European CSF Symposium

on

Laboratory Diagnosis of Human Brain Injury and Inflammation: New and Old Markers in Cerebrospinal Fluid (CSF) and Blood

September 29 and 30, 2000
Marburg an der Lahn

Symposium Abstracts

Med. Zentrum für Nervenheilkunde der Universität
Funktionsbereich Neurochemie - Klinisch-chemisches Labor
Rudolf-Bultmann-Straße 8
Lecture Hall

Organization and Co-Editor:
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Preface

The CSF (Cerebrospinal Fluid) Symposium in Marburg, recurring every 5 years, has become a tradition. In September 2000, on behalf of the 30th anniversary of the clinical chemical laboratory in the Centre for Nervous Diseases, the CSF Symposium was held again with invited European scientists.
Professor R. Moosdorf, member of the board of directors of the University Clinic of Marburg, gave the welcome address mentioning the achievements of the laboratory in providing a growing spectrum of routine analyses: less than 100,000 per year in the seventies and almost 600,000 per year in the nineties, about 50,000 CSF analyses included. He also appreciated the research activities in the laboratory on CSF, neurochemical and biochemical fields.
The CSF Symposium started with the best wishes of Mrs. Minister Ruth Wagner from the Ministry of Science and Art in Wiesbaden, sent by a letter.
The compact CSF Symposium had two main topics:
Topic I: Human brain injury markers;
Topic II: Humoral and cellular inflammation markers of the central nervous system (CNS).
In detail, scientists from the United Kingdom, Sweden, Denmark, Belgium, the Netherlands, Poland, Czech Republic, Austria, Italy and Germany, presented 24 oral review contributions and 7 posters dealing with
- humoral and cellular markers of human brain injury, e.g. Alzheimer's disease and Creutzfeld-Jakob disease in relation to other inflammatory or destructive CNS disease processes;
- humoral and cellular process markers of CNS inflammation, e.g. multiple sclerosis, in relation to other CNS inflammations.

There was time enough to exchange knowledge among the scientists, (many of them are leaders in this field of research), and to discuss how to discriminate these CNS diseases with markers in CSF and/or blood with relevant methods for the benefit of the patients.
A night reception in the Fürstensaal of the Marburg castle further highlighted the Symposium.
I would like to express my gratitude to the sponsors and - last but not least - to the co-workers who contributed substantially to the smooth running of the Symposium.

October, 2000 *T. O. Kleine*

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Abstracts

Topic I
Human Brain Injury Markers

Searching for Needles in Haystacks - the Genetics of Common Neurologic Diseases

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Recent years have witnessed considerable advances in our understanding of monogenic neurodegenerative diseases, such as hereditary motor sensory neuropathy and *Huntington's Chorea*. Progress has been slower in the genetic dissection of other more common neurological diseases with a complex mode of inheritance. The identification of relevant genes in some, such as *Alzheimer's disease* or *Parkinson's Diseases*, has been facilitated by characteristic pathological findings and autosomal dominant inheritance in a proportion of early onset families.

Attempts to identify relevant genes for *multiple sclerosis* have highlighted the role of the major histocompatibility complex, but so far failed to unequivocally implicate other immunologic or structural candidate genes. Six linkage based whole genomes have been completed in *multiple sclerosis* and several regions of interest have been identified. As technology and progress in the human genome project advance, it has become clear that future studies of common neurological diseases will depend critically on the availability of large sample sizes and will have to address issues of disease heterogeneity.

Keywords: Human Genetics; Neurologic Diseases, Mutiple Sclerosis.

The Relevance of CSF Proteins in the Diagnosis of *Dementia*

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The three main determinants of levels of brain proteins found in body fluids in *dementia* or other destructive lesions of the central nervous system (CNS) parenchyma are:

1. The mass effect which relates to the total volume of tissue, which is seen to the greatest degree in *Creutzfeldt-Jakob* disease and to a lesser degree in variant CJD.
2. The time effect due to the longitudinal progression of, say, *stroke* – with the inner area of complete neuronal loss surrounded by an outer penumbra due to either a spreading depression from excitatory neurotransmitters such as glutamate and/or cerebral oedema.
3. The pathological effect due to the release of different proteins from various different cell types including the astrocyte (acutely, S-100 and chronically, GFAP) and the neurone (with 14-3-3 and/or the tau protein).

It is important to compare the sensitivity and the specificity for various diseases, looking for any additive effects which may improve diagnostic specificity. One would therefore compare the Boolean "and" with the "or", looking for an increase in the specificity and hopefully not too much loss in sensitivity (greater than the background noise of about ±5%). In the case of *Creutzfeldt-Jakob* disease we have found that specificity increased from 50% to 100% whilst the sensitivity dropped from 93% to 76%, but the overall efficiency went from 76% to 86% in comparisons of the levels of S-100 plus tau protein.

Keywords: *Dementia*; Brain Proteins; Cerebrospinal Fluid.

CSF Markers for Pathogenic Processes in Alzheimer's Disease: Diagnostic Implications and Use in Clinical Neurochemistry

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In view of current (acetylcholine esterase inhibitors) and future (e.g. γ-secretase inhibitors) therapeutic compounds for treatment of *Alzheimer's disease* (AD), development and evaluation of cerebrospinal fluid (CSF) biomarkers for AD has become a rapidly growing research field. Diagnostic biomarkers for AD would be especially valuable as aids in the diagnosis early in the course of the disease, when correct diagnosis is difficult, and when therapeutic compounds have the greatest potential of being effective. CSF biomarkers are reviewed for AD, with emphasis on their role in the clinical diagnosis.

Today, two biochemical markers, CSF-tau and CSF-Aβ42, perform satisfactory enough to have a role in the clinical work-up of patients *dementia*, if used together with the cumulative information from clinical information and brain-imaging techniques. These markers are especially useful to discriminate early or incipient AD from age-associated memory impairment, depression, and some secondary *dementias*.

Keywords: Alzheimer's Disease (AD); β-Amyloid (Aβ); tau; Phosphorylated tau; Biochemical Markers; Cerebrospinal Fluid (CSF); Diagnosis.

The CSF tau and β-Amyloid (1-42) in the Diagnosis of Alzheimer's Disease

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Aim: We evaluated the diagnostic validity of the combined measure of CSF tau and β-Amyloid 1-42 (Aβ42) in *Alzheimer's disease* (AD) in a clinical setting, with special emphasis on the incipient, predemential AD.

Background: The levels of the tau protein and Aβ42 were found abnormally high and respectively abnormally low in the CSF of AD patients, in several studies since the early '90 [1, 2]. In terms of AD's diagnostic validity, each of the two measures taken alone showed moderate sensitivity, with a tendency to overlap with normal aged and non AD *dementias*. Recently, the combined measure of CSF Aβ42 and tau was shown to meet the requirements for clinical use in discriminating AD from normal aging and specific neurological disorders [3]. The question is how early in the AD development the alteration of each marker occur. Two recent studies seemed to show that both are indeed pathological at the time of the first clinical manifestation of some cognitive impairment [4, 5].

