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Rheumatic diseases

New opportunities in diagnosis

CD74 antibodies a biomarker of axial spondyloarthritis

MMP-3 – A new prognostic and activity marker for the therapy management of rheumatoid arthritis

The gut-joint axis and autoimmunity

1st AESKU Latin America workshop

10th anniversary of AESKU.KIPP Institute

THE WORLD INCIDENCE AND PREVALENCE OF AUTOIMMUNE DISEASES IS INCREASING

Dr. Torsten Matthias, Head of AESKU.KIPP Institute



It gives me real pleasure to commission the renewal of AESKU.SCIENCE. I consider this a crucial part of our effort to reach out to both patients and autoimmunity professionals. By helping them stay up to date with news regarding pathogenesis, laboratory diagnosis and new therapeutic strategies, we hope to bolster their will and further their ability to fight autoimmune diseases, thereby staying faithful to our own commitment.

The AESKU.KIPP Institute's aim is to understand autoimmune genesis in general and improve the laboratory diagnosis of autoimmune diseases in particular. As part of this endeavour we have recently calculated the long-term surge in autoimmune disease frequencies worldwide, analysed the differential increases of autoimmune diseases per country/disease and identified geo-epidemiological trends:

Generally, autoimmune disease frequencies increased globally significantly in the North and West compared to the South and

East. Taking various autoimmune diseases into account, celiac disease increased the most and the highest incidence was allocated to myasthenia gravis, when comparing old surveys to new. Significant annual percentage increases could be shown for neurological (3.7%), gastrointestinal (6.2%), endocrinological (6.3%) and rheumatic autoimmune diseases (7.1%).

We decided to dedicate this edition to rheumatic autoimmune diseases as the annual percentage increase of autoimmune rheumatic diseases is highest.

It is our strong hope that the ongoing surge in autoimmune diseases will boost the drive of scientific communities to raise awareness and heighten efforts to unravel the foundations of this evolving phenomenon.

If we can add positively in any way with the reboot of AESKU.SCIENCE, then every ounce of our work will have been put to good use.

Happy reading!

Editorial

| | |
|---|---|
| The world incidence and prevalence of autoimmune diseases is increasing | 2 |
|---|---|

Dr. Torsten Matthias, AESKU

Focus:

| | |
|---|---|
| CD74 antibodies: a biomarker of axial spondyloarthritis | 3 |
|---|---|

Prof. Torsten Witte, Medizinische Hochschule, Hannover

| | |
|---|---|
| The gut-joint axis and autoimmunity | 5 |
|---|---|

Prof. Aaron Lerner, Technion-Israel Institute of Technology, Haifa, Israel

| | |
|---|----|
| MMP-3 – A new prognostic and activity marker for the therapy management of rheumatoid arthritis | 10 |
|---|----|

Dr. Sandra Reuter, AESKU

News:

| | |
|-----------------------------------|----|
| And the AESKU.AWARD goes to... .. | 13 |
|-----------------------------------|----|

| | |
|--|----|
| 1 st AESKU Latin America workshop | 15 |
|--|----|

| | |
|---|----|
| Questionnaire for laboratories currently using the automated Helios equipment | 16 |
|---|----|

| | |
|--------------------------------------|----|
| New strategies for US business | 17 |
|--------------------------------------|----|

| | |
|---------------------------------|----|
| Automation in the IFA lab | 17 |
|---------------------------------|----|

Profile:

| | |
|---|----|
| 10 th anniversary AESKU.KIPP Institute | 19 |
|---|----|

CD74 ANTIBODIES: A BIOMARKER OF AXIAL SPONDYLOARTHRITIS

Prof. Torsten Witte

Twenty to thirty percent of Europeans suffer from chronic and even more from acute back pain. In 5% of patients chronic back pain is caused by the inflammatory rheumatological disorder axial spondyloarthritis (axSpA), which has a prevalence of 0.5-1.9%. axSpA may lead to new bone formation, ankylosis and thus loss of mobility in the spine (Fig. 1).



Figure 1: Ankylosis of the spine

In view of the extremely high prevalence of chronic back pain due to non-inflammatory disorders, the diagnosis of SpA is still made many years after the onset of the symptoms. Therefore, efficient therapies with non-steroidal anti-inflammatory drugs (NSAIDs) or with TNF inhibitors are initiated too late.

Referral strategies

In order to diagnose axSpA earlier, rheumatologists have developed strategies that general practitioners can use to identify patients at risk. In particular, inflammatory low back pain has been defined as the leading symptom of SpA. Inflammatory back pain is characterised by the following features:

1. Age at onset < 40 years
2. Insidious onset
3. Improvement with exercise
4. No improvement with rest
5. Pain at night (with improvement on getting up)

Inflammatory back pain is however not very specific for SpA, and also identifies only 70% of axSpA patients. Clinical symptoms associated with SpA such as arthritis, dactylitis, uveitis, psoriasis or inflammatory bowel may also help to identify patients with a rheumatological disorder, but are not very sensitive.



Figure 2: Radiological abnormalities in the sacroiliac joints: Sclerosis on both sides of the joint, erosions and widening of the joint space

Making the diagnosis of axSpA at present requires the identification of either HLA-B27 or sacroiliitis using imaging procedures.

HLA-B27 is quite sensitive (approximately 80%) but unspecific (6-15% of European blood donors carry the HLA-B27 antigen).

Conventional X-ray may reveal ankylosis of the sacroiliac joints (Fig. 2).

However, the radiographs are often normal when the first symptoms start and it usually takes years or even decades until definite sacroiliitis is visible.

MRI of the sacroiliac joints is meanwhile being considered as a gold standard in the diagnostics of SpA. MRI findings were described to be the most sensitive and specific for sacroiliitis. MRI was shown to visualise bone marrow oedema and osteitis of the sacroiliac joints and it detected radiographic sacroiliitis with a sensitivity of 80% and a specificity of more than 90%. Nonetheless, interpreting MRI of the sacroiliac joints requires a great deal of experience and may be unspecific, when non-specialised radiologists are involved. In addition, MRI is expensive.

Both HLA-B27 and MRI were incorporated into the „Assessment of SpondyloArthritis international Society“ (ASAS) criteria (Sieper et al, Ann Rheum Dis 2009; 68 (Suppl 2) 1-44.). According to the ASAS criteria, the classification of axSpA in patients with chronic back pain is based on the presence of sacroiliitis on imaging (including MRI) plus at least one SpA feature or on the presence of HLA-B27 plus at least two of the SpA features:

- | | |
|--------------------------|--------------------------------------|
| - Inflammatory back pain | - CED |
| - Arthritis | (Crohn's disease/ulcerating colitis) |
| - Enthesitis (heel) | - Good response to NSAIDs |
| - Uveitis | - Family history of SpA |
| - Dactylitis | - HLA-B27 |
| - Psoriasis | - Elevated CRP |

In summary, axSpA is very common, but is still difficult to diagnose. MRI of the sacroiliac joints may be the best procedure, but this requires experienced interpreters and is too expensive to be used for every patient complaining of chronic back pain. Therefore, laboratory markers of axSpA would be extremely helpful to facilitate the early identification of the patients.

Discovery of antibodies against CD74

In order to identify autoantibodies that may be associated with SpA, we initially screened sera of 5 SpA patients using protein arrays loaded with 27,000 human proteins. We identified antibodies against CD74 in the sera of 4/5 SpA patients but not in 5 blood donors.

CD74 is a molecule which is expressed on the surface of all antigen-presenting cells. CD74 is the MHC class II associated invariant chain, but also serves as a receptor for the chemokine macrophage migration inhibitory factor (MIF), a proinflammatory molecule which has been found to be increased in the sera of SpA patients.

Initially an ELISA was established with peptides derived from the CD74 sequence as an antigen. Later on AESKU.DIAGNOSTICS developed an ELISA that uses the complete CD74 protein as an antigen.

Prevalence of antibodies against CD74 in sera of SpA patients with established disease

In order to evaluate the new ELISA, we obtained the sera of 117 patients with axSpA with disease duration of at least 10 years and with definite disease. Patients suffering from pain syndromes or other diseases mimicking the symptoms of axSpA were excluded. Sera of 38 blood donors served as controls.

The sensitivity of IgG and IgA anti-CD74 antibodies together for diagnosing axSpA was 77%, specificity 90%. Remarkably, IgA autoantibodies against CD74 alone had a sensitivity of 67% and a specificity of 97% (Fig. 3). Furthermore, IgA anti-CD74 antibodies significantly correlated with more advanced radiological sacroiliitis and reduced spinal mobility.

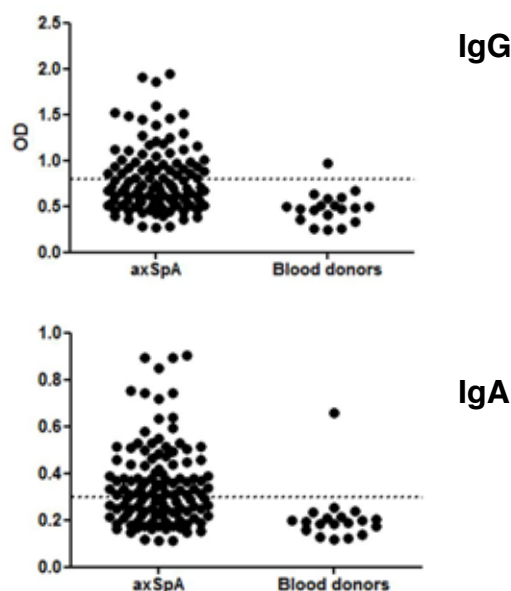


Figure 3: Association of anti-CD74 with established axSpA

Comparison of HLA-B27 and antibodies against CD74 in SpA of recent onset

Making the diagnosis of SpA is particularly difficult in the early phase, when conventional X-ray is not yet pathologic. We therefore conducted the International SpA autoantibody trial (InterSpA). The study was designed as an international multi-centre prospective study. The goal was to measure the sensitivity and specificity of antibodies against CD74 for axSpA in the diagnostic evaluation of inflammatory back pain. In addition, it was studied whether antibodies against CD74 are superior to HLA-B27 in early SpA.

