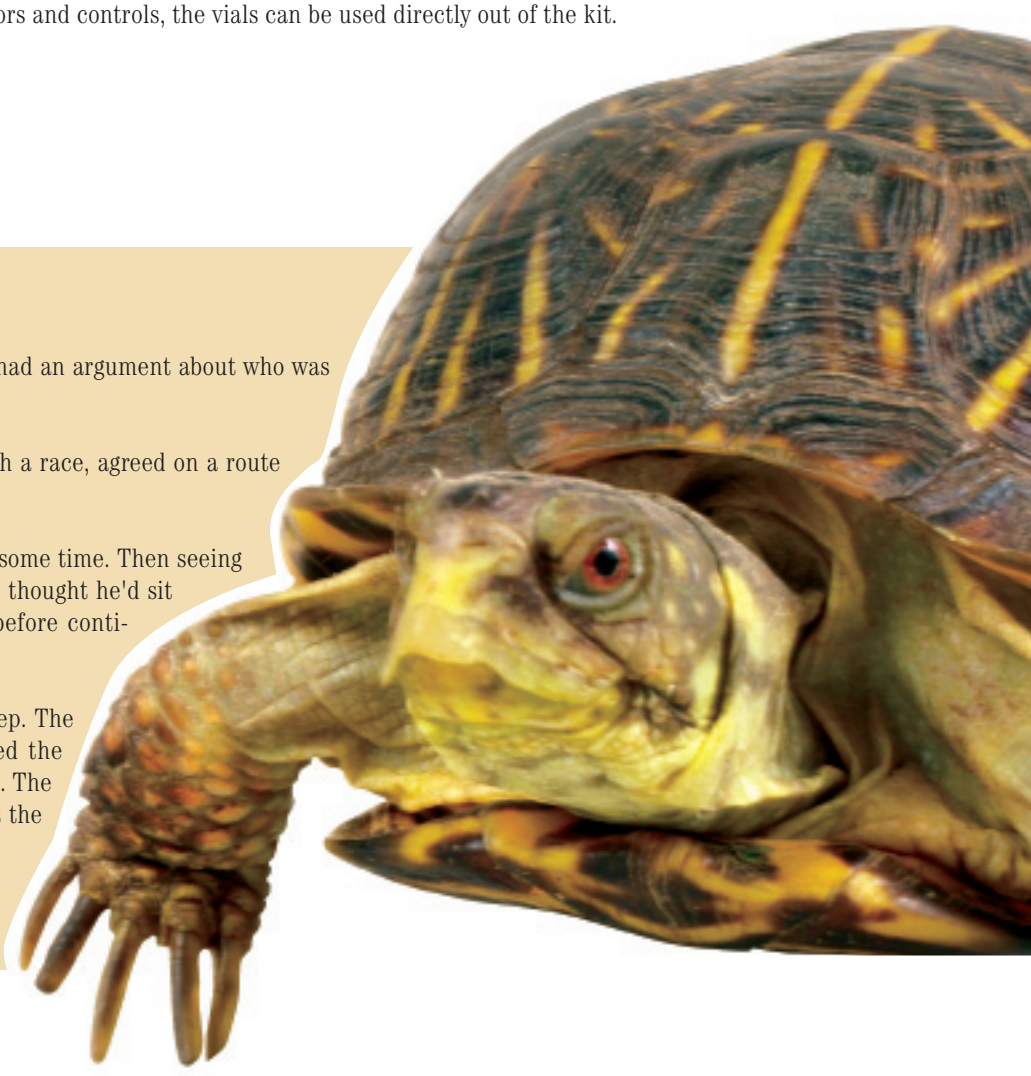
Once upon a time a tortoise and a hare had an argument about who was faster.

They decided to settle the argument with a race, agreed on a route and started off the race.

The hare shot ahead and ran briskly for some time. Then seeing that he was far ahead of the tortoise, he thought he'd sit under a tree for some time and relax before continuing the race.

He sat under the tree and soon fell asleep. The tortoise overtook him and soon finished the race, emerging as the undisputed champ. The hare woke up and realized that he'd lost the race.

And the moral of the story is: Slow but steady wins the race.