Study design: 129 subjects evaluated in our department, were included in this study. 35 were young controls (33±10 years), 25 aged controls (64±8 years), 46 AD patients (69±8 years) and 23 non AD *dementias* (70±5 years). As there was a slight but significant difference of age between the aged controls and the *dementia* groups, the age was used as covariant in the analysis. Within the AD group, 29 were "probable" AD and 8 were "possible" AD, according to the NINCDS-ADRDA criteria. The remaining 9 patients met at the first examination, which was within the two month of their lumbar puncture, the criteria of "mild cognitive impairment" - MCI [6]. Their initial MMSE was of 26.9±1.5 (24-29). Subsequently, all developed full AD, after 12 month of follow-up. Within the probable AD group, 13 were mild (MMSE = 23±2), 9 moderate MSE = 18±3) and 7 severe AD (MMSE 7±2). All patients were evaluated by a trained neurologist, underwent a thorough neuropsychological testing and had a brain imaging by CT scan of MRI. All controls had peripheral and mechanical neurological troubles. The CSF samples collected by lumbar puncture were analysed with the kits for the Aβ42 and tau from Innogenetics NV (Ghent, Belgium).

Results: No signficant difference was observed between the young and aged controls, for both markers. The tau and Aβ42 were significantly higher and respectively lower in AD patients when compared to the aged controls, consistent with the literature data. The non AD *dementia* occupied an intermediate position, being significantly different from normal aged but showing only a trend to difference compared to the AD group, for both markers. Considering our control values as normal values and using the Z-scores method we found a sensitivity of 89% / specificity of 87%, for the combined "either tau or Aβ42" method, whereas each marker alone had sensitivities of about 70%. We then compared our results with those of the (3) study, using their equation relating the tau and amyloid levels for separating the groups (Aβ = 240 + 1.18 TAU). The results were very similar, with a sensitivity of 89% / specificity of 90%. Incipient AD (MCI) were all pathological for one or other measure, showing 100% sensitivity (7/9 for tau, 6/9 for Aβ42 and 4/9 for both). We found no difference between "probable" and "possible" AD. When we compared the incipient to mild, moderate and severe AD groups, we observed no significant difference, either for tau or for amyloid levels.

Conclusions: The combined CSF tau and Aβ42 measure confirm its utility in the diagnosis of *Alzheimer's disease*, in a clinical setting. The marker alterations are already present in patients with *predemential* "mild cognitive impairment", which will subsequently develop AD. By contrast, this method doesn't seem appropriate to monitor the disease progression.

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Keywords: CSF tau; CSF β-Amyloid(1-42); Alzheimer's Disease; Sensitivity; Specificity.

CSF Total and Phosphorylated tau in Alzheimer's Disease: Perspectives for Early Detection, Differential Diagnosis and Mapping of Disease Progression

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Background: One of the major neuropathological hallmarks of *Alzheimer's disease* (AD) are neurofibrillary tangles composed of paired helical filaments (PHF). The principal protein subunit of PHF is abnormally hyperphosphorylated tau (p-tau). An elevation of the unspecific total tau protein level (t-tau) in the cerebrospinal fluid (CSF) has been reported in AD. However, there is still considerable overlap of values between AD and relevant controls. Promising efforts are under way to establish biological markers with sufficient diagnostic accuracy. A major line is combining tau with other disease related proteins and analysing the more specific p-tau in CSF.

Study design: For t-tau, gp130 and neuronal thread protein (AD7C-NTP) we used commercially available enzyme-linked immunosorbent assays (ELISA) [1-3]. P-tau was measured by a newly developed ELISA specific for tau phosphorylated at threonine 231 [4].

Results: T-tau was increased in AD compared to healthy controls (HC). Based on ROC-analysis, as well as on a previously established cut-off of 260 pg/ml, diagnostic accuracy of t-tau was significantly higher in younger AD subjects (<70y) (1). A stepwise multivariate discriminant analysis showed that t-tau and soluble gp130 maximized separation between AD and HC (2). The combined evaluation of t-tau and AD7C-NTP with discriminant analysis raised specificity, but not sensitivity (3). CSF levels of p-tau were significantly increased in AD compared to HC/other neurological disorders (OND) (sensitivity 100%; specificity 91.7%) (4). A linear decrease was found for p-tau during the course of AD. In addition, p-tau was inversely correlated with the MMSE-score at baseline, accelerating with AD progression. Moreover, the majority of subjects with mild cognitive im-

pairment (MCI) showed p-tau levels above the cutoff that discriminated best between AD and HC.

Conclusion: Diagnostic accuracy could be improved by combining t-tau with age and additional proteins. Further improvement could be achieved measuring CSF p-tau. Preliminary evidence suggests that CSF p-tau may serve as a biomarker in early detection and mapping disease progression in subjects at risk and AD patients.

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Keywords: Alzheimer's Disease; Cerebrospinal Fluid; tau-Protein.

Cerebrospinal Fluid Findings in Amyotrophic Lateral Sclerosis (ALS)

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With a prevalence of 0.9-2.8% (half of that of *multiple sclerosis*) ALS is a much more common disease than generally realized. Loss of motoneurons in the cortex, brainstem and spinal cord causes fasciculations, progressive limb and facial motor weakness with atrophy of voluntary muscles, culminating in respiratory failure and death after 2 - 3 years. The diagnosis of ALS is one of exclusion and no specific laboratory test exists. Biological markers for diagnosis, monitoring of disease progression and evaluation of therapeutic effects are urgently needed. Little work has been carried out on destruction markers in the CSF of this neurodegenerative disease, but neurofilament and transglutaminase have been found elevated.

Major hypotheses for ALS pathogenesis include (1) glutamate excitotoxicity, (2) oxidative stress, (3) viral infection, (4) autoimmune. Indicators for each theory have been detected in the CSF with (1) glutamate elevation, aberrant mRNA for the EAAT2 glutamate transporter, (2) raised Cu/Zn-superoxide dismutase, increased nitrate, free nitrotyrosine and 4- hydroxynenal, (3) enterovirus RNA, (4) expansion of T- cells and antibodies against gangliosides.

Information on routine analysis of CSF in ALS is scarce and not up to modern standards. A retrospective analysis from 1993-1998 discovered 128 (86 men) patients (57±11 years) with ALS. Compared to an age and sex matched control group no differences existed in cell count (7/128 each with max. 8 cells/µl) or quantitative intrathecal IgG synthesis (0/128). The albumin quotient was significantly higher (> 8.0) in ALS (27/128) than in controls (6/129). Surprisingly it was not related to age and the elevation was confined to men in both groups. 11 ALS and 9 control patients displayed mostly weak (2-3) oligoclonal bands (OB). The presence of OB in the CSF of ALS patients seemed ($p = 0.06$) to confer a longer survival time.