Patients between 18 and 45 years suffering from inflammatory back pain for a maximum of 2 years were recruited. MRI of the sacroiliac joint was performed in all patients; HLA-B27 was detected by genotyping and anti-CD74 using the CE certified kit of AESKU.DIAGNOSTICS (Wendelsheim, Germany). The sensitivity of anti-CD74 and HLA-B27 were calculated in patients fulfilling the imaging arm of ASAS criteria and in 100 blood donors used

as control group. Both the MRI reading as well as the laboratory procedures were performed blinded.

205 patients suffering from inflammatory back pain were recruited. There were 40 recruiting failures. The remaining 165 patients had a mean age of 29.4 years, a mean duration of IBP of 12.6 months, 50% of the patients were female. Sacroiliitis was diagnosed by one local and one independent expert reader in 71/123 (57.7%). So far complete data sets are available for 91 patients. Of these, 16 fulfilled the ASAS criteria of axSpA by a pathologic MRI only, 40 by both MRI and presence of HLA-B27 and 20 by HLA-B27 only. The sensitivities of IgA anti-CD74, IgG anti-CD74 and HLA-B27 were 71.4%, 26.8% and 75% in the 56 axSpA patients with a pathologic MRI, 73.7%, 23.7% and 81.6% in all 76 patients fulfilling ASAS criteria, and 3%, 5% and 8% in the blood donors. The likelihood ratios are 23.8 (IgA anti-CD74), 5.4 (IgG anti-CD74) and 9.4 (HLA-B27) when considering the patients with a pathologic MRI only, and 24.6 (IgA anti-CD74), 4.7 (IgG anti-CD74) and 10.2 (HLA-B27) when considering all patients fulfilling ASAS criteria.

Considering their specificity and sensitivity, IgA anti-CD74 antibodies are a major breakthrough and will be important diagnostic tools for axSpA. In addition, the combination of HLA-B27 positivity and the presence of IgA anti-CD74 antibodies was observed in 44% of the axSpA patients but not in the blood donors. This combination of the two laboratory tests may therefore replace MRI in at least a subset of the patients.

For further information on the CE-marked *AESKULISA®* SpA Detect kit (for the detection of anti-CD74 antibodies), please contact sales@aesku.com

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In 1994 he worked as Postdoc at the Dana-Farber Cancer Institute, Division of Immunology, Harvard Medical School, Boston, USA. After his appointment to senior physician, he qualified in internal medicine and was appointed to professor of rheumatology at Hannover Medical School.

Since the AESKU.KIPP Institute was founded in 2006, Prof. Witte has collaborated in several projects.

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THE GUT-JOINT AXIS AND AUTOIMMUNITY

Prof. Aaron Lerner

1. Introduction

1.1 The surge in rheumatic diseases frequency

In the introduction of the current AESKU.SCIENCE, Dr. Matthias described the increased incidence/prevalence of autoimmune diseases (ADs) in the last decades. Autoimmune diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and celiac disease (CD) are a heterogeneous set of diseases that share common features including multifactorial etiologies, involvement of T cell-mediated autoimmune pathways, shared genes and a chronic clinical course with increased morbidity requiring life-long disease management. A striking increase of ADs in recent decades is apparent, whereas the genetic basis in affected populations has remained arguably constant (1). The current review will expand on rheumatic ADs, zooming in on the relationship between intestinal ecosystem events and their link to joint autoimmune inflammatory manifestations. Some of those aspects were recently reviewed (1-7).

Considering the frequencies, a review of available literature reveals that incidences of CD have increased significantly over the last 6 decades (2). In parallel, the incidence and prevalence of IBD is increasing worldwide (8). A similar trend can be observed for rheumatic diseases in females aged 50 years, when studied between 1995 and 2010 (9). The authors suggested three potential etiologies: increased awareness, increased disease or increased survival. It seems that the trend towards a higher prevalence often coincides with a high pace of socio-economic improvement and westernisation in these countries.

When we reviewed and analysed the literature, taking in account only long-term evaluations, the mean net increase/year of autoimmune rheumatic diseases (RA, SLE, SARD) is higher than that of gastrointestinal ADs (Thyroiditis, IDDM, Crohn's, celiac), 7.14%, 6.2%, respectively (1). Anyway, many of those ADs are genetically and epidemiologically shared in high risk individuals and families. IBD (mainly Crohn's disease), systemic lupus erythematosus, primary biliary cirrhosis, autoimmune hepatitis, rheumatic diseases and CD are several examples [3, 10-13].

Below are several luminal cross talks that potentially connect the intestine to the joints, in autoimmunogenesis.

1.2 Nutrients and RA, CD and IBD autoimmunity

Nutrients are only one part of the exposome that human body is facing. However, the association between diet and the risk of developing ADs was proposed half a century ago and was reviewed

recently (14, 15). Despite our increasing knowledge, little is known about the interplay of diet and gut microbiota in human immune-mediated diseases. It is obvious that dietary habits in Western societies ("too much", "too fatty", "too salty", low fibre) and a high body mass index constitute risk factors for autoimmune diseases. Dietary milk, carbohydrates, fats, protein, fibre, fruit, vegetables or animal proteins have been studied as potential etiological factors in IBD. A comparable pattern of dietary risk factors was also suggested in RA (4, 16). It has been shown that IBD patients that underwent an exclusion diet, by means of an elemental diet, maintained remission for longer periods. The commonly identified food sensitivities were cereals, milk, eggs, vegetables and citrus fruits. Studies on gut mucosal antigen behaviour have shown higher rectal blood flow, in response to specific food antigens, in Crohn's disease patients over healthy subjects. The exclusion of sugar shows little evidence of amelioration in CD. Omega-3 fatty acids are promising in the treatment of IBD but await larger randomised controlled trials (17). Nevertheless, the majority of studies have been equivocal or circumstantial and do not yet support any of these macronutrients as causal factors (9). Several more specific nutritional factors like; vitamin A, D, selenium, zinc, omega-3 fatty acids and flavanols were associated with immune responses involved in ADs (14). Even the more beneficial nutrients like polyunsaturated fatty acids, plant fibre, fish oils or the intake of vegetables and fresh fruits are far from establishing causality in prevention or therapy of ADs (14, 15). Researchers learned a lot about the effects of diet on the mucosal immune system, epithelial function, and the intestinal microbiome. These findings could have significant practical implications. It seems that the nutritional exposome is far from explaining human reactome. It is foreseeable that in future there will be a place for engineered diets able to restrict deleterious components but supplement beneficial nutrients. These diets will be used to modify the luminal intestinal environment of patients with different diseases.

1.3 The microbiome in rheumatic and gastrointestinal autoimmunity

Recent studies show that the complex communities of commensal species that occupy our mucosal tissues influence not only the development and homeostasis of the host's immune system but also confer susceptibility to immune-mediated disease. Alterations in the fine-tuned but fragile microbiome-host relationship can result in community inhabitant change, whereby the dysbiota is overriding the microbiota, setting the stage for immune dysregulation and potential autoimmunogenesis (18-20). In animal models of IBD and RA no disease develops under germ free conditions, supporting the notion of "no bugs, no disease", while in some others

| Autoimmune disease | Dysbiotic characteristics | Microbial species | Action |
|----------------------------|---|---|--|
| Inflammatory bowel disease | Reduction of Firmicutes+Bacteroides, overgrowth of Proteobacteria | Proteus mirabilis, Klebsiella pneumoniae Segmented filamentous bacteria B. fragilis B. thetaiotaomicron | Induce Induce Attenuate Attenuate |
| Rheumatoid arthritis | Increased diversity, Increase in Porphyromonas, Prevotella, Leptotricha and Lactobacillus species | Porphyromonas gingivalis, Prevotella nigrescens, Segmented filamentous bacteria Lactobacillus bifidus, Clostridium | Induce Induce Induce Induce |
| Celiac disease | Increased diversity | Lachnoanaerobaculum, Prevotella, Actinomycetes Lachnoanaerobaculum umeaense | Induce Induce |

they are only attenuated (21). Causality is strengthened by the reintroduction of specific pathobiont restoring the AD severity. Most recently, we reviewed the microbial species used in defined animal models of specific ADs and their functions (5). Table 1 shows the dysbiota associated with RA, IBD and CD, even though established causality is far from being substantiated.

Evidence that links microbiota-associated pathways with spondyloarthropathy exist and several lines of implicating pathways related to the microbiota pathogenesis in Spondyloarthritis (SpA) were recently described (22). Except for epidemiological similarities between gut and joint conditions, IBD shares dysbiotic similarities with SpA. Decreased numbers of Firmicutes, a major phylum of gut commensals, especially the species *Faecalibacterium prausnitzii* and *Clostridium leptum*, are found in both conditions. These bacteria could be an important link in the gut-joint axis. Multiple studies in ankylosing spondylitis, psoriatic arthritis, juvenile SpA, and animal models of SpA reveal common microbial associations among these diseases as well as IBD (23). The environmental trigger of SpA may be related to gut microbiota, familial transmission of gut microbes may contribute to “heritability gap” in SpA and other ADs. Shared amino acid sequences between HLA-B27 and enteric pathogens, T cell clones from joints with reactivity to enteric microbes, evidence for bacterial products in joints and finally, HLA-B27 expression that alters bacterial handling were described (22). Several mechanisms of microbial involvement in driving autoimmunity have been suggested (24): Molecular mimicry, bystander activation during infection, or the “amplification of autoimmunity by cytokines” - elicited by microbial activation of professional APCs and the innate lymphoid cells to produce proinflammatory cytokines by T cells. The fourth suggested mechanism, involving the dysbiota originated post-translational modification of protein (PTMP), generating various neo-epitopes, will be discussed in the next paragraph.