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Mikroforum Ring 2
D-55234 Wendelsheim

New assays for the US market

With FDA approval, now new assays from the unique *AESKULISA*® product line with maximum specificity and sensitivity are at hand for in vitro diagnostic use in the US. Same protocol, ready to use reagents, consistent incubation times – every new assay shares all the benefits that established the *AESKULISA*® product line as an ideal partner for laboratory automation systems.

AESKULISA® Gliadin-A and Gliadin-G for the combined or separate quantitative and qualitative determination of antibodies to alpha-gliadin in human serum complete AESKU.DIAGNOSTIC´S portfolio for the diagnosis of celiac disease.

Recently AESKU.DIAGNOSTICS also received FDA approval for two assays for a secure diagnosis of Cohn's Disease and differentiation from ulcerative colitis: *AESKULISA*® ASCA-A and ASCA-G for the quantitative and qualitative determination of IgA and IgG antibodies to cell wall proteoglycans of Saccharomyces cerevisiae in human serum.

Numerous products from the wide *AESKULISA*® product portfolio from the fields of rheumatology, thyroid, vasculitis, thrombosis, hepatology and gastroenterology are available in the U.S. market for in-vitro diagnostic use: ANA HEp-2, ANA-8Pro, ENA-6Pro, SS-A, SS-B, Scl-70, CenpB, Jo-1, U1-70, Sm, snRNP-C, dsDNA-G, ENA 6S, Cardiolipin A, Cardiolipin GM, Cardiolipin Check, tTg-A, tTg-G, Glia-G, Glia-A, ASCA-A and ASCA-G.

The whole *AESKULISA*® product line is of course available for research purposes.

AESKU's 3rd Autoimmunity Workshop Presents Two Premières

On 18 September 2006, AESKU held its meanwhile third Autoimmunity Workshop in the Wendelsheim facility.
In its workshop for interested laboratory practitioners, AESKU.DIAGNOSTICS intentionally combines plain "laboratory issues" and the discussion of the consequences of the addressed autoimmune diseases to hospital and affected patients.

Participants from all parts of Germany and from Austria were welcomed by Petra Löffler, sales manager Germany. Then Dr. Torsten Matthias, AESKU.DIAGNOSTICS' general manager shortly presented the AESKU.KIPP INSTITUTE initiated by AESKU.DIAGNOSTICS itself (see p. 17 of this issue), followed by a discussion of current issues: Which aspects do autoimmune diseases like multiple sclerosis demonstrate not only in the laboratory, but also in the hospital and the patients' day-to-day life? Which quality criteria do apply to immunofluorescence diagnostics of HEp2 cells? What is needed to implement screening and risk stratification based on serological parameters into the diagnosis of chronic inflammatory diseases?
The program was completed by the presentation of new options in the automation of autoimmune diagnostics and more.
Intended as a complementation to AESKU.DIAGNOSTICS' newly launched infectious diseases assays, also a lecture was held on new approaches in diagnostics of lyme disease.

Multiple sclerosis - facts of a disease in daily life, hospital and laboratory

Dr. Stefan Langel of the Rheinhessen Fachklinik Alzey, Germany, gave a clear introduction into neuroanatomy, epidemiology and pathogenesis of multiple sclerosis (MS); he also explained, how MS is diagnosed, and which course the disease may take.

Current diagnosis still relies most on clinical symptoms (type and number of acute attacks), the detection of lesions by magnetic resonance tomography and liquor analysis (increase in protein and IgG levels). Reliable serological markers for diagnosis and follow-up are still missing.

Also Langel's presentation of current strategies for management and treatment of MS attracted wide interest. Current therapeutic strategies range from the use of corticosteroids over immune modulation with novel drugs like interferon or the active compound natalizumab to surgical interventions to stimulate the cerebral function.

Dr. Stefan Langel of the Rheinhessen Fachklinik Alzey, Germany

Diagnosis of Lyme disease - are new aspects available?

