

Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes

Project: Clinical Pattern Assessment and Sample Acquisition in Rheumatoid Arthritis and Spondyloarthropathies

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Introduction

In NGFN-1, patient acquisition, documentation and sample collection protocols as well as a database of about 500 entries of arthritis patients have been established with corresponding patient samples of DNA, serum, whole blood, blood for purification of subpopulations, synovial tissue and/or synovial fluid. Cell type specific expression patterns in active disease have been defined and long-term studies with defined treatment protocols have been performed for anti-TNF therapy in RA and extended to steroids and MTX. In collaboration with the NFGN bioinformatics platform in Heidelberg (Eils) and the SME oligene GmbH (Berlin, Germany), the database structure has been refined and adapted for integration of core information into the database and exchange platform iChip. In NGFN-2, the clinical database will be expanded to a network of centres beyond the NGFN-1 clinical partners including now also partners from the BMBF funded Competence Network systemic Inflammatory Rheumatic Diseases "Kompetenznetz Rheuma". The major focus in NGFN-2 will be on the identification of disease triggering molecular events in early arthritis, treatment studies for biomarkers of therapeutic responsiveness, and on longitudinal studies for markers of disease activity. To accomplish this task continuous recruitment of defined patients with RA and SpA is required which will be greatly facilitated by access to the inception cohort of spondyloarthropathies (Kompetenznetz Rheuma) with 500 patients with early disease. Collection of DNA, whole blood, serum, synovial fluid and synovial tissue biopsies will provide samples for linkage analyses (Schreiber), virtual diagnostic signatures (Grün, Häupl), diagnostic FACS profiling (Grützka), immunomics (Skinner) and diagnostic tissue analysis (Häupl) as well as functional pathophysiological studies by cell separation and identification of cell type specific signatures (Grützka, Radbruch), proteomics (Thiesen) and complex inflammation pathways in tissues (Häupl). Finally, the network of industrial collaborations will be intensified with ongoing and new clinical treatment studies.

Results/Project Status

Patient recruitment and datamanagement

Coordinated acquisition of blood and tissue samples requires systematic collection of accompanying anamnestic, clinical, laboratory and imaging information. A constantly updated database established for this purpose stores all available information about disease specific and also concomitant diseases and therapies. A brief overview is presented in figure 1.

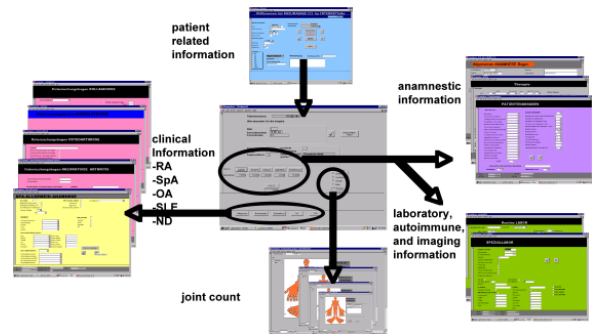


Fig 1: Scheme of the clinical database for storage of patient related, anamnestic, clinical, laboratory and imaging data of rheumatoid arthritis, spondyloarthropathy, osteoarthritis, and systemic lupus erythematosus with pseudonymized encryption of the patient identity.

Gene expression profiles in early disease

Pending the final and detailed analysis of gene expression profiles which clearly differentiate between RA and SpA a genetic profile study in early disease is in preparation. Profiles in early disease in RA and SpA are of great relevance since this may aid in making the diagnosis. To accomplish this an extensive and multi-centre recruitment of early arthritis patients is currently being performed. The "Inception cohort for SpA" initiated in 2000 as a large cohort within the Kompetenznetz Rheuma comprises already 500 patients and serves as a major source of samples and clinical data from SpA patients. More than 200 SpA patients of the cohort are cared for in Berlin allowing easy access to patient's material. About 250 RA patients were recruited in NGFN-1 and more are being enrolled from the large outpatient department at the Charité with more than 6000 visits per year in the Department of Rheumatology. Furthermore, partners of the BMBF funded "Kompetenznetz Rheuma" in the rheumatology outpatient departments in Regensburg and Hannover will contribute. Samples will include serum (autoreactivity screens, marker proteins), whole blood samples and blood samples for cell sorting procedures (RNA and protein expression analysis), and DNA for genotyping of candidate genes. The expression analysis in large cohorts of patients will focus on pivotal findings of expression profiling results from NGFN-1. Sample collection protocols have been exchanged and standardized between all participating centres of the infection and inflammation network to ensure comparability of data in subsequent analyses. Furthermore, the quality management task force of the NGFN will be asked to approve the protocols as suggested standards for operating procedures within the NGFN.