1.4 Intestinal luminal post-translation of peptides and autoimmunity

The immune system carefully distinguishes between self and non-self-components. Therefore, any small deviation of this balanced function may result in an autoimmune activity and harm against self-antigens (autoantigens). One of the ways to transform a self-tolerated antigen to a non-self autoantigen is PTMP. The microbiota, the dysbiota and the ingested probiotics secrete multiple enzymes. The activity of these enzymes spans a plethora of chemical and biochemical reactions that can change a naive peptide into a neo-peptide. Peptides crosslinking or deamidation in CD, de/glycolation or ubiquitination in IBD and citrullination and carbamylation in RA, are some examples of PTMP taking place in the intestine (5). We hypothesise that the spectrum and activities of enzymes, which are normally involved in PTMPs, become biased when the intestinal microbiota ecosystem is replaced by dysbiotic microbial communities. In CD, the autoantigen is tissue transglutaminase (tTg), capable of deamidating or transamidating gliadin peptides (25, 26). The result are neo-epitopes of gliadin docked on the tTg, inducing anti-tTg or anti-tTg neo-epitope autoantibodies. These are the well-known serological markers of CD (26, 27). More recently microbial Tg (mTg), which is heavily used in the food industry, has been shown to form complexes with gliadin peptides resulting in epitopes similar to the recently described tTg neo-epitope. By studying CD patients antibodies against these mTg neo-epitopes were found correlating to the intestinal atrophy of the disease progression (3, 10, 28). Interestingly, the same food additive has been suggested as a new environmental trigger and potential inducer of CD (10, 28). The second example is rheumatoid arthritis, where citrullination is a major post-translational modification of arginine, which converts naive peptides into the immunogenic neo-epitopes. This PTMP constitutes the basis for

the specific prediction of disease activity due to the production of anti-citrullinated protein antibodies (4). Recently, it has been suggested that infectious agents that release toxins such as lipopolysaccharides at mucosal surfaces may trigger the inflammatory response with a potential to cause citrullination of various proteins such as fibronectin, fibrinogen and collagen (29).

The PTMP contribute substantially to the adaptability, cell cycle regulation and survival of the microbes (30). On the other hand, the microbial PTMP has a paramount pathogenic potential to the host. Their enzymatic machinery is capable of transforming naïve/self or non-self-peptides into autoimmunogenic ones, as shown in CD, IBD and RA.

1.5 The leaky gut and autoimmunity

Increasing amounts of evidence supports the concept of increased intestinal permeability as an intrinsic characteristic of several ADs in both humans and animal models of the disease. Often referred to as a 'leaky gut', its mechanistic impact on the pathogenesis of ADs remains unclear. Is it a cause, consequence or co-evolutional phenomenon? (31). Data is accumulating that intestinal ecosystem cohabitants might perturbate the regulation of the tight junctions (TJ), resulting in a leaky gut thus breaking equilibrium between tolerance and immunity to non-self-antigens. Nutrients, toxins, allergens, carcinogens, intestinal infections, dysbiotic bacteria, drugs, stress and the recently described industrial processed food additives can breach the TJ integrity (3-6, 31). In fact, TJ dysfunction seems to be a primary defect in AD (31). Intestinal permeability is increased in many ADs including: Ulcerative colitis, Crohn's disease, CD, inflammatory joint disease, ankylosing spondylitis, juvenile onset arthritis, and psoriatic arthritis, thus contributing to the gut-joint axis. The end result of the passage of these non-self proteins, from the luminal compartment to the subepithelial one, initiates the autoimmune cascade. The richness of the mucosal milieu in immune components, cells and systems, blood and lymphatic vessels, entero-neuronal and endocrine network and mural endo-mesoderm cohabitation, constitutes an ideal place to initiate, maintain and propagate the autoimmune cascade. The mucosal and articular committed immune cells, intestinal post-translational modified proteins, proinflammatory cytokines and lymphokines have the capacity to circulate via the local vessels, to bring the autoimmune message to remote organs, thus creating gut-joint axes.

2. The gut-joint axis and autoimmunity

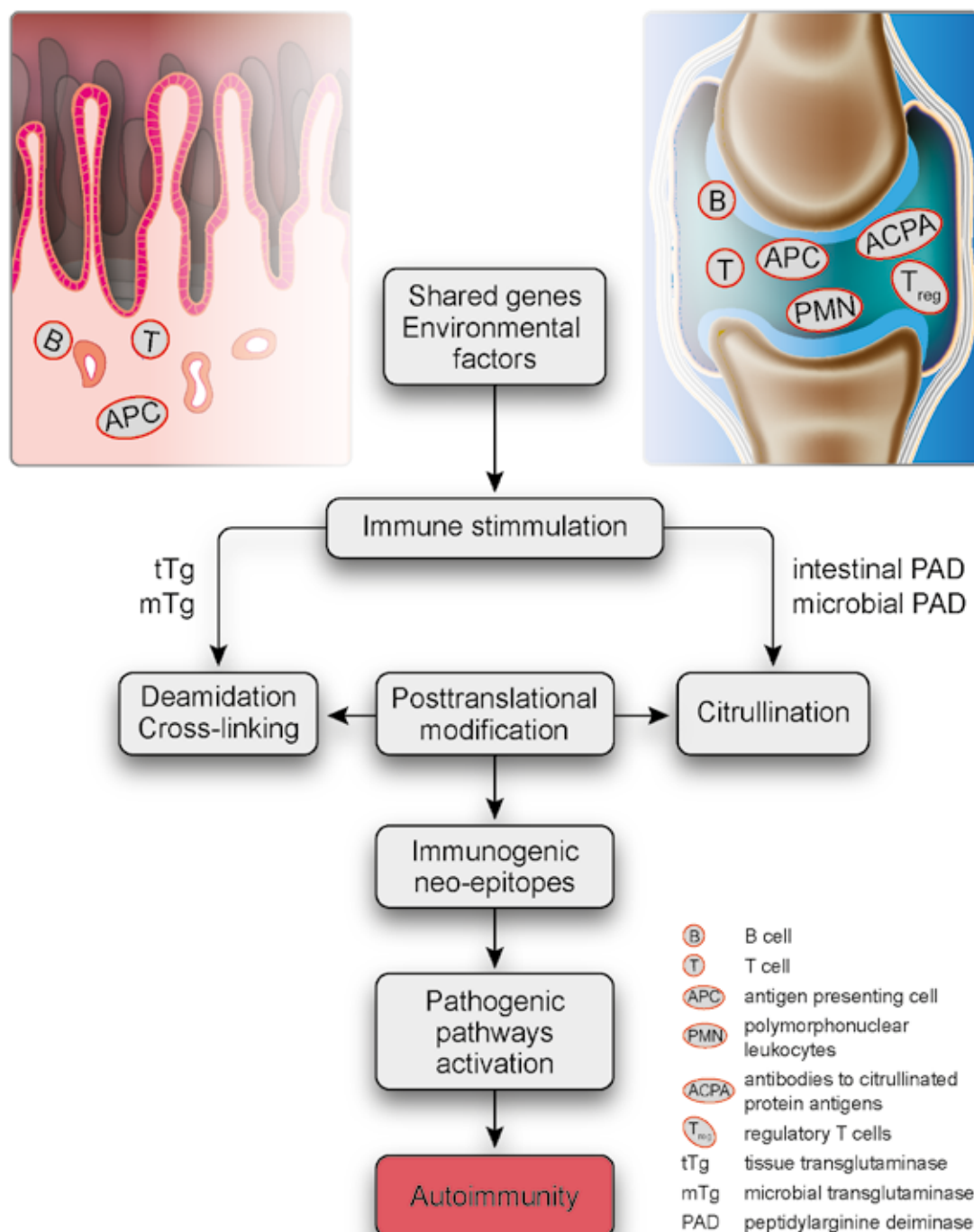
Multiple observations strengthen the gut-joint axis in rheumatologic and gastrointestinal diseases. It appears that autoimmune inflammation starts in the gut mucosa, years prior to the onset of detectable joint manifestations suggesting that rheumatoid arthritis and spondyloarthritis are a gut initiated inflammatory state (4, 32, 33). Gut inflammation is associated with age, sex, disease activity and degree of MRI inflammation on sacroiliac joints, and is predictive for disease course, therapeutic decision-making

and prognosis (32, 33). Specific alterations in gut bacteria have been shown to enhance or attenuate susceptibility to experimentally induced arthritis and, in humans, increased relative abundance of various microbes in rheumatoid arthritis/CD and spondyloarthritis/IBD patients have been detected (4, 32). Taking into account common dysbiota, the wide potential of luminal PTM of naïve peptides, the increased intestinal permeability and the multiple communications between the gut and joints by the blood vessels, one can foresee how the two compartments are interrelated (4).

Several mechanisms may be involved in the breaking of tolerance to self-antigens at the intestinal mucosa initiating rheumatologic manifestations in gastrointestinal condition or participating in rheumatologic disease (4, 34).