It is not trivial to perform a reliable diagnosis of lyme borreliosis. Prof. Brigitte König, Otto-von-Guericke University Magdeburg, Germany, illustrated this fact convincingly in the beginning of her lecture by presenting a classic case and a more complicated case with unclear symptoms and uncharacteristic serological findings.
Whenever laboratory diagnosis is indicated due to the presence of clinical cardinal symptoms or neurological symptoms, which might be due to borreliosis, a number of different tests are available. In addition to the detection of Borrelia antibodies by initial screening with an ELISA test (IgM, IgG) and confirmation by immunoblotting (pathogen-specific IgM-/IgG-antibodies), the pathogen can be alternatively detected by microscopy or PCR.

Prof. Brigitte König, Otto-von-Guericke Universität Magdeburg, Germany

Many different test systems are available for the serology of borreliosis. Therefore, König recommends selecting a suitable ELISA test in particular by the type of antigens used and the test parameters. Are the right antigens used? Are the antigens of recombinant origin? Which antigen coating density is used in the wells? How is the ELISA test designed? Competitive or non-competitive, µ-capture? Which cut-off value is used? It decides the quality of the result.

New automation options in autoimmune diagnostics

Paul Ballieux of the Diagnostic Division of the Spanish enterprise Grifols reported about current automation options in autoimmune diagnostics, referring to the benefits of the Immunoassay analyzer TRITURUS®: high accuracy and precision, unlimited flexibility and perfectly user-friendly software.

Then the participants of the 3rd AESKU Autoimmunity Workshop experienced a novelty. Dr. Christine von Landenberg, responsible for AESKU.DIAGNOSTICS' sales and marketing, presented for the first time the new test format 30/30/30 of the *AESKULISA*® product range, which will be officially launched at the MEDICA and will be available from 2007. The new test format ensures uniform incubation conditions, a significantly larger temperature tolerance and even more reduction in operating effort (see p. 12 of this issue).

Quality criteria in the immunofluorescence diagnostics of HEp2 cells

The troubleshooting of immunofluorescence diagnostics of HEp2 cells was the topic of Prof. Dr. Philipp von Landenberg's (Klinikum der Johannes Gutenberg Universität Mainz, Germany) presentation. His lecture did not only take a closer look at the technically correct performance of immunofluorescence tests and the required quality control measures, but he also invited his audience to a riddle tour through a number of slides to find out "Which fault causes which picture?"

Screening and risk stratification in chronic inflammatory intestinal diseases

What do serological markers achieve in the diagnosis of chronic inflammatory bowel diseases? This was the central question discussed in the lecture of Prof. Dr. Dr. Jürgen Stein, Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Germany.
The number of patients with Crohn's disease or ulcerative colitis rapidly increases in whole Europe. This is due to a large variety

Lecturers in an interdisciplinary discussion: Prof. Dr. Dr. Jürgen Stein, Klinikum der Johann Wolfgang Goethe-Universität Frankfurt, Germany, and Paul Ballieux, Grifols, Spain (left to right)

of reasons. A high hygiene status is considered a risk factor, infectious factors are discussed. Like in all autoimmune diseases, also genetic factors appear to play a role.
Although many differences exist in the clinical pictures of Crohn's disease and ulcerative colitis, differential diagnosis is demanding. Often, diagnosis is not more specific than "indeterminate colitis".

Stein said that a clear differential diagnosis is pronouncedly supported by established serological markers like ASCA (anti-Saccharomyces cerevisiae antibodies) or p-ANCA (perinuclear antineutrophil cytoplasmatic antibodies). He further said that in particular the analysis of subtypes allows to draw conclusions on localization, severity and complications. He stated that AESKU.DIAGNOSTICS' ASCA assays demonstrate particularly high specificity and sensitivity.

Stein concluded his lecture with a forward look of the future of the laboratory diagnostics of chronic inflammatory bowel diseases: according to current research, there might be new serological markers which allow the prediction of severe developments or developments with many complications. They would be of inestimable value for the treating physician in the selection of an adequate therapy.

To be continued!

The date of the 4th AESKU Autoimmune Workshop is already fixed to September 2007.

The response to the 2006 workshop was excellent; however, the number of participants was limited. Therefore, all lectures of the 3rd AESKU Autoimmune Workshop are published on the AESKU homepage www.aesku.com.