Keywords: ALS; CSF; Pathogenesis.

Protein S-100B, Neuronspecific Enolase (NSE), Myelin Basic Protein (MBP) and Glial Fibrillary Acidic Protein (GFAP) in Cerebrospinal Fluid (CSF) and Blood of Neurological Patients

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Increased CSF levels of the brain specific protein markers NSE, S-100B, MBP and GFAP have been observed in acute neurological disorders such as inflammatory diseases of the central nervous system (CNS), cerebrovascular accidents (CVA), head injuries and exacerbating *multiple sclerosis* (MS) and in chronic progressive disorders such as metabolic disorders, tumour cerebri and *Creutzfeldt-Jacob*. Various biological factors such as distance between the affected brain area and the CSF compartment can influence the levels of these proteins in CSF. Also the time of lumbar puncture is important especially in diseases with acute *brain damage*. Measurement of these proteins in CSF might be relevant for assessing severity and activity of the disease process and for monitoring treatment effects.

NSE, S-100B and GFAP can also be found in increased amounts in blood specially in disorders with acute *brain damage* such as *stroke*, head injury and cardiac arrest. Measuring the release of these proteins in blood might be promising for establishing severity of the primary and secondary *brain damage* for prognosis and clinical outcome and for monitoring therapeutical interventions.

Keywords: Neuronspecific Enolase; Myelin Basic Protein; Glial Fibrillary Acidic Protein; Cerebrospinal Fluid; Blood.

Monitoring S-100B, Creatine Kinase (CK) Isoenzymes, and Neuronspecific Enolase (NSE) in Blood Sera and Cerebrospinal Fluid (CSF) of Neurological Patients and Patients with Intracerebral Bleeding

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Objective: S-100B and NSE contents were monitored with CK and CK-MB activities together with other laboratory parameters in blood sera from neurological patients to discriminate *brain damage* from injuries of other tissues.

Study design: In 210 serum samples showing CK activities >150 U/L, respectively, in 15 ventricular drainages and

serum samples pairs from patients with accute intracerebral bleedings, contents of S-100B and NSE (µg/L) were determined with 2 luminometric immunoassays with Liaison^R analyser (Byk-Sangtec Diagnostica). CV of intra- and inter-assay ranged between 1 and <11%.

CK-MB-activity (U/L) was determined with anti-CK-M antibodies, CK-MB mass (µg/L) with an ELISA; activities of aspartate transaminase (AST), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (HBDH) as well as leukocytes, platelets and Na, K, Ca contents were measured with routine analyzers.

Results: Cut offs of injury parameters were set up

- for *brain damage:* CK-MB >6% of total CK activity, CK-MB mass <5 µg/L, NSE >12.5 µg/L, S-100B >0.15 µg/L;
- for *myocardial injury :* CK-MB >6%, CK-MB mass >5 µg/L, elevated AST, LDH and HBDH activities;
- for injury of other tissues: CK-MB <6%, CK-MB mass <5 µg/L, elevated AST, LDH and HBDH activities.

Some patients did not show the parameters of *brain damage*, but elevated S-100B contents correlating positively with CK-MB and leukocyte counts, weakly with NSE contents, and negatively with Na, K, and Ca contents (impairing cell mebranes e.g. from adipocytes).

NSE contents correlated positively with CK-MB and other parameters of myocardial injury (indicating indirectly brain hypoxia); but CK-MB mass did not correlate with S-100B and NSE contents in blood serum.

Evaluating low NSE and S-100B contents in serum (x) from their high ventricular CSF ones (y), significant correlations were only found for NSE (r=0.688) when assuming equilibrium conditions in blood; for S-100B (r=0.980) when considering half life periods of 60 min in blood.

Conclusions: Low NSE and S-100B contents in serum arise from high ones in CSF (released by *brain damage*), when 0.4 ml CSF/min equilibrate with 3000 ml blood plasma (half life 1 min). Only elevated S-100B concentrations in serum may arise from other injured cells containing high S-100B contents, e.g. adipocytes and chondrocytes [1], which may be injured with acute care and stroke patients (intubated) or patients with violent status epilepticus.

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Keywords: Brain damage; S-100B; Neuron Specific Enolase (NSE); Blood Serum; Cerebrospinal Fluid; Adipose Tissue.

Topic II
Humoral and Cellular Inflammation Markers I

Intrathecal Pro- and Anti-inflammatory Cytokines in Dementias

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The knowledge regarding putative inflammatory component(s) participating in *Alzheimer disease* (AD) and vascular *dementia* (VAD) is scarce.

The aim of this study was to investigate the intrathecal pattern of cytokine release, by measuring the cerebrospinal fluid (CSF) levels of interleukin (IL)-1ß, IL-1ra, IL-6, tumour necrosis factor (TNF)-α and -ß in patients with AD and VAD as well as to assess the extent of TNF-α and TNF-ß gene polymorphism in these conditions. The CSF levels of above cytokines were related to cerebral neuronal damage, apoptosis, and to clinical variables of *dementia*.

Patients with both AD and VAD displayed significantly higher CSF levels of TNF-α but not of TNF-ß compared to controls. In addition, patients with AD showed significant negative correlations between the intrathecal levels of TNF-α and the levels of Fas/APO-1, a measure of apoptosis, as well as the levels of tau protein, a measure of neuronal damage. The levels of these cytokines did not differ significantly in patients displaying different alleles of TNF gene. CSF levels of the other two proinflammatory cytokines studied, IL-Iß and IL-6 were not significantly altered in any of the *dementia* groups whereas the CSF levels of the anti-inflammatory cytokine IL-1ra were significantly decreased compared to controls.

Our study demonstrates an intrathecal production of TNF-α in patients with *dementias*, suggesting that this cytokine may have a neuroprotective role in these neurodegenerative conditions. In addition, our results indicate that increased intrathecal production of TNF-α in AD is preferentially controlled by environmental stimuli rather than by genetic make-up.

Keywords: Cytokines; Gene Polymorphism; Dementia.

Activation of Alternative (APW) and Classical (CPW) Complement Pathways and Proteolytic Activity and in Cerebrospinal Fluid (CSF) and Blood from Patients with Inflammatory Brain Disorders

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Objective: The effect of inflammatory brain disorders on activation of alternative (APW) and classical (CPW) complement pathways was studied here with respect to different proteolytic activities in CSF.

Study design: In 460 EDTA-CSF and EDTA-plasma samples activation of CPW (kU/L) and APW (kU/L) were measured by quantifying the C1rsC1 Ina complex with anti-C1 Inactivator antibody (fixed), anti-C1s and peroxidase (P0) labelled anti-IgG, respectively, the C3bBbP complex with anti-properdin (fixed) followed by biotinylated anti-C3c streptavidin-PO [1]. C3a-desArg (µg/L) was determined using anti-C3a-desArg neodeterminant (fixed) and PO labelled anti-C3a [2]. Proteolytic activity (µmol/24h, 37°C) was measured in CSF and plasma samples using casein, resorufin-labelled, and inhibitors (Roche Diagnostics) [3].