1. Toll-like receptors, that are part of intestinal innate immunity and are triggered by endogenous nuclear material aberrantly released during cell death, are associated with induction of autoimmunity. Anti-tTg antibodies are known to decrease apoptosis clearance and apoptosis generates citrullinated endogenous protein in CD and rheumatoid arthritis, respectively.
2. Mucosal microbial constituents can lead to inflammation by molecular mimicry, generating autoantibodies.
3. Migrating mucosal neutrophils may induce tissue citrullination, induced by local peptidylarginine deiminases (PAD).
4. Activated neutrophil extracellular traps (NETs), can generate anti-citrullinated protein antibodies via release of citrullinated peptides.
5. Any local factor may lead to an altered balance between autoreactive and regulatory T cells.
6. Elevation of the rheumatoid factor, even in the absence of arthritis, is associated with mucosal inflammation.
7. Emerging evidence indicates that blockade of TNF- α by biologics not only ameliorates rheumatoid inflammation, but can affect the secretion and action of gut hormones on appetite, body composition, energy expenditure, muscle catabolism and bone remodelling. A link between the gastrointestinal endocrine axis-immune system and joints may be established through the interaction of proinflammatory cytokines, including TNF- α and gut hormones (35, 36).
8. Additionally, some immunological pathways may be actively involved in the transition of autoimmunity from the intestinal mucosa to the joint: Citrullinated immune complex formation deposition, shared antigenic targets (tTg) in the joint or intestinal PAD, gut-joint epitope spreading, and migration of activated effector T cells from the gut to the joints, mimicking the mucosal vaccine's effects on remote organs. Very recently, citrullination of the epithelial neutrophil-activating peptide 78/CXCL5 chemokine resulted in activation of the monocyte-recruiting chemokine (37). In fact, epithelial neutrophil-activating peptide 78/CXCL5 is expressed in intestinal inflammation, citrulline is preferentially synthesised in the gut epithelium and PADs reside in the local microbiome and the intestinal mucosa. This may explain how mucosal citrullinated chemokines recruit monocytes to inflamed joint tissue. Most recently,

The gut-joint axis



A scheme of the gut-joint axis. Shared genes and environmental factors between rheumatoid arthritis and celiac disease allow foreign luminal antigens to stimulate the local immune system. Posttranslational modification of proteins, induced by microbial or intestinal specific enzymes through deamidation, cross-linking and citrullination, can lead to immunogenic neo-epitopes that activate systemic inflammatory immune pathways, resulting in autoimmunity and end organ damage.

IL-17 and IL-22 were suggested to bridge between the gut and synovial fluid in ankylosing spondylitis (38).

9. The hypothesis of a food additive (like: mTg) inducing CD (3, 10) is strengthened by the observation that the production of cross reactive antibodies is strikingly increased in the gut of many rheumatoid arthritis patients. Their food related problems might reflect an adverse additive effect of multiple modest hypersensitivity reactions mediated, for instance, by food originated immune complexes promoting autoimmune reactions in the joints (39)

3. Conclusions

Human beings assemble and maintain a diverse but host-specific gut microbial community along the longitudinal axis of the intestines. However, due to genetic background and changing environment, the physiological microbiota can turn into dysbiota inducing major alterations in the gut ecosystem. It is accepted that the rapidly changing environment impacts ADs susceptibility much more in the last decades. The gut-joint axis is a part of multiple gut-remote organ axes that play a pivotal role in systemic autoimmunity (6). The major contributors and drivers of this axis are the daily ingested nutrient, specific dysbiota. The luminal transformation of naive peptides to immunogenic ones by PTMP and breached intestinal permeability is associated with rheumatic as well gastrointestinal diseases. It is current knowledge that nutrition, the intestinal microbiota and its capacity of PTMP, the gut mucosal immune system, the leaky gut and autoimmune pathology are deeply intertwined in rheumatic and gastrointestinal ADs. Better understanding of the gut-joint axis might unravel novel predictive, preventive and therapeutic strategies to combat those diseases.



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Prof. Aaron Lerner graduated from the Faculty of Medicine at Tel Aviv University, Israel. He specialised in paediatrics, paediatric and adult gastroenterology, and got a MHA degree in medical management. He spent several years of clinical and basic research in Chil-

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He is currently an associate professor in the faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, and is on an extended Sabbatical at the AESKU.KIPP Institute, Wendelsheim, Germany, involved in multiple research projects on novel bio-markers, pathophysiology, diagnosis and prediction of autoimmune disease and the involvement of industrial food additives in chronic disease induction. The attached 24 recent publications summarise his scientific diversity and productivity.

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MMP-3 – A NEW PROGNOSTIC AND ACTIVITY MARKER FOR THE THERAPY MANAGEMENT OF RHEUMATOID ARTHRITIS

Dr. Sandra Reuter

Rheumatoid Arthritis – A great socio-economic burden

Rheumatoid Arthritis (RA) is a severe autoimmune condition that affects 20 million people worldwide through all age groups. RA is characterised by joint swelling, joint tenderness and finally destruction of synovial joints (Figure 1). This leads to severe disability and a dramatic loss of life quality for RA patients due to chronic pain and functional restrictions. RA patients also often suffer from additional clinical manifestations of the disease like arteriosclerosis, pneumonia or lymphomas which probably develop due to the constant chronic inflammatory processes in the patient's body. Combined with the more or less severe side-effects of common drug regimens this leads to premature mortality of RA patients. Altogether, aside from the personal restraints of each patient, RA is generating high costs for the health-care system (diagnostics, anti-rheumatic drugs, physical therapies, etc.) and for the economy (loss of working ability).

However, during the last decade it has also been shown that early therapeutic intervention is able to control inflammation process-

Drawbacks in the diagnosis and prognosis of RA

A very critical parameter in RA therapy management is time, because bone erosions and development of joint lesions can start in the early stages of RA. Treating patients at a stage in which development of joint damage can still be prevented would be ideal, but diagnosis of RA can be a long journey that still depends greatly on how quickly a patient is referred to the rheumatology division. Especially in its early phase RA is difficult to discriminate from other joint affecting diseases and it can be accompanied by many unspecific symptoms. Moreover, there are different types of onset and progression of the disease and even if RA is diagnosed early there is still no common consensus about how to identify patients that have a severe progression and a high risk of developing bone erosions. Still, this differentiation is very important because drug therapy is strongly dependent on it. Due to severe side-effects and high costs only those patients with a high risk for joint destruction should receive aggressive drug therapy while patients with a milder course of the disease might do better with less aggressive

drug therapy. Since the prognosis of RA is still very difficult there is another great loss of time and costs due to "trial and error" therapeutic management.

The need for early differentiation of RA patients is also expressed in the new ACR (American College of Rheumatology) diagnostic criteria from 2010 which have been revised to improve their sensitivity especially in the early phase of RA, and to detect patients that would benefit from early therapeutic inter-

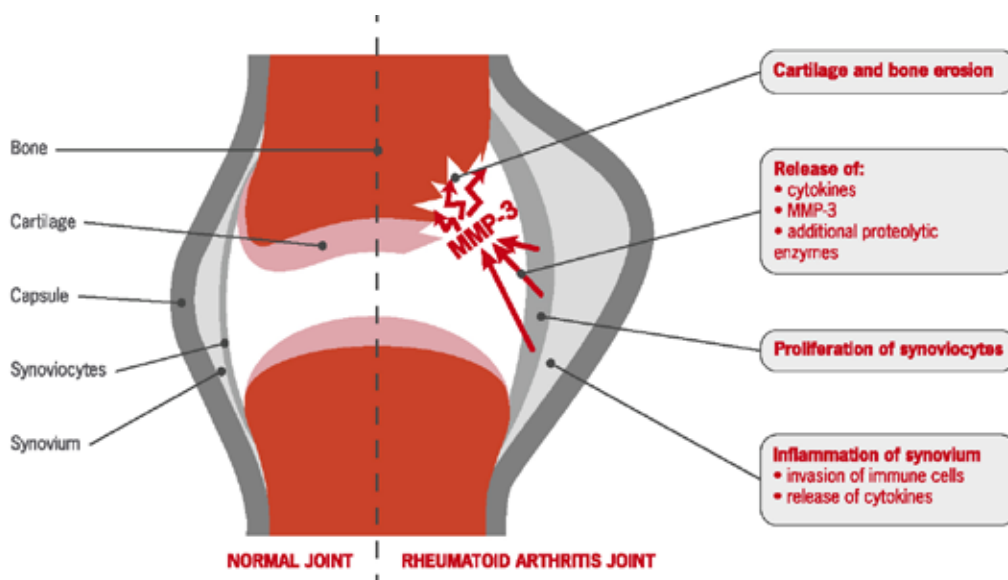


Figure 1: A potential model of events in the joints of RA patients

es effectively when appropriate drugs are administered, thereby reducing or even preventing the destruction of joints. Therefore, early diagnosis of RA and prognosis of disease progression and outcome are mandatory for therapeutic success.

vention (1). Although the measurement of ACPAs (anti-cyclic citrullinated protein antibody) has been included in the ACR criteria there is still a lack of good prognostic markers. ACPAs are very good markers for diagnosis due to their high specificity and ACPA positive patients have been shown to usually have a higher risk for severe disease progression. But there are still no good

serological markers for the actual risk of bone erosions at a given point in time. Conventional radiographic imaging techniques are not sensitive enough to detect small changes at the beginning of an erosive process. Furthermore, progression and activity of RA are currently evaluated with different activity scores like DAS (disease activity score), or radiological scores like Larson's score or Lansbury's index. These scores are time consuming, laborious, and require the observation of patients over a longer time period. They are also not useful when making quick decisions to start or adapt therapy. There is an urgent need for good serological markers that aid decision-making in therapy management of RA.

MMP-3 and Rheumatoid Arthritis

MMP-3 (matrix-metalloproteinase-3, stromelysin-1) has been shown to be overexpressed in RA patients. MMP-3 is a member of the matrix-metalloproteinases family and has a wide range of substrate specificity, e.g. tissue matrix proteins such as cartilage proteoglycans, fibronectin, various collagens, and laminin (2;3). In addition to being directly involved in cartilage and bone destruction processes it also activates further degrading enzymes like proMMP-1 (procollagenase) and proMMP-9 (progelatinase B) and is therefore thought to be a key player in joint destruction in RA patients (4-9). In conclusion with this it could be shown that MMP-3 is produced by articular synovial cells, fibroblasts, and chondroblasts. It is secreted as an inactive zymogen (proMMP-3) which has to be activated by endopeptidases (8;10-12). The synovial fluid of RA patients contains large amounts of MMP-3. Serum MMP-3 levels of those patients are also strongly elevated and correlate with the amount of MMP-3 in the synovial fluid (12-14) which is important for the measurement and interpretation of MMP-3 serum levels.