Please contact us if you want to participate in the next AESKU Autoimmune Workshop!
Phone.: +49 (0) 6734 9627-0; e-mail: loeffler@aesku.com

AESKU.KIPP INSTITUTE:

First Research Projects on the Starting Line

The inauguration of the AESKU.KIPP INSTITUTE on 5 May 2006 in Wendelsheim, Rhineland-Palatinate, was the starting point of a novel platform to support continuous interdisciplinary research and training in autoimmune diseases on an international level.

The AESKU.KIPP INSTITUTE is intended to initiate and coordinate international research cooperation and developmental projects. At the same time it offers the spatial and personnel requirements for the implementation of joint interdisciplinary projects at the Wendelsheim facility. The AESKU.KIPP INSTITUTE has consciously positioned itself as an international interface between basic and clinical research, allowing recent research results to be integrated into clinical applications.
Consequently, representatives of science, clinical practice and industry participated in the inauguration, in particular Prof. Yehuda Shoenfeld, Tel Aviv, Israel, Prof. Dr. Klaus Helmke, Klinikum Munich-Bogenhausen, Prof. Dr. Karl Lackner, University Mainz and Prof. Dr. Gerd Schnorrenberg, Boehringer Ingelheim, all from Germany; they presented their personal views on the necessity and relevance of an institution like this.
Vice minister Walter Strutz of the Ministry of Economic Affairs of Rhineland-Palatinate, Dr. Wilfried Bechtolsheimer representing the investors family Kipp-Bechtolsheimer and Dr. Torsten Matthias, gave the approximately 150 guests of the inauguration an overview of their intentions to open new paths in current and future autoimmunity research by their financial support of the AESKU.KIPP INSTITUTE.
Presentations of Dr. Auerheimer, undersecretary of the Ministry of Health of the Rhineland-Palatinate and Dr. Ulrich Link, ISB, completed the program.
The AESKU.KIPP INSTITUTE also serves as a forum for the international exchange of information. With its events and publications, the institute actively commits itself to supporting the public awareness of prognosis, diagnosis and therapy of autoimmune diseases, allowing patients and treating physicians to take advantage of most current research to make year-long odysseys of affected individuals hopefully be a thing of the past soon.

Exciting prospects for the future of autoimmunity research: the 1st International AESKU.KIPP INSTITUTE SYMPOSIUM

International AESKU.KIPP INSTITUTE symposium “Autoimmunity 2006”

As part of its continuing commitment to supporting interdisciplinary exchange of knowledge between researchers and clinical practitioners, the institute intentionally organized the “1. International AESKU.KIPP INSTITUTE Symposium Autoimmunity 2006“, in addition to the official opening ceremony on 5 May 2006. 11 renowned scientists, most of them members of the scientific advisory board of the institute, gave lectures on the current state of research in Wendelsheim on Saturday, 6 May 2006,

Lecturers, members of the AIRA advisory board and management: Prof. Dr. Steiner, Prof. Pier Luigi Meroni, Prof. Dr, Klaus Helmke, Brigitte Pfeiff, Yehuda Shoenfeld MD FRCP, Dr. Torsten Matthias, M. Eric Gershwin, Loic Guillevin PU-PH, Prof. Dr.Markus Maeurer, Allan Wiik, MC DSc (left to right)

The calm before the storm

A reason for celebration: Together with 150 invited guests, the institution AIRA e.V. celebrates the official inauguration of the AESKU.KIPP INSTITUTE.

Interdisciplinary cooperation will be the future, not only in research – M. Eric Gershwin MD, and Dr Torsten Matthias on their way to new challenges.

and they discussed with approximately 80 participants from over the world its impact on diagnosis, prognosis and therapy of autoimmune diseases.
The contents of the lectures covered exciting views of the prospects of the future of autoimmunity research (Autoimmunity towards the second millennium; Prof. Yehuda Shoenfeld, Israel) and also existing results on individual pathologies.
Current information on general immunological mechanisms provided valuable background information and the intended look beyond the nose.
Just in time for the "5th International Congress on Autoimmunity" in the end of November 2006 in Sorrento, Italy, the scientific journal "Clinical Reviews in Allergy & Immunology (CRIA)" will publish the lectures of 1st International AESKU.KIPP INSTITUTE Symposium in a special issue. Many thanks to the editors!