Results: In CSF, C1rsC1 Ina complex correlated with granulocyte and monocyte counts, total protein and L-lactate contents (similarly as C3a-desArg content); C3bBbP complex did with total protein and L-lactate contents only. Activation appears not to be triggered by proteolytic activity in CSF, although C3a-desArg content correlated weakly with native and Ca^{2+} activated protease or elastase activities indicating direct cleavage of C3.

Conclusions: Although with acute bacterial and abacterial (viral) meningitis both pathways were activated in blood to a higher extent than in CSF, indications for direct APW and CPW activations in CSF including cell lysis were presented thus implying a bad outcome of those patients whose nerve and glia cells have been attacked by APW and CPW.

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Keywords: Complement Activation; Cerebrospinal Fluid; Brain Inflammatory Disorders.

Complete Proteinogram of Cerebrospinal Fluid and its Contribution to the Diagnostics of Inflammatory and Autoimmune Diseases of Central Nervous System (CNS)

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Nowadays, complete evaluation of cerebrospinal fluid proteinogram represents a routine request of a clinician in analysis of CSF in Czech Republic. It comprises measurement of concentrations of acute phase reactants (CRP, orosomucoid, haptoglobin, transferrin, prealbumin), immunoglobulins (IgA, IgG, IgM), compressive markers (albumin, fibrinogen), markers of tissue destruction (Apo A-I, Apo B), components of complement (C3, C4) and proteinase inhibitors (antithrombin III, alpha$_1$- antitrypsin).

So, 16 CSF proteins of precisely verified clinical relevance became routine parameters for assessment of the functional state of blood-CSF barrier, presence of intrathecal synthesis of immunoglobulins, inflammatory changes and verification of tissue destruction. Evidence of these clinically relevant and independent parameters enables to detect the presence of autoimmune and neuroinfective diseases of CNS even in clinical cases where basic routine CSF parameters do not express relevant changes or they are only of bordering character.

And so, clinically typical and the most significant abnormalities in the CSF proteinogram represent themselves a new acces to a contemporary CSF analysis. In spite of the fact that assessment of CSF proteins and their analysis is quite a difficult field of laboratory medicine, it is now routinely requested and routinely done in our country.

Keywords: Cerebrospinal Fluid Proteins; Autoimmune Diseases; Inflammatory Diseases of CNS.

Clinical Relevance of PCR and Antigen-Driven Immunoblots for the Diagnosis of Neurological Infectious Diseases

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Objective: With the advent of the PCR technique, examination of the CSF has become the single most important laboratory test for diagnosis of many infectious diseases of the CNS. This is especially true for viral infections due to herpes viruses. However, in some cases, as in neuroborreliosis and neurosyphilis, the detection of an intrathecal synthesis of antibodies still remains the most sensitive test for diagnosis of the causal infectious agent. A combined approach, that is the detection of infectious genomes by PCR on one hand, the search for an intrathecal synthesis of specific antibodies on the other, could be the most reliable way to estab-

lish a definite diagnosis. The detection of an intrathecal synthesis of antibodies can be performed either by a quantitative ELISA technique and calculation of an antibody index, or by an antigen-driven immunoblotting technique in order to detect CSF-specific oligoclonal antibodies.

A difference in the antibody pattern between CSF and serum indicates an intrathecal synthesis of antibodies.

Study design: We used nested PCR, and multiplex PCR for herpes simplex virus 1 and 2. The primers were selected within the glycoprotein D sequence of HSV 1; within the glycoprotein G sequence of HSV 2; within the XbaI M region of VZV; within the immediate early gene of CMV; within the gene encoding for large and small T antigens of JC virus and within the gene coding for protein MBP 64 of Mycobacterium tuberculosis.

Native CSF samples are directly injected into the reaction tube, except for Mycobacterium tuberculosis DNA first extracted by phenol/chloroform precipitation.

The expected PCR product size is 138 bp for HSV 1; 101 bp for HSV 2; 266 bp for VZV; 146 bp for CMV; 173 bp for JC virus, and 200 bp for Mycobacterium tuberculosis.

Results: We analyzed 28 patients with herpetic meningitis (N=4, all type 2) and herpetic encephalitis (N=24, all but one type 1); 1 patient with an HSV-2 induced recurrent meningitis; 18 patients with varicella zoster-induced neurological complications; 2 with a suspected zona sine herpete; 4 with CMV encephalitis; 8 with progressive multifocal leukoencephalopathy; and 12 with tuberculous meningitis.

Conclusions: Longitudinal studies of a large number of patients are necessary to establish typical profiles of PCR positivity and of intrathecal antibody synthesis in such infections. These studies are also necessary to assess the impact of treatments. The (un)ability to mount an antibody response in immunodeficiency states must be reevaluated as an intrathecal synthesis of antibodies occurs even in such states. Brain biopsy may be avoided in many cases by these new CSF diagnostic methods.

Keywords: Polymerase Chain Reaction; Intrathecal Immunity; Infections of the Nervous System; Herpes Simplex; CMV; VZV; JC Virus; Mycobacterium Tuberculosis.

Old and New Inflammation Markers in Cerebrospinal Fluid (CSF) and Blood

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Objective: Old inflammation markers (leukocyte counts, total protein, L-lactate) and new ones (TNF-α, IL-1ß, IL-6, IL-8, lipopolysaccharide binding protein (LBP)) were compared with inflammatory brain disorders to find out relevant laboratory constellations.

Study design: In 100 CSF and serum samples from acute bacterial and abacterial meningitis cases, contents of TNF-α, the interleukins (ILs) (ng/L) and LBP (µg/L) were determined with solid phase sandwich chemoluminescence immunoassays using the Immulite System DPC. CV of intraassay and interassay precision ranged between 3 and <11%. Contents of old markers were measured by routine procedures.

Results: In CSF, TNF-α, IL-1ß, IL-6, IL-8, and LBP correlated mostly with granulocyte counts, total protein and L-lactate contents. As IL and TNF contents were strongly lower in serum compared to CSF, a transfer from blood into CSF can be exluded with different inflammatory brain disorders; rather a lower turnover of TNF and IL pools in CSF obviously produces the high CSF values. However, serum LBP content was strongly higher than in CSF where it was below the dection limit as it was found with controls.

During courses of bacterial meningitis contents of IL-1ß, IL-6, IL-8, TNF-α decreased in CSF; the pattern changed with viral meningitis and encephalitis; thus indicating different time courses of their release from CSF and brain cells by the agents. LBP, an acute phase hepatic glycoprotein, showed elevated CSF levels depending upon the degree of barrier impairment evaluated by total CSF protein contents.