MMP-3 is also expressed in healthy people but to a much lesser extent. MMP-3 is thought to play a role in the physiological, constantly ongoing remodelling processes in cartilage and bone tissue. Its activity is controlled by TIMPs (tissue inhibitors of metalloproteinases) that bind to MMPs thereby inhibiting their enzymatic activity. In healthy people the expression of both protein classes is precisely balanced to allow for necessary extracellular matrix turnover processes but to impair destructive processes (15;16). Figure 1 shows a potential model of what is happening in the joints of RA patients. For reasons that are still unknown first there is a massive inflammation of synovial tissue with invasion of immune cells and the release of proinflammatory cytokines. This results in a vast proliferation of the synovial lining cells and the overexpression of MMP-3 which is stimulated by cytokines like TNF and IL-1 and which has been shown to depend on the degree of inflammatory cell infiltration of the sublining cell layer (8;11;17). The overgrown synovium is a major source of proinflammatory cytokines and proteolytic enzymes. Although many enzyme classes and other factors such as free radicals and mechanical factors are implicated in the progressive damage of the joint, the large amount of MMP-3 is considered to play an important role. In conclusion, proteoglycan core protein cleaved at a site susceptible to MMP-3 has been found in the synovial fluid of RA

patients. The surplus of MMP-3 in the joints of RA patients cannot be balanced by TIMP proteins anymore and leads to further activation of degrading enzymes and destruction processes resulting in deformity and disability of joints (18-20).

MMP-3 as a predictor of joint damage in Rheumatoid Arthritis

Many studies have shown the potential of MMP-3 to predict bone erosions and to reflect disease activity. First of all, it has been shown that serum MMP-3 levels are significantly higher in RA patients compared to osteoarthritis patients and to normal controls (12-14;21-23). It has also been shown repeatedly that serum MMP-3 levels correlate nicely with the inflammation markers CRP and ESR (13;14;21;22). Moreover, it could be shown that MMP-3 levels rose with increasing joint affection and destruction in RA (13;14;22;24;25) and that MMP-3 elevation in serum represents disease activity regardless of age or disease duration (26) which led to the conclusion that serum MMP-3 is a useful marker of inflammatory activity in the joints of RA patients. Even more encouraging are the results from a study by Yamanaka et al. (2000) who compared MMP-3 levels and joint destruction measured by Larsen score in a prospective study with 82 patients. The study found, that as a predictor of joint destruction MMP-3 was superior to Larsen score. Serum levels of MMP-3 at entry into the study showed correlation with Larsen score at 6 and 12 months after entry into the study. In conclusion, elevated levels of serum MMP-3 reflect joint destruction that will occur in the near future, namely within a period of 6-12 months and therefore MMP-3 is a useful marker to predict bone damage (21). Another longitudinal study from Tchetverikov et al. (2003) included 109 recent onset RA patients over a time period of two years. They also found that serum MMP-3 levels at disease onset were predictive of joint damage progression, whereas CRP levels were not. Since MMP-3 is directly involved in joint degradation they also conclude that MMP-3 may be seen as a constitutive activity marker of the pathological process underlying joint tissue degradation in RA (25). Another study supporting this idea has been conducted by Kobayashi et al. (2007). There, MMP-3 levels were monitored in 29 RA patients having total knee arthroplasty. It could be shown that levels of MMP-3 dropped to low values 1 and 2 weeks after the surgery, but not CRP (increased at one week) and ESR (unchanged) (23). Furthermore, the studies by Posthumus et al., 2002 and Young-Min et al., 2007 showed that serum levels of MMP-3 decreased in patients who responded to DMARDs (27;28). Ally et al. (2013) found that MMP-3 is significantly correlated with disease activity, inflammatory mediators and cartilage breakdown (29).

Altogether, this leads to the conclusion that serum MMP-3 is a potential marker for first of all the prediction of bone erosions and second of all the control of disease activity and therapy success.

AESKULISA® MMP-3

In cooperation with the group of Prof. Egerer from the Charité, Berlin, the relevance of MMP-3 for disease progress and prognosis in patients with RA was evaluated. Follow-up analyses of serum MMP-3 levels with Aeskulisa® MMP-3 were performed in comparison to control groups over an observation interval of more than 1 year.

At least 6 serum samples per patient from 21 patients with RA, 12 patients with SLE and 10 patients with pSS were tested.

The highest MMP-3 levels were measured for female patients with SLE and RA, followed by patients with pSS and blood donors. In RA patients, follow-up analyses showed a correlation between MMP-3 levels and DAS28 ($r=0.43$, $P<0.0001$), radiological score ($r=0.35$, $P<0.0001$) as well as clinical and laboratory findings.

In conclusion, MMP-3 cannot be used for diagnostic differentiation, since serum levels were increased in different systemic autoimmune diseases. However, MMP-3 levels correlate with clinical, laboratory and radiologic findings in patients with RA. It shows that increased expression of MMP-3 is linked to RA activity and prognosis and therefore, may be a very useful biomarker.

In order to explore the correlation between MMP-3 and histological synovitis in RA, a study with the group of Prof. Lie Dai from China was performed by using the Aeskulisa® MMP-3 (30). Sixty-two patients with active RA were tested by ELISA and in parallel serial synovial tissue sections from all RA patients, 13 osteoarthritis, and 10 orthopaedic arthropathies patients were stained immunohistochemically for MMP-3. The percentage of both lining MMP-3+ cells and serum MMP-3 were significantly higher in RA patients, especially with high grade synovitis compared to control groups. Therefore, it was concluded that serum MMP-3 might be a noninvasive biomarker of histological synovitis.

was determined by Aeskulisa® MMP-3 and clinical data were collected at 0, 1st, 3rd, 6th and 12th month. The results showed that radiographic progressive patients had significantly higher serum MMP-3 levels for 3~6 months compared to the non-progressive group (see Figure 2). The data showed that continuously elevated serum MMP-3 levels for 3~6 months predicted one-year radiographic progression. Therefore, monitoring of dynamic serum MMP-3 in combination with core disease activity indicators is helpful for predicting radiographic progression and treatment decision in RA.

MMP-3 as a new prognostic tool

There are two major strategies in drug therapy management of RA:

1. Early introduction of anti-rheumatic drugs and
2. Strict control of disease activity and therapy success.

Although in recent years a lot of attention has been given to the prognostic value of ACPAs, due to its features MMP-3 may be an even better aid for both strategies. Firstly, elevated MMP-3 levels identify people who need aggressive therapy regardless of their disease phase. Since it can already be elevated in the early phase of RA, MMP-3 is also a valuable tool in classifying early RA patients. Furthermore, unlike ACPAs whose occurrence is dependent on the genetic background of the patients and can be quite different in individuals or even be undetectable in as much as 40% of RA patients, MMP-3 can be measured and evaluated in every patient.

Secondly, MMP-3 is a marker of ongoing inflammation in the joint. MMP-3 is directly involved in the joint destruction processes by resolving bone and cartilage tissue and it also activates further

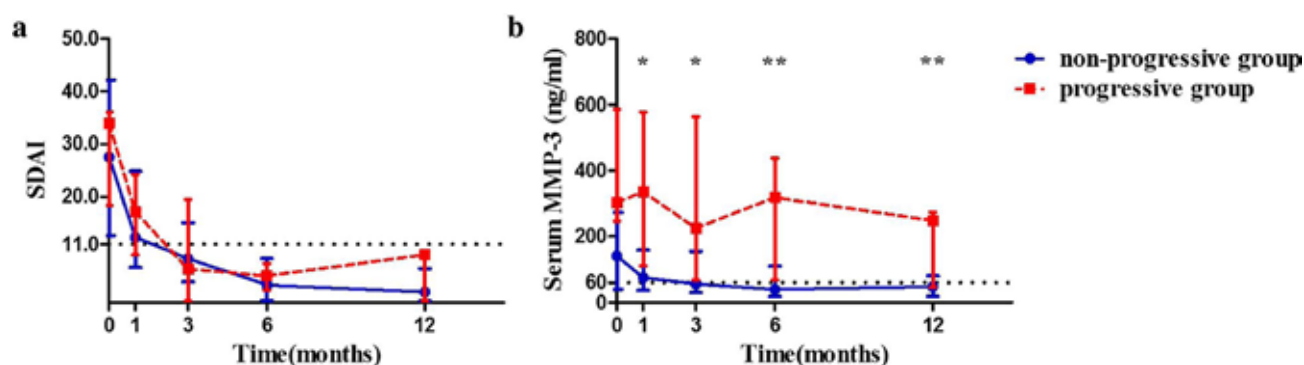


Figure 2: (a) Dynamic disease activity defined by simplified disease activity index (SDAI) and (b) serum MMP-3 between non-progressive and progressive patients

A recent prospective cohort study from the same group confirmed that monitoring of serum MMP-3 is helpful for predicting radiographic progression and choosing correct treatment in RA (31). In this study 56 patients with active RA were followed up for one year. Based on X-ray assessment of hand/wrist at time point 0 and 12 months the patients were subdivided into a progressive (16 patients) and a non-progressive group (40 patients). Serum MMP-3

degrading enzymes. MMP-3 levels directly reflect the situation in the joint in terms of inflammation and destruction and are a valuable tool to monitor therapy success. Although quite a few studies have already confirmed the potential of MMP-3 as prognostic and activity marker, studies with larger patient cohorts are necessary to evaluate the usefulness of MMP-3 in the daily routine of risk assessment in RA patients.

Conclusions

Markers indicative of disease activity and bone erosions help to identify RA patients that would benefit from early aggressive therapy and provide a valuable tool to monitor disease activity and therapy success in later phases of RA. MMP-3 testing is a good and necessary tool in addition to other established markers like RF (rheumatoid factor) and ACPA. With MMP-3 there is a chance to predict ACPA negative patients also, and to specify prognosis in ACPA positive patients. ACPAs may predict a higher risk for aggressive disease courses, but they do not tell us anything about the patient's current situation. Is there already destruction going on and to what extent? Or is the disease still inactive? MMP-3 may be able to provide answers to these questions and greatly enhance information for physicians for finding the appropriate therapy.