Patient forum "Autoimmune Diseases"

On Sunday, 7 May 2006, the AESKU.KIPP INSTITUTE addressed patients and their families and the interested public with the patient forum on autoimmune diseases providing current information in German on the diagnosis and therapy of rheumatoid arthritis, multiple sclerosis, Crohn's Disease, ulcerative colitis and autoimmune liver disease.
Up to 100 affected individuals took the opportunity to get current information on diagnostic and therapeutic options and to contact the speakers for discussing more details.

AESKU.KIPP INSTITUTE supports first projects

The opening ceremony of the AESKU.KIPP INSTITUTE did not only emphasize the uniqueness of this institution, but also impressively demonstrated the broad support by science, policy, sponsors and the public, ensuring forward-looking development of the institute.
Thanks to this support, first of all due to the dedicated commitment of the 10-member scientific board, it was possible to initiate a number of cooperations since its opening in May 2006. e.g. with the ZAFES in Frankfurt, Germany, and the Karolinska Institute in

INTERNAL INFORMATION: AFTER THE GAME IS BEFORE THE GAME

On 10 November 2006, Prof. Dr. Klaus Helmke, a committed clinical immunologist and member of AESKU.KIPP INSTITUTE's scientific board celebrated his retirement together with many friends and colleagues during a symposium. In the last 19 years, he worked as the senior physician of the 4th Medical Department in the Krankenhaus München Bogenhausen.
On this occasion, we thank Prof. Helmke for his motivating words spoken when the institute was founded. We would like to return the favor now!
Dear Professor Helmke, many thanks for your - often spontaneous - support, not only in your function as a member of the board, but also as a true friend. It was a pleasure to initiate the AESKU.KIPP INSTITUTE project together with you.
Soccer players say: "After the game is before the game". This means for you that your new life will surely be full of new challenges, interesting projects and human contacts. We wish you all the best, success, happiness, health and of course luck.
Although we know that pensioners never have time, we wish that we can rely on your time, competence, and commitment also in the future!

Your AESKU.KIPP INSTITUTE team

Stockholm, Sweden. The Wendelsheim facility already started initial interdisciplinary research projects, which are also publicly granted.
In addition, AESKU.KIPP INSTITUTE's invitation for grant applications financed by own resources generates a new basis for the specific support of - in particular interdisciplinary - research projects in autoimmunity.
The first exciting results are expected to be already presented on the next scientific symposium intended for the end of April 2007.

Please find application documents for grants provided by the AESKU.KIPP INSTITUTE, more information on the 2nd International AESKU.KIPP INSTITUTE Symposium and objectives and background of the institute starting November 2006 at: www.aesku-kipp.com

And the winner is …

AESKU.AWARD 2006 will be awarded to three renowned researchers

On 29 November 2006, AESKU.DIAGNOSTICS' "AESKU.AWARD for life contribution to autoimmunity" will be already awarded for the second time.

Prof. M. Eric Gershwin, MD, will present the awards to the prizewinners together with Dr. Torsten Matthias during the opening ceremony of the "5th International Congress on Autoimmunity", which will be held in Sorrento, Italy, from 29 November to 3 December 2006.

The AESKU.AWARD includes prize money of 30,000 Euro. This makes it one of the highest endowed scientific awards in medicine, and it is also represents a unique honor in the field of autoimmune research.

AESKU.DIAGNOSTICS considers the AESKU.AWARD an expression of its company philosophy. Since its foundation almost seven years ago, AESKU.DIAGNOSTICS specifically supports interdisciplinary research in autoimmune diseases.

One of the goals of the AESKU.AWARD is to stress the importance of research in autoimmune diseases and the progress in this field. AESKU.DIAGNOSTICS also intends with the donation of this award to foster interdisciplinary research and to establish autoimmunity as an independent field of research, as before with the establishment of the first chair of autoimmunity worldwide in 2002 and the continuing commitment for the establishment of the AESKU.KIPP INSTITUTE.

This uncommon synthesis of networked scientific competence and clear orientation to practical issues makes AESKU.DIAGNOSTICS an outstanding enterprise, and it offers the ideal environment for the quick implementation of current research into new diagnostic and therapeutic options.

And the winner is ...