Conclusions: Comparing old with new inflammation markers, 3.5 mmol/L L-lactate content, cut off for discrimination between acute bacterial and abacterial meningitis [1] correlated with increased IL-1ß > TNF-α > IL-6 > IL-8 contents in CSF indicating L-lactate to be a still relevant inflammation marker, easy and quick to determine. Similary, total CSF protein, indicator of barrier impairment, correlated with TNF-α and ILs contents in CSF of inflammatory disorders. LBP appears to act on the LPS-dependent inflammatory cascade in CSF only when its content becomes substantial with barrier impairments.

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Keywords: Cerebrospinal Fluid; TNF-α, IL-1ß, IL-6, IL-8; Lipopolysaccharide Binding Protein; Leukocyte Counts; Total Protein; L-Lactate.

Topic III
Humoral and Cellular Inflammation Markers II

Leukocyte Counts in Cerebrospinal Fluid (CSF) Determined with the Hematology Analyzer Cell Dyn 4000

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Introduction: In most laboratories counting of leukocytes in CSF is still performed microscopically, caused by unsatisfying evaluation results of several types of automated cell counters especially in the lower range of cell counts. But the microscopic method is cumbersome and associated with high variation coefficients depending on the qualification of the staff. We evaluated the Cell Dyn 4000 (Abbott Diagnostics), in which the multiangle polarisation scatter separation

technology (MAPSS) is used in the optical channel to count and differentiate leukocytes.

Study design: 280 samples were examined for leukocytes in a pair comparison on the CD4000 and with the *Fuchs-Rosenthal*-chamber. Additionally, an artificial CSF (preparation of lymphocytes and monocytes in PBS) was used to check the variation coefficients between 10 and 1000 cells/µl.

Results: With the artificial CSF preparation CVs were determined between 25% (11 cells/µl) and 2.6% (1000 cells/µl). Assuming that at cell counts >1000/µl the automated counting procedure doubtless exceeds the microscopic chamber counting, in the statistical analysis only samples were included with counts between 0-1000/µl with the manual method (n=260). In the total range 0-1000/µl a good correlation was found (r=0.90). A detailed analysis of the critical range between 0-100/µl provided the same good result. Prerequisite for this agreement is the obligatory measurement of the blank before analysis of the CSF sample. This procedure leads to a cleaning of the CD4000 avoiding cell contamination from previous samples.

Conclusion: In our hands the automated determination of leukocytes counts in CSF samples with the CD4000 is a reliable alternative to the microscopic chamber counting, whereas the determination of the leukocyte subpopulation needs further evaluation. For differential diagnosis of acute viral or bacterial meningitis the automatically determined subpopulations are sufficient, but for detection of plasma or tumor cells the microscopic examination is necessary.

Keywords: Leukocyte Count; CSF; CellDyn4000.

Transport of Lymphocyte Subsets from Blood into Cerebrospinal Fluid (CSF) Studied with Flow Cytometry

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Objective: Transport of lymphocyte subsets from blood into CSF was studied with flow cytometry to reveal immune surveillance of human central nervous system (CNS) under normal and diseased conditions through different CNS barriers.

Study design: Flow cytometry was adapted to low white cell counts in CSF, e.g. in lumbar, cisternal, ventricular CSF, which was collected from controls and patients with various CNS inflammation disorders. Venous EDTA-blood was collected simultaneously. To analyse lymphocytes and 9 subsets, flow cytometry was performed with 2 fluorescein isothiocyanate- (FITC)-conjugated and 6 phycoerythrin- (PE)-conjugated monoclonal antibody reagents using LDS-751 to restrict the collected data to nucleated cells only [1]. Adaptation proved to be sufficiently precise, sensitive and accurate yielding CVs of imprecision between 2 and <29%.

Results: CNS barrier effect on the subsets was revealed by blood/CSF ratios of the control group: ratios of subsets in lumbar.CSF increased in the order: $CD8^+4^+ << CD3^+4^+ \leq CD3^+$ HLA-$DR^- < CD3^+8^+ < CD3^+$ HLA-$DR^+ < CD3^+16^+56^+ < CD16^+56^+3^- \leq CD8^+3^- << CD19^+3^-$ subsets indicating a facilitated transfer of immature T cells ($CD4^+8^+$) through the barriers. Transfer of activated T cells ($CD3^+$ HLA-DR^+; $CD3^+69^+$) appeared to be hindered to some extent; transfer of NK cells, NK subset $CD8^+3^-$ and of B cells was barred as revealed with Spearman correlation to the blood/CSF ratio of total $CD3^+$ cells.

Blood/CSF ratios of lymphocyte subsets were altered in CSF from patients suffering from various CNS disorders indicating different leakage effects of the barriers which, however, preserve their selective effects on lymphocyte subsets found with controls. CNS barriers were almost abolished with acute meningitis pointing to a non-selective lymphocyte transfer.

Conclusions: Immune surveillance of human CNS takes place through barriers indicating non-selective and selective lymphocyte transfers. With cases of neuroimmunologic inflammations only selective transfer of lymphocyte subsets was found with a similar pattern as detected with controls. Transfer of $CD8^+4^+$ T cells appears to be facilitated under normal and diseased conditions pointing to importance of the immature T subset to induce immune responses in human CNS.

Reference

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Keywords: Flow Cytometry; Cerebrospinal Fluid (CSF); Lymphocyte Subsets; Blood-Brain-Barrier; CNS Inflammations.

Chitotriosidase Activity in Cerebrospinal Fluid as a Marker of Inflammatory Processes in Neurological Diseases

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Objective: Activated macrophages contribute to tissue damage in the central nervous system. The aim of the presented work was the application of cerebrospinal fluid (CSF) chitotriosidase activity as diagnostic tool, namely as a marker of activated macrophages.

Study design: In our study we compared chitotriosidase activity in CSF in different neurological diseases: *multiple sclerosis* [MS; (n=39)], other acute inflammatory neurological diseases [OIND-1; (n=13)] and other chronic inflammatory neurological diseases [OIND-2; (n=18)], ischemic *stroke* [*Stroke*; (n=34)] and other non-inflammmatory neurological diseases [ONIND; (n=52)]. Chitotriosidase activity was measured fluorimetrically. We calculated index: the CSF/serum chitotriosidase activity ratio (CSF chitotriosidase: serum chitotriosidase x 100).

Results: In cases of OIND-1 the enzyme activity was within control range; on the contrary in OIND-2 the enzyme activity was significantly increased when compared either with ONIND or with MS. The MS group was heterogeneous. In 50% of cases the enzyme activity was very high exceeded the median control value and in 41% of cases it

was within control range. In *Stroke* patients the enzyme activity was slightly higher than that in controls, but the serum chitotriosidase activity was increased in this group as well - in all cases index was within control values.

Conclusion: This finding confirms the view of increased macrophage and immune activity in some neurological diseases eg. *multiple sclerosis* and OIND-2. It seems that overproduction of chitotriosidase in *Stroke* patients is a generalized process.