For further information to the CE-marked *AESKULISA*® MMP-3 kit, please contact sales@aesku.com



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Dr. Sandra Reuter studied Biology at the Johannes Gutenberg-University of Mainz and at the Ruprecht-Karls-University of Heidelberg. After her graduation from the "Institute of Virology" at Heidelberg University Hospital, she worked there as a postdoctoral research fellow. She has been part of the AESKU.KIPP Institute since 2006.

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AND THE AESKU.AWARD GOES TO...

For the 7th time the "AESKU.AWARD" for life contribution to autoimmunity" will be presented during the Opening Ceremony of the 10th International Congress on Autoimmunity in Leipzig. The Award pays tribute to individuals who have contributed in a significant way to the field of autoimmunity.

This year we are pleased to announce the following three distinguished nominees:

Dr. Robert Morris, USA

After graduating from Rutgers University, Dr. Robert Morris attended Hahnemann Medical School and was accepted for the re-

nowned UCLA Rheumatology Fellowship. Dr. Morris has taught at UCLA medical school and other institutions for over 25 years.

As he started his private practice in 1974, he was troubled by the paucity of quality autoimmune testing for his patients. In 1977, after much effort, he and Dr. Allan L. Metzger established RDL Reference Laboratory, with the goal of providing the most reliable and cost effective laboratory testing for Rheumatologists throughout the USA. Since its inception RDL has been a leader in scientific research and always at the forefront of new assay development, for example introducing anti-CCP testing in the USA. In 1999, Dr. Morris retired from private practice in order to be able to devote his full energies to RDL. Late in 2003, Dr. Morris was diagnosed with a rare form of hydrocephalus, resistant to conventional

treatments. After two years of hospitalisation and rehabilitation, Dr. Morris returned to work full-time at RDL.

From the time of his Fellowship to the present day, Dr. Morris' vision has never been limited to his Practice and RDL. He was the primary investigator in studies that proved the link between HLA-B27, Ankylosing Spondylitis and Reactive Arthritis. This discovery was published in the New England Journal of Medicine in 1974. Dr. Morris was the first clinician to make a finding of legionella infection in synovial fluid. In addition to many other contributions, Dr. Morris has been instrumental in the development of the helminth compound as a potential treatment and/or vaccine for autoimmune diseases. In 2014, Dr. Morris was made Master of the ACR.

Prof. Angela Tincani, Italy

Angela Tincani is Professor of Rheumatology, University of Brescia and Head of Rheumatology and Clinical Immunology, Brescia General Hospital, Italy. After attaining her MD at the University of Milan in 1974, Prof. Tincani continued postgraduate studies in Allergology and Clinical Immunology, Haematology and Rheumatology, before taking up senior positions at the University of Brescia. She has been Head of the Rheumatology and Clinical Immunology Unit of Spedali Civili, Brescia since 2010.

Her main clinical and research areas include: pathogenesis, diagnosis and treatment of systemic autoimmune diseases in particular SLE and APS; management of pregnancy in patients with inflammatory arthritis and systemic autoimmune diseases; biomarkers of outcome and use of anti-rheumatic drugs in pregnant patients; evolution of autoantibody determinations and reliability and the clinical significance of emerging new technology.

Prof. Tincani has been included on the organisational boards of many national and international congresses, including the International Congress on Autoimmunity since 1999 (Tel Aviv). She has served on many international committees for research and education in lupus, most recently the EULAR committee "Points to consider for use of anti-rheumatic drugs before pregnancy and during pregnancy and lactation" (2013–14) and, as co-chair, "Recommendations for the management of family planning, assisted reproduction, gestation, delivery and menopause in patients with Systemic Lupus Erythematosus".

Prof. Tincani is a widely published author: 300 publications indexed by PubMed with nearly 11000 citations and H-index 50 (by WOS). She is the regional Editor (Europe) of the journal Autoimmunity and sits on the Editorial Boards of Clinical and Experimental Rheumatology and Autoimmunity Reviews.

Mr. Poju Zabłudowicz, UK

Poju Zabłudowicz is a global investor, entrepreneur and philanthropist. Mr. Zabłudowicz is Chairman of Tamares which he has led since 1990. Tamares makes long-term investments in real estate, technology and the primary sector and is a long-standing contributor to medical facilities. Over the years Mr. Zabłudowicz has had an increasing interest in the field of autoimmunity. He

created the Zabłudowicz Center for Autoimmune Diseases, one of the world's most advanced centres for research into autoimmune diseases. Mr. Zabłudowicz has contributed to research projects on subjects like IVIg applications in autoimmunity, immunomodulating drugs and basic research on the aetiology and pathogenesis of autoimmune models.

In 1994, Mr. and Mrs. Zabłudowicz established The Zabłudowicz Collection, a dynamic collection of contemporary art works. The Zabłudowicz Collection supports hundreds of new artists, many of which have achieved significant success.

Poju Zabłudowicz was born in Finland and remains a passionate supporter of his home country. Alongside Tamares' investment in Outotec, a leading Finnish mining technology company, Mr. Zabłudowicz serves on its board of directors as well as on the board of the Kiasma Museum of Contemporary Art in Helsinki. He is also a key supporter in the initiative to establish Guggenheim Finland.

The AESKU.AWARD for life contribution to autoimmunity not only shows the importance of research on autoimmune diseases, it also aims to establish the significant field of autoimmunity as an independent research area and to foster inter-disciplinary cooperation. AESKU continuously invests in research and development to create new opportunities substantially improving diagnosis and therapy of autoimmune diseases.



1ST AESKU LATIN AMERICA WORKSHOP

In November 2015, AESKU.DIAGNOSTICS organised the first Latin America workshop in Punta Cana (Dominican Republic). We were delighted with the overall response and welcomed participants from Colombia, Ecuador, Mexico, Peru, El Salvador, Paraguay, Spain and Brazil.

The objective of this event was to meet and motivate our distributors to achieve greater brand loyalty from our end customers.

After a warm welcome by Marcela Tafur, AESKU's Area Manager Latin America, AESKU's CEO Dr. Torsten Matthias gave a short introduction concerning the new AESKU.GROUP. In the course of the following days the participants were informed about the trends in autoimmune diagnostics, recent automation, new products and strategies. In addition to the presentations given by AESKU.DIAGNOSTICS staff, it was not only a pleasure but an honour for us to be able to welcome several renowned external speakers:



Dr. Nancy Barrera, member of rheumatological society of Colombia with two very interesting lectures.

In her presentation "Vitamin D and its relation to Autoimmunity" she went over the history, the first measurements, the production, as well as the synthesis and metabolism of Vitamin D. Moreover, she went into greater detail on the causes of the deficiency, its consequences and its link to autoimmune diseases with an extensive review of diverse publications. At the end she emphasised the importance of proper measurement as a diagnostic aid from the laboratory.

In her second talk, "International recommendations in IFA", the importance of unifying concepts in the area of immunofluorescence was mentioned, because it is currently influenced by

too many factors, including local differences in performance. Moreover, she presented a review of the regional consensus, for example those in Brazil and the international recommendations for the validation of antinuclear antibodies and the unification of nomenclature.

Dr. Aresio Plaza (Immunology Area, Puerta De Hierro University Hospital, Majadahonda) from Madrid, Spain, talked about new perspectives in serological Celiac Disease testing. He passed on information about the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Guidelines for the Diagnosis of Celiac Disease (CD) and the diagnostic value of tTg. Dr. Plaza gave a report on the implementation of screening protocols using *AESKULISA*® CeliCheck new Generation kits and results obtained by the laboratory from 2009 to 2012. He emphasised the assays' usefulness for laboratories with a high throughput of serological CD testing since it allows the exclusion of all negative results from further testing. Moreover, it provides a good correlation between *AESKULISA*® CeliCheck screening results (positive or inconclusive) in children or adults and positive biopsy for CD. In the last part of his presentation, Dr. Plaza talked about new perspectives and new antibody markers for gluten intolerance with a seronegative result for conventional markers but with gastrointestinal symptoms or nonspecific intestinal inflammation.

Two of our distributors from Colombia and Chile shared their success stories in their countries and one of our Colombian end-users gave a speech on the implementation of HELIOS® in their daily lab work routine. Our Peruvian distributor recounted his experiences implementing our products into the work routines of a specialised laboratory.



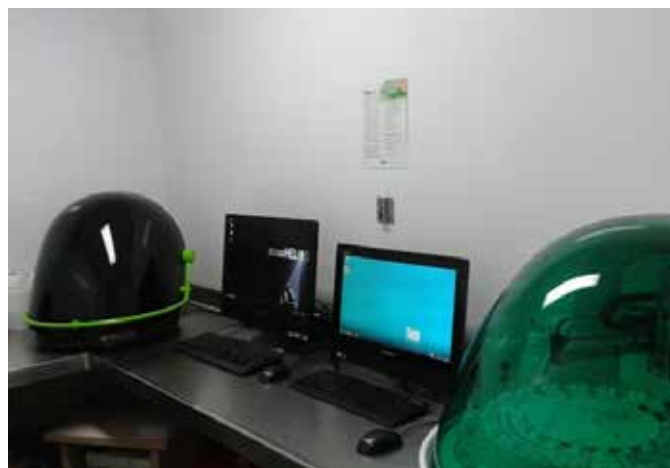
The aim of the workshops was to create an interactive, dynamic, productive atmosphere, to give us and the participants the opportunity to exchange experiences and knowledge. As feedback was overwhelmingly positive, we seemed to have achieved our goals.

Comments like, "The event surpassed our expectations" or "Excellent for sharing experiences" are strengthening AESKU's conviction and will ensure that this type of workshop will become a regular feature.



QUESTIONNAIRE FOR LABORATORIES CURRENTLY USING THE AUTOMATED HELIOS* EQUIPMENT

Laboratory: Dinamica Laboratory
Experience in years: 20 years



Which are the main features that you would highlight of the performance of the Helios?