Also in 2006, the AESKU.AWARD will be awarded to three renowned researchers, who distinguished themselves by yearlong research in autoimmunity:

- **Prof. Dr. Dr. Joachim R. Kalden, Erlangen, Germany**
- **Prof. Graham R. V. Hughes, MD FRCP, London, UK**
- **Prof. Irun R. Cohen, MD, Rehovot, Israel**

Prof. Dr. Dr. Kalden will be presented by Prof. Dr. G. Burmester from Berlin; he will talk in his lecture about "Apoptosis in Autoimmune Disease".

Then the congress president Prof. Yehuda Shoenfeld, MD FRCP, will outline the scientific career of Prof. Graham R. V. Hughes, London, UK, who will present then in his lecture "Lupus – A Story of Failure and Success" the proverbial ups and down of scientific day-to-day work.

The third prizewinner, Prof. Irun R. Cohen, MD, Rehovot, Israel, will be introduced by Prof. Yaakov Naparstek, MD, Jerusalem, Israel; his lecture "Tending Adam's Garden" will refer to his book carrying the same name.

Current information on the award ceremony in Sorrento, the prizewinners and their lectures will be available on AESKU.DIAGNOSTICS' website www.aesku.com from 30 November 2006.

Challenge Lyme Disease

Lyme disease is the most common infectious disease of the Northern hemisphere transmitted by ticks. Its pathogen is the spirochete Borrelia burgdorferi sensu lato, occurring in Europe in three human pathogenic genospecies, i.e. B. burgdorferi sensu stricto, B. afzelii and B. garinii. Up to 30% of the ticks are infected in Germany; the ratio is even higher in some areas. It is estimated that sixty thousand new infections every year occur in Germany alone.

Stages of the disease

Lyme disease is characterized by a very complex combination of symptoms, and is subdivided into three clinical stages based on characteristic clinical pictures. Stage I, occurring within days or few weeks, is characterized by erythema chronicum migrans (EM), a circular skin lesion around the bite, which is the most common manifestation of Lyme disease, occurring in around 70% of the infected individuals.

Weeks to months following the infection, neurological symptoms like neuritis, facial paresis and Bannwarth's syndrome may occur in stage II. Cardiac symptoms (Lyme carditis) are less common. Late manifestations, which develop years after the infection, include acrodermatitis chronica atrophicans (ACA) and Lyme arthritis. However, the course of the Lyme disease may vary individually, thus resulting in differing intensities of the individual stages, which sometimes may be even completely missing. In addition, a number of unspecific, influenza-like symptoms occur, which are similar to other clinical pictures. As non-treated patients may suffer from severe consequential damages, the early diagnosis and immediate therapy of Lyme disease contribute to the complete recovery of the patients. This means that high demands are made on its diagnosis.

Diagnosis

The interpretation of serological findings requires extensive anamnesis and knowledge of the clinical picture of the patients. Following international guidelines, Borrelia diagnosis is performed in two steps. First, patient sera undergo an ELISA test for Borrelia-specific antibodies; then an additional test, an immunoblot, is performed, allowing the detection of individual antibody specificities.
The presence of antibodies differs from patient to patient. IgM antibodies are detectable in stage I; however, some patients will stay IgM-negative. In the further course of the disease, mostly IgG antibodies are present; they may occur alone or together with IgM antibodies. While only 20%-50%of the antibodies are detected in stage I, their detectability increases to 70%-90%in stage II and to almost 100% in stage III.

Therefore, the thorough assessment of the results requires follow-up examinations, in particular in early stages of the infection. If neurological symptoms develop, an additional diagnosis in CSF should be considered.

Borrelia antibodies

Borrelia possesses a very complex antigen structure. The majority of the antigens are membrane-bound proteins, the expression of which depends on the stage of the disease. The longer the infection continues, the larger is the range of antibody specificities. Outer surface proteins (Osp) cause an excellent antibody response; in particular, OspC plays a role in the early phase of Lyme disease. The reactivity against OspC is rather polyspecific, a reason for the use of OspCs of different Borrelia strains. Antibodies are also directed against flagellin, the major protein of the flagellum. However, cross reactions may occur, because also other spirochetes have flagellin epitopes. VlsE is one of the most sensitive antigens for the detection of IgG antibodies. In addition, it is also essential for the detection of antibodies in patients, who have only VlsE antibodies.

