

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

Project: Prospective Evaluation of Clinical Sepsis Phenotypes by Focussed Gene Expression Profiling ("Sepchip") and Association with Genetic Resistance Factors

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Project: Cellular Pathways of Infection and Inflammation. Role of TNF Producing Dendritic Cells in Human Models of Sepsis

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Introduction

Mortality due to sepsis, septic shock and septic organ failure has not improved substantially over the past 20 years and represents a leading cause of mortality in critically ill adults despite best life supportive interventions available. According to a recent study in US hospitals, more patients die from sepsis than from acute heart attack, making sepsis one of the most underestimated killers in developed countries (1, 2). Current techniques of disease monitoring are insufficient to assess the individual risk for sepsis development in critically ill patients and fail to reliably predict the individual clinical course in septic patients regarding rapid deterioration, recovery or persistent septic organ failure. Moreover, conventional patient monitoring is unable to discern injury from repair cascades in the course of critical illness. A major cause of mortality in posttraumatic critically ill patients is a resistance to classical modes of intensive care therapy. The role of the patient's genetic background and predisposition and the extent of the activation of the inflammation/coagulation appear to be crucial. Rationally guided therapeutic intervention strategies in both adults and preterm neonates are, however, critically dependent on early, sensitive and potent surrogate markers for the individual patient's clinical course and probable response to therapies targeting inflammatory/coagulation cascades.

In NGFN-1, comparative transcriptome analysis of blood and bronchoalveolar lavage samples, sequentially obtained from patient cohorts prone to sepsis development (multiple trauma, severe pneumonia, preterm infants) and from volunteers after endotoxin inhalation, was undertaken. Sets of genes were identified, which are differentially regulated in response to microbial challenge and in the course of clinical sepsis. Notably, significant changes in gene expression profiles were found to occur much earlier than clinical deterioration as detected by sepsis scores. In NGFN-2, customized microarrays containing gene sets stepwise derived from these investigations (first and second generation 'sepchips' for adults and preterm neonates) are evaluated prospectively in patient cohorts of critically ill adults and preterm neonates for their potential to provide earlier diagnosis of sepsis and septic organ failure, to contribute to etiologic classification and to allow more precise clinical course or therapy response prediction than conventional tools like scores and serum markers. In addition, this prospective study evaluates whether differential host gene expression profiles assessed by 'sepchip' in highly purified peripheral blood and bronchoalveolar lavage cell populations correlate with distinct patterns of organ failure (kidney, lung, liver, gut, heart) or clinical host defense phenotypes like hyperinflammation or anergy. The cell-specific gene expression profiling approach by focussed microarrays in the prospective multicenter studies of adult and neonate patient cohorts is combined with high throughput SNP screening and extended haplotype mapping of selected candidate genes (PAI-1, protein C,

thrombomodulin, tissue factor, Factor V, TNF, IL-1 β , IL6, MBL, SPA/SPD) to confirm the association of genetic variants identified in NGFN-1 with sepsis development and outcome.

Results/Project Status

The clinical studies on sepsis focus on the genome-wide analysis of the gene expression pattern associated with the severity of the syndrome. Blood samples are taken from 3 large cohorts (hospitalized patients suffering from polytrauma or severe pneumonia and preterm infants below 32 weeks gestational age) and microarray analyses are carried out. The patients' clinical data, findings and laboratory parameters are stored online and correlated with the gene expression data.

In polytrauma patients, the gene expression analyses have already revealed a very clear distinction between the gene expression patterns of septic and non-septic patients at time point zero, admission to the ICU. In comparison to non-septic patients, in septic patients genes involved in the inflammatory response are significantly upregulated at time point zero. Analyses in preterm infants show a clear distinction between the gene expression patterns of infants suffering from congenital sepsis in comparison to those of infants without infection and thus would make the detection of a fetal inflammatory response (FIRS) at transcription level possible already at birth. In addition, a variant of the SFTPD-gene was detected, which was correlated with a low surfactant protein D (SP-D)-serum level, thus affecting lung maturity and resistance against pulmonary infection (3).



Fig 1: In Germany, severe sepsis has an annual incidence of approx. 200,000 cases.

Outlook

The next step in the analysis will be the correlation of gene expression profiles with clinical phenotypes. Secondly, the prediction analysis will follow. The ultimate goal of the study is the development of a focussed 'sepchip' to provide the

earliest possible diagnosis and treatment of the syndrome

Lit.: 1. Rangel-Frausto. The epidemiology of bacterial sepsis. Infect Dis Clin North Am. 1999 Jun;13(2):299-312 2. Martin et al. The epidemiology of sepsis in the United States from 1989 through 2000. N Engl J Med. 2003 Apr 17; 348(16): 1546-54. 3. Heidinger et al. Polymorphisms in the human surfactant protein-D (SFTPD) gene: strong evidence that serum levels of surfactant protein-D (SP-D) are genetically influenced. Immunogenetics. 2005 Apr;57(1-2):1-7.