It is a functional, compact, fast, with a good design, a friendly software and comfortable machine.

What are the main benefits that the laboratory has by having the Helios?

It allows the standardization on the reading and classifying of patients, of easy access of the information, the file of images offers competitive advantages, the possibility of communication between AESKU.DIAGNOSTICS and the commercial house. And reagents are of a very good quality.

How does the Helios help to improve processing times in the section?

When the reading and sorting the photos of patients on a monitor and not on a microscope located in a dark room, the welfare of users end is improved by reducing eye fatigue.

How has the Helios helped to standardize the results in the lab?

When the software makes the discrimination between positive and negative, it allows the user to have a consensus in order to define the result according to the already established criteria, and it makes it a more objective reading.

Is the level of automation from the Helios the required one by the laboratory?

The level of automation of the system is appropriate.

How do you think it helps the discrimination between positive and negative results on laboratory efficiency?

The discrimination results improves laboratory efficiency because low false positives are reduced, which before they were re-processed, allowing a better use of resources and it improves the opportunity on results.

With how many photos do you make the immunofluorescence pattern discrimination? Is it enough the photo capacity that the Helios can generate?

The discrimination of the pattern is made with three photos, and we believe this is an appropriate and sufficient number.

What is the main utility of the library of patterns within the software Helios?

The library of patterns offers easy access information and good understanding, and it is educational.

What do you like the most about working with Helios?

I like to work with Helios because the opportunity on the results is better, is easy to use, it makes bar codes readings and the results are reliable, reproducible and standardized.

** Please be aware that Helios is a registered trademark.*

NEW STRATEGIES FOR US BUSINESS

On the 25th of June 2015 a contract was signed between AESKU and Grifols Diagnostic Solutions, represented by Dr. Torsten Matthias (CEO AESKU) and Carsten Schröder (President of Grifols Diagnostic Solutions Inc). The central point of this agreement was to assign exclusive distribution rights for AESKU products in the USA to Grifols Diagnostic Solutions.



Left to right: Bruno Larida (Vice President - Country Manager AESKU. INC), Dr. Torsten Matthias (CEO & President AESKU.GROUP), Carsten Schröder (President of Diagnostic Solutions Inc.) and Raul Alvarez Grifols (Vice President Global Marketing Diagnostic Division)

In 2002 Grifols and AESKU entered into a long standing business partnership. Grifols already distributes AESKU's products in Italy, Portugal, Chile, Spain and Great Britain. In 2014 AESKU assigned the exclusive distribution rights in Mexico - for its diagnostic products - to Grifols.

The US market holds a 47% share; it is one of the main global autoimmune diagnostic markets. With this strategic partnership, AESKU gains the ability to increase US-customer support to 24/7, using the distribution and service network of Grifols US. Additionally a budget for FDA approval procedures, which are essential to gain US marketing permits for future products, was set up.

Grifols, with headquarters in Barcelona, Spain, is an important global player in the field of laboratory medicine, active in over 100 countries, with more than 16,000 employees worldwide. Grifols itself is global market leader in the field of plasma preparation, with a turnover of EUR 3.4 billion. They are developing, producing and distributing a wide range of products for transfusion medicine.

AESKU is looking forward to a successful and productive collaboration.

AUTOMATION IN THE IFA LAB

Indirect immunofluorescence (IIF) is still confirmed to be the gold standard for the detection of antinuclear antibodies (ANA) and antineutrophil cytoplasmic antibodies (ANCA). Therefore, this method has to be accurate, highly specific and reproducible. Conversely, IIF has some undesirable drawbacks that are the subjective interpretation, the need for experts, the poor standardisation and the intra- and inter-laboratory variability. To avoid these factors the call for automation was growing. With the HELIOS® AESKU launched the first fully-automated IIF processor that performs all IIF steps - from slide preparation to reading - automatically. Processing without human intervention is enabled by a unique feature of mounting medium dispensation combined with the non-requirement of a cover slip. "Since the process is fully automated and it does not require the intervention in the process then all runs are made with the same characteristics, therefore that it is completely reliable", notes Dr. Constanza Sánchez (laboratory AnaLIZAR, Colombia) on how HELIOS® helps to standardise the results. The HELIOS® consists of two built-in barcode readers for scanning sample and slide IDs to ensure full traceability and an integrated auto-focus epifluorescence microscope unit that incorporates Nikon optics controlled by an AESKU engineered positioning unit. The image capture works automatically after the

slide processing is completed. The HELIOS® software image processing is analysed with a mathematical algorithm which analyses every single image and suggests a pre-classification result (positive/negative) based on different variables (structure analysis, fluorescence intensity, background/cells ratio, etc.). The cut-off settings can be easily adjusted for the lab requirements during the installation and validation process.

Recently, new features have been developed for the HELIOS®: the end-point titre determination and the pattern recognition modules that make it possible to analyse images captured by the HELIOS® device.

In screening mode, an estimated end-point titre is calculated for wells with previous positive pre-classification, which is time-saving and cost-effective.

The following estimated pattern can be suggested by the software:

For Rheumatology

- Homogeneous
- Speckled
- Nucleolar
- Nuclear membrane
- Nuclear dots
- Centromere
- Cytoplasmic

For Vasculitis

- P-ANCA
- A-ANCA (X-ANCA)
- C-ANCA
- Undefined positive

During result confirmation, the patterns can be assigned manually by the IFA expert and a follow-up decision can be made, ensuring secure management of results.

The HELIOS® system works with the standard FITC fluorochrome (excitation wavelength 465 to 499 nm) and no additional stain is needed for focusing the objects. This feature allows the expert to manually review the slides using a traditional microscope, if desired.

In a first study by Platzgummer and colleagues it was shown that the HELIOS® has an overall sensitivity of 96% and a specificity of 94%. Besides a reliable pattern recognition, a good precision within the acceptable range of $<>1$ titre step was revealed. The results of this study will be published soon and presented at the 10th International Congress on Autoimmunity in Dresden.

Sánchez summarised main benefits of the HELIOS® as follows, "This device has many good features: No darkroom is required, a digital file is obtained from all of the results, standardise, reproducible process, easy maintenance, easy access to software and high quality in the pictures."

Due to the fact that fewer labs are responsible for an increasing number of hospitals and practitioners, automatic solutions with



a high throughput, low hands-on time and easy handling are the basis for success in the market.

The systems from AESKU are able to process several million patient samples per year.

The HELIOS® was awarded the innovation prize and the ISB success award in 2014.

For further information please visit our website www.whatishelios.com

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10TH ANNIVERSARY AESKU.KIPP INSTITUTE

The AESKU.KIPP Institute, a non-profit organisation, has been initiated and supported by AESKU.DIAGNOSTICS. It is primarily active in basic research and interdisciplinary knowledge transfer in autoimmunity by initiating and coordinating international research cooperation and development projects in autoimmunity. At the same time it offers the spatial and personnel requirements for the implementation of joint interdisciplinary projects at the Wendelsheim facility.

As early as 2003 Dr. h.c. Karl-Heinz Kipp and the company AESKU.DIAGNOSTICS, in cooperation with the Laura Schwartz-Kipp Foundation, set a fresh milestone in autoimmunity research

with the establishment of this first chair for autoimmunity.

A future-oriented event forum is being created at the AESKU.KIPP Institute, including meetings between leading scientists, contribution to PhD degree programs, further training of doctors and laboratories, as well as information events for patients.

The AESKU.KIPP Institute has consciously positioned itself as an international interface between basic and clinical research, so that recent research results may be integrated into clinical applications.

Some of the research projects and their results are introduced below.

Research topic: Autoimmune liver diseases and Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is a relatively rare autoimmune disease of the liver and in 90% of cases women are affected. In an advanced stage the whole liver can be inflamed and this can result in cicatrization to the point of cirrhosis.

If untreated, PBC can be lethal within 12 years. However, the course of the disease is very variable from patient to patient and in early stages of the disease the life expectation does not seem to be restricted significantly. Today, the standard therapy for PBC is to take Ursodeoxycholic acid in tablet form. Usually the therapy is well tolerated and is started directly after diagnosis.

If, despite therapy, the disease progresses or symptoms such as pruritus become intolerable, a liver transplant will be necessary. After successful transplantation 75% of patients are cured. However, in 25% of the patients similar symptoms can still appear.

In co-operation with Prof. Dr. Pietro Invernizzi, Associate Professor of Gastroenterology, Director, Program for Autoimmune Liver Diseases, Section of Digestive Diseases, International

Center for Digestive Health, Department of Medicine and Surgery, University of Milan-Bicocca, Italy, the AESKU.KIPP Institute is searching for a new therapeutic approach to PBC. The aim of this approach is to adapt specific therapy to the patient and to interfere with the pathomechanism inhibiting the progress of the disease. Thus, the number of liver transplants can be minimised and the quality of life will be improved.

The aim of the research project is to identify peptides for possible cell based immunotherapies in PBC. Therefore, blood from PBC patients will be investigated in several cell-based assays.

One method used is the iTOPIA binding assay to measure the binding ability of individual peptide candidates to the HLA molecules under standardised optimal binding conditions. Peptides identified as binders are characterised in terms of affinity and dissociation experiments and scored for further use in individualised cell based assays.



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Research topic: Rheumatic diseases and ankylosing spondylitis

In the ADAPThera research project several new and interesting markers could be identified that are related to rheumatic diseases. One of these markers was Matrix metalloproteinase (MMP)-3. Since the aim of rheumatoid arthritis (RA) treatment is to reduce synovial inflammation and to prevent joint destruction, early diagnosis, prognosis of progression and disease activity control are crucial to therapy success. MMP-3 is thought to be a key player in cartilage and bone destruction. Due to its broad substrate specificity MMP-3 is capable of degrading connective tissue matrix components and activating other destructive enzymes. Its expression is considerably enhanced in rheumatoid arthritis, even in the early phase. It was shown that in the serum of RA patients the concentration of MMP-3 was significantly elevated compared to healthy controls. Serum MMP-3 levels correlate with MMP-3 levels produced by the synovium, thus reflecting disease activity and enabling prognosis of progression. Moreover, MMP-3 levels drop as a consequence of efficient therapy and therefore, it is an excellent marker of successful therapy. Due to its multifaceted informative value, MMP-3 helps physicians to create and adapt an individualised drug therapy for each patient.