The method is rapid, cheep and needs minute of material. The estimation of chitotriosidase activity can be a very useful diagnostic test for estimation of inflammation and cellular immune activation in central nervous system.

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Keywords: Chitotriosidase Activity; Cerebrospinal Fluid; Multiple Sclerosis; Stroke.

Extending the Role of the Humoral Immune Response by the Study of Free Light Chains

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Free light chains are those which are not bound to heavy chains and occur as a natural part of the inflammatory response when the sequential switching of heavy chain genes takes place initially from IgM and IgD through IgG ultimately to IgA and IgE. The light chains (either κ or λ) continue during the period in which one heavy chain stops synthesis and eventually a second heavy chain begins its synthesis to complement the light chain.

Among the three body fluids which we have collected, CSF is relatively stagnant in that it takes at least 6 hours to renew the fluid within the ventricles, and depending upon the degree of movement (or relative lack thereof) the lumbar fluid is principally shifted about by pressure waves within the torso (hence the recumbent patient would have much more restricted movements).

The 2nd fluid is blood which, given one per second heartbeat, can complete its transit through the brain in well under 10 seconds. Given the other filters along the way, such as the liver, spleen and reticulo-endothelial system (as well as the kidneys), various proteins may have a relatively short half-life in this particular compartment.

The 3rd compartment is the urinary bladder. This can collect fluid for up to 8 hours, during which time it performs a kind of integration due to the filtering effects of the kidneys, which remove proteins from the blood and thus functionally concentrated these proteins in the bladder. We also know that overnight there is a 6-fold increase in the amounts of CSF produced, and these transported proteins will ultimately find their way through the blood into the bladder.

Keywords: Immunoglobulins; Cerebrospinal Fluid; Urine.

Heterogeneous Humoral Immune Responses with Light and Heavy Immunoglobulin (Ig) Chains Revealed by Isoelelctric Focussing (IEF) with Specific Detection in Cerebrospinal Fluid (CSF) and Blood

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Objective: To reveal Ig microheterogeneity in CSF, especially of IgG, denoted as oligoclonal bands (OBs), various antisera directed against different parts of the IgG molecule were used taking the molecular IgG structure as basis for classification of OBs.

Study design: The PhastSystem™ was applied to IEF on in-house-made polyacrylamide microgels with carrier ampholytes (pH 3-10) [1]. 5 CSF/serum sample pairs, the IgG contents of which were diluted to ≥8 mg/L with saline, were applied to the gel using a home-made 4.3 µl sample applicator [1]. Immunofixation was performed using Ig fractions of commercially available anti-human sera such as anti-Fd, anti-Fc, anti-C_H2, anti-IgG-Fab, anti-IgG1, anti-IgG2, anti-IgG3, anti-IgG4, as well as anti-lambda and anti-kappa light chains. OBs were detected with silver stain [1]. The CV of band detection ranged between 4 and 18%.

Results: The high resolution of the applied IEF technique indicates a polyclonal background pattern of Ig bands in CSF and serum samples which differed, because different epitopes were detected by the antibodies against different segments of the gamma chain and because of the higher kappa chain content in CSF. Additional bands on the gamma chain and the light chains only in CSF indicate Obs, and thus a local synthesis of kappa and lambda IgGs in CNS, which are specified as IgG1s, IgG2s, IgG3s, and some IgG4s. Thus, heterogeneous IgG subclasses are distinguished which differ in their pI values at acid, neutral and basic pH ranges.

However, some OBs were only detected on the light chains. Applying antisera against heavy chains of IgA and IgM indicates some additional OBs with anti-IgA only in the acid region.

Conclusions: Our data point to a heterogeneity of B cell clones producing different OB patterns in CNS with different inflammatory disorders. "Free" kappa and lambda chains have to be identified with care because detection of corresponding bands on heavy Ig chains depends upon the capacity of the antibodies applied to detect epitopes.

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Keywords: IgG Heterogeneity; Cerebrospinal Fluid; Isoelelctric Focusing with Immunofixation; Free Light Chains.

Neuropathology of Multiple Sclerosis - New Concepts

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Multiple sclerosis is a chronic inflammatory disease of the central nervous system with profound heterogeneity in clinical course, neuroradiological presentation and response to therapy. The pathological analysis of actively demyelinating *multiple sclerosis* lesions revealed different structural and immunological features suggesting that different pathogenetic mechanisms are involved in lesion formation. Although most lesions share in common a T cell- and macrophage-dominated inflammatory reaction, myelin and oligodendrocytes may be destroyed by distinct immunological mechanisms.

Four fundamentally different patterns of demyelination were found in a large sample of biopsy and autopsy material, defined on the basis of myelin protein loss, the extension of plaques, the patterns of oligodendrocyte destruction, and the immunological evidence of complement activation.

Patterns I and II closely resembled autoimmune encephalomyelitis.

In *pattern II* lesions the pronounced deposition of immunoglobulins and complement at sites of active demyelination suggested an important role of antibodies.

In *pattern I* lesions, the destructive process may be induced mainly by products of activated macrophages, such as tumor necrosis factor-α.

Patterns III and IV lesions were highly suggestive of a primary oligodendrocyte damage, reminiscent virus- or toxin-induced demyelination rather than autoimmunity.

A profound heterogeneity in the immunopathological profiles was found between individual patients, whereas lesions of the same patient showed the same subtype of lesion.

The reasons for the different patterns of demyelination are not clear, and there is no direct evidence for pathogenetic mechanisms. However, this pathogenetic heterogeneity of plaques from different MS patients may have fundamental consequences for the diagnosis and therapy of the disease.

Keywords: Multiple Sclerosis; Pathomechanisms; Patterns I, II, III, IV.

Complement Pathway Activation, Cytokine Levels, and Humoral Immune Response in Cerebrospinal Fluid (CSF) from Multiple Sclerosis (MS) Patients

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Objective: Together with humoral immune response, activation of classical (CPW) and alternative (APW) complement pathways as well as chemokine and cytokine (IL) levels were determined in CSF and blood from MS patients to detect possible MS subgroups.

Study design: In CSF and serum samples from 30 MS patients, contents of TNF-α and 3 ILs (ng/L) were determined with solid phase sandwich chemoluminescence immunoassays using the Immulite System DPC. Activation of CPW (kU/L) and APW (kU/L) were measured by quantifying the C1rsC1 Ina complex and the C3bBbP complex with solid phase sandwich immunoassays as well as C3a-desArg (µg/L). CV of precision ranged between 3 and <18%. Oligoclonal bands (OB) were determined with IEF with immunofixation of IgG bands.

Results: In CSF, TNF-α contents correlated with lymphocyte (r=0.451) and monocyte counts (r=0.504); IL-8 levels did with CSF IgG ones (r=0.668). APW activity correlated with L-lactate content (r=0.535) and C3a-desArg with total protein content (r=0.461).