AESKULISA® Borrelia

The different coatings of the Borrelia-G and Borrelia-M tests allow the specific detection of early and late Borrelia antibodies. Both tests involve highly purified antigens from the Borrelia strains relevant for Lyme disease. The IgM-ELISA test is coated with purified OspC and Borrelia-specific truncated p41 (i.e. p41i). Therefore, it allows the specific detection of early Lyme disease antibodies.

As the antigen spectrum is broader, when the disease is more progressed, the IgG-ELISA is coated with an antigen mixture of highly purified native antigens. The mixture is also enriched with VlsE, which is highly specific for IgG antibodies.

The special selection of antigens allows for maximum sensitivity; this applies in particular to the IgG test.

Therefore, *AESKULISA*® Borrelia is a particularly reliable assay ideal for diagnosis and follow-up control of Lyme disease.

Both assays, i.e. Borrelia IgG ELISA and Borrelia IgM ELISA are quantitative tests; the results are recorded in U/mL. This allows monitoring and in particular the evaluation of antibody kinetics following therapy. In addition, each test is equipped with a cut-off control.

AESKULISA® Borrelia IgG ELISA

It was demonstrated in an extensive evaluation study* that the IgG ELISA had a higher sensitivity compared to other assays (Tab.1). Seroconversions were earlier recognized than by competitors' tests. Also persisting infections, patients with neuroborreliosis and arthritis samples were clearly detected. In particular isolated VlsE positive samples were reliably recognized.
Therefore, the selection of the antigen panel and the coating type allow optimum sensitivity in detection.

AESKULISA® Borrelia IgM ELISA

The evaluation study demonstrated for the IgM ELISA a more specific recognition of relevant antibodies of patients with acute EM (Tab. 2). In addition, antibody titers could be monitored well following therapy.
Persisting antibodies were specifically detected.

Both the *AESKULISA*® IgG ELISA and also the *AESKULISA*® IgM ELISA are currently being evaluated for their suitability in cerebrospinal fluid diagnostics. Extensive studies on dilution properties demonstrate the suitability of both tests for cerebrospinal fluid.

The evaluation study was performed by Prof. Dr. Hagedorn, Medizinaluntersuchungsstelle Herford, Germany.

(cl)

IgG				
Assay	Mikrogen			
AESKU	negative	positive	equivocal	total
negative	9	6	-	15
positive	28	53	3	84
equivocal	-	-	-	-
total	37	59	3	99

Table 1: Comparison of the Borrelia IgG ELISAs of Mikrogen and AESKU.DIAGNOSTICS (EM panel)

IgM				
Assay	Mikrogen			
AESKU	negative	positive	equivocal	total
negative	15	16	-	31
positive	4	63	1	68
equivocal	-	-	-	-
total	19	79	1	99

Table 2: Comparison of the Borrelia IgM ELISAs of Mikrogen and AESKU.DIAGNOSTICS (EM panel)

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AESKU.DIAGNOSTICS on the road!

Capabilities and motivation of the employees are essential prerequisites to cope with continuously increasing requirements in the laboratory environment, i.e. new tests, new techniques and new working processes. Practically oriented training on a continuing basis is necessary.

However, the steadily increasing workload does almost leave no spare time for one or even several days of training to keep the staff updated and to allow essential exchange of experiences. Therefore, in cooperation with renowned experts, AESKU.DIAGNOSTICS will establish the AESKU.RoadShow starting in 2007 to offer all lab practitioners a convenient opportunity to update their knowledge in the most current aspects of autoimmune diagnostics and the serological diagnosis of infectious diseases.

The AESKU.RoadShow offers: local practically oriented issues, training in current issues requiring minimum time to be spent, experienced lecturers and concentrated exchange of experiences with others.

Information on program and time schedule and locations of the AESKU.RoadShow will be available from December 2006 on at our website:
www.aesku.com

MEDICA 2006 - DISCOVER NEW OPPORTUNITIES

Automation

- Format 30-30-30, the new test format of the *AESKULISA*® product range, will be launched in the beginning of 2007. It ensures uniform incubation conditions, a significantly extended temperature range and even more reduced effort.
- AESKU.Seven-up, the solution for automation in autoimmune diagnostics and the diagnosis of infectious diseases. It is ideally suited for small, medium and large labs, and it comprises a broad range of innovative, practically oriented ELISA tests.