In another project an assay for a new marker was developed that detects anti-CD74 antibodies. It is a tool to differentiate between spondyloarthritis and non-inflammatory back pain. Axial spondyloarthritis (axSpA) is a common chronic rheumatoid systemic disease that is characterised by predominant involvement of the spine and/or sacroiliac joints. It appears in 5% of patients with chronic back pain and has a prevalence of 0.45-1.8%. Within axial SpA two groups can be differentiated: Ankylosing Spondylitis (AS) and non-radiographic axial spondyloarthritis (nr-axSpA) without definite sacroiliitis on X-ray. axSpA may lead to new bone formation, ankylosis and thus irreversible structural damage and functional impairment. Diagnosis of SpA is difficult, particularly in the early phase, since abnormalities in conventional X-ray develop with a latency of several years. Currently, the diagnosis of axSpA requires the identification of either HLA-B27 or of sacroiliitis by imaging processes. HLA-B27 is a quite sensitive but unspecific marker, since 5-6% of the Caucasian population carries the HLA-B27 antigen. Conventional X-ray may or may not reveal ankylosis of the sacroiliac joints. Magnetic resonance imaging (MRI) of the sacroiliac joints is meanwhile being considered as a gold standard in the diagnostics of SpA. MRI findings were described to be the most sensitive and specific for sacroiliitis. There is a considerable delay of 7-10 years between the onset of inflammatory back pain and the diagnosis of axial SpA. To prevent destructive effects of the disease, early diagnosis and intervention in patients with SpA is important. But often, efficient therapies with non-steroidal anti-inflammatory drugs (NSAIDs) or with TNF inhibitors are initiated too late. The new marker might help to facilitate diagnosis and to start therapy at an earlier stage of the disease.



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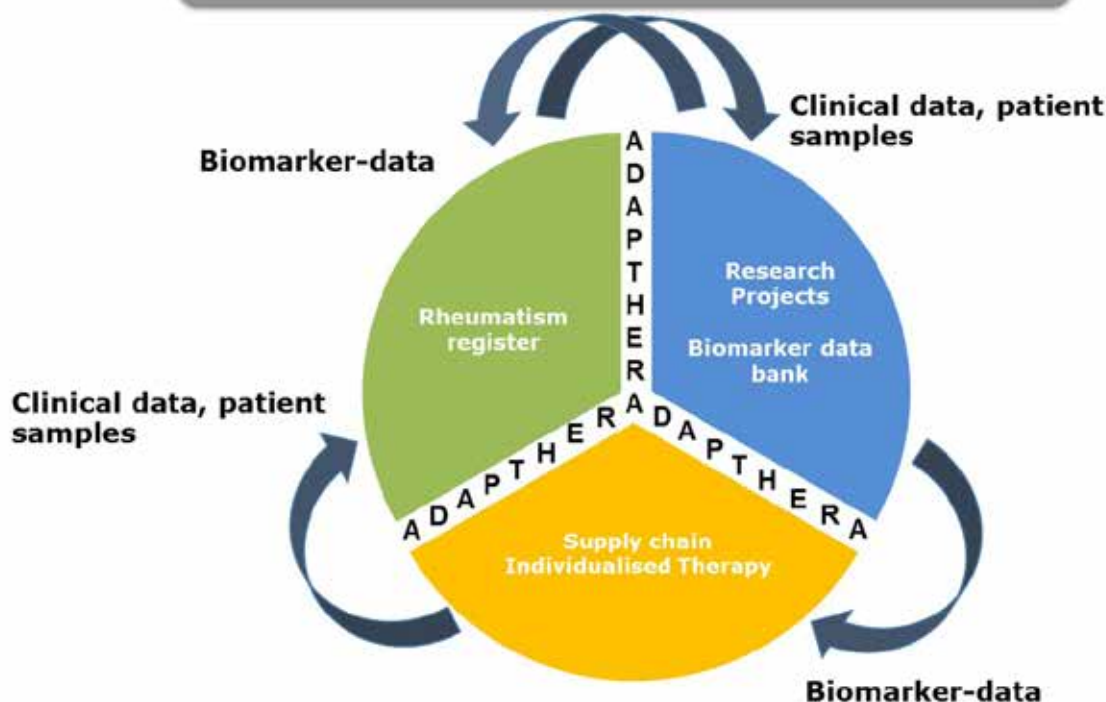
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Research topic: Celiac disease

Celiac disease (CD) is an HLA-dependent disease that is characterised by atrophy of the small intestinal villi leading to a so-called flat mucosa. It is caused by a pathological intolerance to gliadin, the alcohol-soluble fraction of gluten in wheat, rye and barley. Diagnosis of celiac disease is made by small intestinal biopsy (demonstrating flat mucosa) supported by serological markers. Antibodies against gliadin and anti-endomysium antibodies (EMA) are of major significance. They are detected so far by indirect immunofluorescence, which is restricted to subclass IgA only. The identification of tissue transglutaminase (tTg) as the major target antigen of EMA provided the opportunity of a more easy and reliable diagnosis of celiac disease.

Transglutaminases are common enzymes in different organisms and responsible for a broad range of processes. Due to their ability to cross-link proteins they are of broad interest in science and have found various applications. In the food industry, microbial transglutaminase (mTg) is used to modulate texture and improve the properties of food products. In contrast to tTg, mTg is a calcium independent transglutaminase and is less substrate specific. Due to their common enzymatic function, the question arose whether complexes of mTg formed by transamidation reactions could be a relevant health risk for celiac patients. This is all the more important as the use of mTg in food needs not be in-

ADAPThERA – The Rheumatism-Network 1 Vision – 3 Projects



licated on the label. Therefore, the antigenicity of mTg-Gliadin complexes (mTg neo-epitopes) was tested in an ELISA format and compared to results obtained by detection of autoantibodies against the tTg complexes (tTg neo-epitopes) and tTg. Evidence for a common epitope is provided by a competition assay wherein the detection of autoantibodies against tTg neo-epitopes can be impaired by the presence of mTg neo-epitopes. Moreover, the structure of both complexes was compared. Despite the absence of sequence homology and 3D congruence between mTg and tTg, the mTg neo-epitopes and tTg neo-epitopes were found to be similar in structure.

Further studies are necessary to prove antigenic potential of mTg neo-epitopes.



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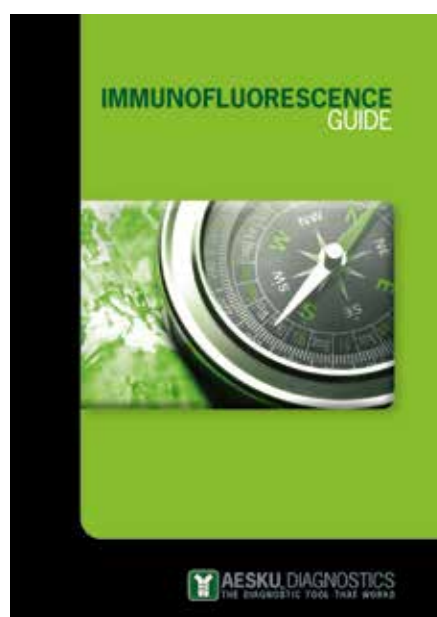
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| A D A P T H E R A | R&D | <p>Enabling of research projects on new biomarkers that will improve diagnostics and the choice of an optimal therapeutical strategy for any individual</p> <p>Organisation of a "RheumaRegister" that collects comprehensive data and samples of RA patients, enabling further research projects for a better knowledge of RA</p> |
| | Patient care | <p>Organisation of a unique medical aid network to raise awareness in general practitioners of the indications of RA</p> <p>To ensure the referral to a specialist within two weeks, assuring minimal time from the first symptom to a clear diagnosis and the start of therapy</p> |
| | Support | <p>ADAPThera was supported as flagship project of the Initiative Gesundheitswirtschaft ("Healthcare business" initiative) of Rhineland-Palatinate. The initiative is the brainchild of the Rheinland-Pfalz Ministries of Labour, Social Welfare, Health, Family and Women (MASGFF) and Economic Affairs, Transportation, Agriculture and Viticulture (MWVLW).</p> |
| | Participants | <p>AESKU.KIPP Institute & AIRA e.V., ACURA Center of Rheumatology, Rhineland-Palatinate, the University Medical Center of the Johannes Gutenberg University, Mainz, Chugai, Abbott, the Association of SHI Physicians, various healthcare insurance companies and the self-help group Rheumaliga Rhineland-Palatinate and many more...</p> |

IMMUNOFLUORESCENCE GUIDE

This immunofluorescence guide provides lab assistants with a quick overview of the complete range of possible patterns. It helps to identify them easily and leads us through the labyrinth of IFA-testing.

The first part of the guide presents good pictures of ANA patterns with a clear description of the interphase, the metaphase, and the nucleoli, cytoplasm and antigens that occur. In addition to disease association, possible follow-up tests are shown for each pattern. In the second part of the immunofluorescence guide there is a trouble-shooting guide for frequently asked questions: defining common problems, listing possible causes and offering helpful solutions. Thanks to the wipe-clean surface and the convenient pocket format of the guide, it is a perfect companion to daily lab routines and it fits into any labcoat pocket. The IFA guide supports a complete range of CE-marked innovative IFA tests for the diagnosis of autoimmune diseases - the *AESKU SLIDES*® Product Line.

If you are interested or need further information, please contact: promo@aesku.com



AESKU.SCIENCE - IMPRESSUM

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