Conclusions: Evaluating the CSF parameters of 30 MS cases, all showing several OBs in CSF, significant relationships between APW activation and L-lactate production in brain as well as increase of C3a-desArg content and small barrier leakage were found, besides relationships between elevated release of TNF-α and IL-8 from small monocyte or lymphocyte clones, respectively of IgG increase. However, only values of some cases stood out against the majority indicating possible MS subgroups where APW may be activated, increased C3a contents may induce anaphylatoxin activities, and TNF-α and IL-8, released from CSF cells, may induce IgG synthesis. Thus, biochemical data indicate some MS subgroups differing in their pathomechanism.

Keywords: Multiple Sklerosis Subgroups; Complement Activation; Chemokines, Interleukins; Cerebrospinal Fluid.

Chemokines and Matrix Metalloproteinase-9 in Leukocyte Recruitment to the CNS

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Chemokines and matrix metalloproteinases (MMPs) are increasingly recognized as playing key roles in the control of leukocyte migration across the blood-brain barrier in infectious and inflammatory diseases. *Multiple sclerosis* (MS) is currently considered as a T cell-mediated, autoimmune disease of the central nervous system. Some chemokine receptors are expressed by an increased percentage of T cells in the peripheral blood, and the CSF concentration of chemokine ligands for these receptors are increased in MS. Furthermore, there is a marked accumulation of T cells expressing relevant chemokine receptors in CSF.

Chemokines and chemokine receptor-expressing cells are readily deteced in and around active MS plaques. Chemokine receptor expression patterns also appear to reflect disease activity and disease stage in MS. MMPs are constitively expressed or induced by proinflammatory cytokines and chemokines in leukocytes and CNS-resident cells.

Several MMPs are expressed in MS plaques, and the CSF concentration of MMP-9 is increased in MS. MMP-9 may be involved in basement membrane and extracellular matrix proteolysis, and in the degradation of myelin proteins in MS.

The CSF concentration of MMP-9 appears to reflect disease activity in MS, and the CSF concentration of MMP-9 is higher in patients carrying the MS-associated DRB1*1501 HLA type. The expression of these molecules may find use as surrogate markers of disease activity in MS.

Keywords: Chemokines; Matrix Metalloproteinase-9; Leukocyte Recruitment; Multiple Sclerosis.

Blood MMP-9 and sICAM-1 as Markers of rIFN-β Treatment Efficacy in Relapsing – Remitting Multiple Sclerosis

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Intercellular adhesion molecule-1 (ICAM-1) and Matrix Metalloproteinase-9 (MMP-9) facilitate activated leukocyte adhesion and migration trough an altered *blood-brain barrier* (BBB) within the CNS [1]. The ICAM-1 soluble form (sICAM-1) has been demonstrated instead to protect BBB by reducing T-cell traffic [2, 3].

Interferon beta (rIFNß) treatment reduces the frequency of gadolinium-enhancing (Gd+) MRI in relapsing – remitting (RR) *Multiple sclerosis* (MS) [4], suggesting that it may inhibit BBB opening, probably by interfering with adhesion molecule and MMP pathways.

In a first cohort of 36 RR multiple sclerosis demonstrated [5] that serum sICAM-1 (*ELISA*) and MMP-9 (*zymography*) increased ($p<0.05$) and decreased ($p<0.01$), respectively, in the first out of 2 years of rIFNß-1b treatment and these changes paralleled a reduction ($p<0.05$) in the accumulated clinical disability and in the percentage of patients with Gd+MRI scans. sICAM-1 induction and MMP-9 reduction may represent additional anti-inflammatory properties of rIFNß.

In a second cohort of 10 RR MS patients during 6 months of rIFNß-1a treatment we investigated the expression of LFA-1, the counter-receptor for ICAM-1, on activated/memory $CD4^+CD45RO^+$ T-cells. We found that rIFNß-1a treatment *in vivo* increased ($p<0.05$) $CD4^+CD45RO^+$ T-cells and especially those expressing LFA-1^{dim}, whereas *in vitro* increased ($p<0.05$) the $CD4^+CD45RO^+$ expressing LFA-1^{bright} only. The low expression of LFA-1 obtained *in vivo* may be mediated by a contemporary observed increase ($p<0.05$) of sICAM-1 serum levels during treatment.

MMP-9 may be evaluated as total and active endogenous forms. MMP-9 is secreted as 92-kDa pro-enzyme and then differently activated depending on its association with tissue inhibitor of MMP (TIMP) and the nature of activating agent. We preliminary reported on serum evaluation (*MMP-9 activity assay system by Amersham Pharmacia Biotech*) in 10 clinically inactive, 9 clinically active RR MS patients and 4 healthy donors (HD). We found lower ($p<0.05$) serum levels of active endogenous MMP-9 in clinically active patients compared to inactive patients. This, if confirmed in a larger number of patients, may be explained by a local sequestration of the enzyme active form at the site of inflammation (namely the cerebro-vascular endothelium). The serum MMP-9 total form and TIMP-1 in active MS patients resulted to be higher and lower, respectively, ($p<0.05$) compared to inactive. Certainly the evaluation and the comparison of the two forms may provide additional insights into the MMP-9 role in MS.

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Keywords: Intercellular Adhesion Molecule-1; Matrix Metalloproteinase-9; Interferon-ß; Multiple Sclerosis.

Immunological Profile in Multiple Sclerosis Patients During Treatment with Interferon-β-1A

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Interferon β (IFN-β) has shown a beneficial effect in *multiple sclerosis* (MS) by a regulatory effect on immune system. The aim of study was to find out whether INF-β-1a / Avonex™ 30μg i.m. weekly/influences immune profile of peripheral blood (PB) in MS patients.

We have studied 21 out-patients with the relapsing-remitting form of MS: 12 women and 9 men; mean age: 37,2 yr. (95%CI 33,7-40,7); mean duration of disease: 10.9 yr. (95% CI 8,53-13,4); mean value of EDSS: 2,59 (95% CI 2,48-2,71).

Phenotyping analysis of PB leukocytes was carried out using two color flow cytometry. The following antigens were determined: $CD3^+$, $CD4^+$, $CD8^+$, $CD14^+$, $CD19^+$, $CD25^+$, $CD38^+$, $CD86^+$ before INF-β-1a administration, after 3, 6 and 9 months of treatment.

In MS patients were found statistically significant decrease of $CD19^+$ cells (lymphocytes B) after 3 months of INF-β-1a administration and an increase of $CD14^+CD86^+$ cells (monocytes with costimulatory molecules presentation) percentage after 3, 6 and 9 months of INF-β-1a therapy. Unexpected increase of $CD3^+CD25^+$ (activated T cells) percentage that occurred after 6 months of INF-β-1a treatment might be due to initial INF-γ activation.