Autoimmune diagnostics

- *AESKULISA*® tTg assays set a new standard in sensitivity in the diagnosis of celiac disease due to the detection of neo-epitopes of tTg, induced by the addition of gliadin-specific peptides. At the same time, the tests are highly specific, because no cross-reactions to gliadin occur.
- *AESKULISA*® RA-CP Detect, a novel specific marker with synthetic citrullinated peptides, represents a new approach to the diagnosis of rheumatoid arthritis.

Serological diagnosis of infectious diseases

- *AESKULISA*® Borrelia, a pronouncedly reliable test system for the early diagnosis and reliable follow-up of lyme disease.
- AESKU.Seven-up Serological Diagnosis of Infectious Diseases offers the complete TORCH panel (toxoplasmosis, rubella, cytomegaly virus, herpes simplex) for the diagnostics of pregnant women; toxoplasmosis, rubella and CMV tests are also available as avidity tests. The product range is completed by Eppstein Barr virus, varicella, measles, mumps, syphilis and Helicobacter pylori tests.

AESKU.DIAGNOSTICS Hall 3, Stand H27

A live interface

AESKU.DIAGNOSTICS meanwhile offers its products and services in more than 75 countries worldwide. The company commits itself to developing products, which can be adapted to the individual needs of any user in the world.
It is also AESKU.DIAGNOSTICS' philosophy to adapt information transfer and education to the requirements of various health systems and cultures to allow the user to take advantage of the unusual commitment of our company to research and development.
In Germany, AESKU.DIAGNOSTICS' own highly qualified staff already performs on-site visits to users in hospital laboratories and laboratory physicians' practices. In the other parts of the world, the company is represented by well-selected, specialized distributors.
Already since summer 2003, AESKU.DIAGNOSTICS is represented in the U.S. by an own marketing organization, AESKU.INC., based in Miami, Florida. Since that time, the FDA (Food and Drug Administration) already approved more than 20 tests of the *AESKULISA*® product range, the worldwide largest portfolio of innovative but also economic ELISA assays in autoimmune diagnostics.
Currently, AESKU.DIAGNOSTICS performs the next step towards customer orientation in the U.S: since August 2006, the expert in autoimmune diseases and an experienced connoisseur of the U.S. market, Jim Radford, works for AESKU.DIAGNOSTICS, a "live interface" between the Germany-based enterprise and the American market.
The objective is to provide users in the U.S. market with the most current knowledge in developments from AESKU research, science transfer and practically oriented training and vice versa to inform AESKU.DIAGNOSTICS about current developments in the U.S. health system and individual requirements in everyday laboratory work.

Most specific and sensitive:

Novel marker for Rheumatoid Arthritis

A number of studies demonstrated within the last years that the citrullination of arginine residues is a general motif in RA-specific antibody response: citrullin is a common epitope of RA-specific antibodies in a number of proteins like keratin, filaggrin or vimentin.
Anti-citrullinated protein antibodies (ACPA) have been established as the most specific serological marker antibodies for RA. They are particularly useful in early arthritis.
Commercially available ELISA tests have adopted this property for serological detection based on coating with citrullinated peptides.
It was demonstrated that the reaction with the antibodies does not require the complete citrullinated proteins but only the citrullin residues. As antibodies of RA patients always react with all sorts of citrullinated peptides and proteins occurring in the body, it was an obvious idea to develop ELISA tests based on synthetic citrullinated peptides.
AESKU.DIAGNOSTICS succeeded in developing a novel ELISA test, *AESKULISA*® RA/CP Detect, using synthetic citrullinated peptides of human immunoglobulin G for coating.
The assay is a most sensitive and specific serological approach to the diagnosis of RA, combining the high specificity of the citrullin antigen with the sensitivity of a rheumatoid factor ELISA test.
Showing comparable sensitivities and specificities *AESKULISA*® RA/CP Detect is an adequate and cost-effective alternative to the known CCP ELISA tests.

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