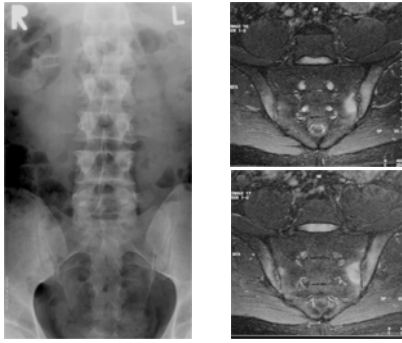


Fig 2: Diagnostic challenge in early ankylosing spondylitis (AS). While the radiographics (left) show no changes of the spine and suspicious changes at most of the sacroiliac joints, the bone edema around both sacroiliac joints as detected by magnetic resonance imaging suggest early AS. Genetic profiles may support the diagnosis.

Biomarkers of therapeutic responsiveness

Therapeutic effectiveness is currently being assessed by follow-up analysis of molecular profiles in patients with defined treatment regimens such as glucocorticoids, methotrexate, leflunomide, and anti-TNF agents in RA and different anti-TNF treatments (infliximab, etanercept, adalimumab) in ankylosing spondylitis. Several anti-TNF trials are currently ongoing in ankylosing spondylitis in Berlin and genetic profiles prior to therapy are already available from 14 of these patients (NGFN1 data). Highly purified cells are currently being obtained from 10 active AS patients entering a new trial with adalimumab. Studies with etanercept in early AS are about to start and will provide important data regarding early disease. In all patients highly purified cells before and 3 months after begin of anti-TNF therapy will be analysed. Comparison of gene expression profiles of these cells will help to identify biomarkers or patterns indicating responsiveness or failure of treatment not only after initiation but hopefully prior to therapy. Moreover, comparison of the profiles induced by different TNF blockers will help to understand the different effect and side effect profiles of these agents. This may provide data to examine the hypothesis that infliximab (and probably adalimumab) may induce apoptosis whereas etanercept does not.

Diagnostic tools and procedures for routine application

To translate the results of the high-throughput screening into applicable procedures and techniques for routine analysis, diagnostic studies are performed. Blood analyses include the testing of monocytes on a customized array platform. Candidate genes which are expressed as cell surface

markers are investigated for their diagnostic value in multi-parameter FACS analyses from blood cells. A bedside test which is currently developed by InVent Diagnostica will be evaluated to screen for immune reactivities against newly identified autoantigens in rheumatoid arthritis. Although analysis of blood samples is more convenient, synovial tissues revealed the most extensive changes in rheumatoid arthritis compared to normal or osteoarthritic controls. Therefore, a minimal invasive arthroscopic technique was established to take biopsies of inflamed tissue under visual control. Currently, a reduced set of less than 50 candidate genes is tested for diagnostic classification based on such biopsies.

Outlook

The clinical project is part of an interacting network collaborating with projects in basic science, research and development. The main task is to provide the different types of patient samples for molecular analyses in various projects of the SIPAGE network and to collect clinical data for combined analysis of clinical and molecular characteristics. Therefore, the long-term strategy is to build an expandable and detailed database and biobanking system for the growing need of well established and characterized cohorts. Including not only typical rheumatological data for these systemic diseases is also important. This became obvious in the recent discussion on the cardiovascular risk associated with long-term administration of cyclooxygenase type 2 inhibitors - compounds, which were frequently subscribed in rheumatic diseases. Here, our strategy to include concomitant diseases and therapies has allowed a first screening of a new cardiovascular risk marker in patients with rheumatic diseases and enabled to compare patients with and without COX-2 inhibitor therapy. In the future, such studies will be increasingly important and demanded to test the numerous candidates evolving from high-throughput screening technologies like microarrays and mass spectrometry. This will promote clinical molecular investigations and our understanding of molecular pathomechanisms to improve diagnostics and therapy of these chronic diseases.

Lit.: 1. Burmester GR, Haupl T. Strategies using functional genomics in rheumatic diseases. *Autoimmun Rev.* 2004;3(7-8):541-9. 2. Haibel H, Rudwaleit M, Braun J, Sieper J. Six months open label trial of leflunomide in active ankylosing spondylitis. *Ann Rheum Dis.* 2005;64(1):124-6. 3. Baraliakos X, Hermann KG, Landewe R, Listing J, Golder W, Brandt J, Rudwaleit M, Bollow M, Sieper J, van der Heijde D, Braun J. Assessment of acute spinal inflammation in patients with ankylosing spondylitis by magnetic resonance imaging (MRI): a comparison between contrast enhanced T1 and short-tau inversion recovery (STIR) sequences. *Ann Rheum Dis.* 2005;64(8):1141-4.