$CD14^+CD86^+$ cells are involved in Th2 lymphocytes activation which secrete immunosuppressive cytokines that possibly limits damage of myelin sheet by inflammation. Decrease of $CD19^+$ cells that are responsible for production of immunoglobulins directed to myelin components, probably play a role in positive action of INF-β-1a in MS.

Keywords: Multiple Sclerosis; Interferon; Flow Cytometry.

Expression of Cell Surface Bound and Soluble Cellular Adhesion Molecules in CSF and Blood in Multiple Sclerosis: Correlation with Disease Burden and Activity Assessed by Cranial Magnetic Resonance Imaging

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Objective: To assess whether there is any correlation of the expression of cell surface bound and soluble ICAM-1 and -3 (c and sICAM-1 and -3) in human CSF and blood with cranial MRI markers of disease severity and activity in patients with MS. sICAM-1 and sVCAM-1 in CSF and blood have been described to correlate with MRI parameters for disease activity and severity.

Study design: 77 patients with relapsing-remitting MS were included into the study. Blood was taken from all, and CSF samples from 33 of the patients. The relative expression levels of cICAM-1 and -3 on mononuclear cell (MNC) subsets were determined by FACS analysis and the concentration levels of the sICAM-1 and -3 by ELISA.

Results: ICAM-1 expression on T cells in blood correlated negatively with the number and cumulated area of all lesions on MRI scans ($p < 0.05$) both for all patients and for the subgroup of patients with active lesions (n=28), respectively. In CSF there was negative correlation of sICAM-1 and number and area of Gd-enhancing lesions ($p < 0.05$) both for all patients and the subgroup of patients with signs of activity in cranial MRI. In serum concentration levels of sICAM-1 correlated negatively ($p < 0.05$) with the area of active MS lesions only in the patient group with activity signs.

Conclusion: This is the first time to show correlation of the c-ICAM-1 on MNC with signs of disease severity and activity on MRI scans in MS patients. cICAM-1 expression on $CD3^+$ T cells of the peripheral blood might be a distinct marker for subclinical disease severity assessed by MRI.

Keywords: Multiple Sclerosis; ICAM-1 and ICAM-3; MRI.

Posters

CSF Leukocytes and Erythrocytes Counting with Bayer H1e Counter, 8 Years of Experience

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Objective: Cerebrospinal fluid.(CSF) cell counting is traditionally made by microscopy with counting chamber; usually 3 populations are investigated: Granulocytes, lymphocytes, monocytes.

Several attempts have been made to use haematological analysers, but several problems occur: In CSF cell number is very small, in CSF cells are the same as in blood but different matrix gives morphological differences that make identification uncertain.

Bayer H1e is the first of a family of haematological analysers that counts leukocytes, erythrocytes and platelets in whole blood and differentiates leukocytes into 6 populations. White Cell differentiation is made using 2 different lecture channels: specific substrate staining for leukocytic peroxidase and nuclear density. Nuclear density channel divides cells in 2 populations: mononuclear cells, and polymorphonucleated cells. Peroxidase channel utilises 2 parameters: cellular volume, cellular peroxidase activity, and identifies 5 populations.

Results: The lower detection limit of Bayer H1e is 10.000 red cells and 10 leukocytes per µL, so that samples with lower values are not detected; in any case reproducibility is very good, so that values are correct without any bias, apart from approximation. CSF cell identification with Bayer H1 analyser is related to channel analysis. Peroxidase channel is not able to identify neutrophils because they are smaller than in blood. Nuclear density channel is able to differentiate polymorphonuclear cells and mononuclear cells, combination of both channel results gives correct identification.

Conclusions: In our laboratory, CSF cells count with Bayer H1 analyser is a very good supporting instrument, especially to confirm cell counts in pleocytosis, where microscopic cell count becomes difficult. Furthermore, in emergency situations, we can easily and quickly support laboratory diagnosis.

Keywords: CSF; Leukocytes; Cell Counting.

Are Antibodies, Cross Reactive with Copolymer 1, Markers of Neo-Epitopes in CNS Inflammation?

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Objective: Antibodies to Copolymer 1 in untreated patients might be artefacts due to crossreactivity with transient, nat-

urally occurring antigens. These might result from the action of tyrosinase from neutrophils.

The enzyme oxidizes tyrosine side chains in (self-)proteins in a specific way to protein-bound dopa and dopaquinone. The latter are then part of neo-epitopes that give rise to immune reactivity.

Study design: Determination of antibody by ELISA microtiter plates which were coated with Copolymer 1, MBP, MOG and the heat shock protein homologue Crystallin and then incubated with 100 IU/mL mushroom tyrosinase for 1 hour at 37° C.

Results: We show that Copolymer 1 does not compete with native myelin antigens in (polyclonal) immune complexes (5%, 15% and 0% inhibition for MBP, MOG and Crystallin), but strongly inhibits immune reactivity of serum and CSF to neo-epitopes (83%, 54% and 60%, ID50 approximately 10^{-7} M and less).

Antibody to Copolymer 1 is found in concentrations of 7.6 mg/L (IgM) and 7.3 mg/L (IgG) in serum of normal persons (blood donors, amyotrophic lateral sclerosis, non-inflammatory cerebral diseases and RF-negative rheumatoid arthritis).

In inflammatory diseases the concentrations of antibody increase to 30.8, 14.0, 11.5, and 20.6 mg/L IgM and to 18.3, 9.3, 13.6, and 14.8 mg/L IgG in cases of acute cerebral inflammation, neuroborreliosis, *multiple sclerosis* and RF(+) rheumatoid arthritis.

In cases of cerebral ischemia the concentration of antibodies to neo-epitopes rose three- to fourfold within two days and declined after day forty, reaching normal values at day eighty.

Conlusion: The reactivity of Copolymer 1 might be representative for neo-epitopes in general, i.e. in various antigens. For several weeks increased concentrations of antibody to Copolymer 1 might be a marker of an inflammation faded away.

Keywords: Copolymer 1; Protein-Bound Dopa; Neo-Epitopes; Inflammatory Diseases; Ischemia.

Cytological and Immunocytological Diagnostics of Cerebrospinal Fluid (CSF)

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Objective: Cell recovery and results of cell differentiation in CSF depend on the method of cell preparation. Therefore, two common techniques, used for cell analysis in CSF, are evaluated: the Hettich centrifuge technique (TH) and the sedimentation chamber procedure (TS).

Study design: Standard conditions for TH are presented: CSF samples with up to 50 leukocytes per µL (M/L) were precentrifuged at 220xg for 20 min. Cell-free supernatant was removed; cell pellet was resuspended in 0.200 mL CSF per cell preparation and 0.050 mL BSA medium was added (final concentration 40g/L BSA). Transfer of cell suspension into the Hettich cytocentrifuge chamber, 5 min centrifugation at 200xg, removal of 0.200 mL cell-free supernatant; after removal of the cylinder of the chamber 1 min dry centrifugation at 820xg. With cell numbers between 50 and 100 per µl (M/L) CSF a smaller CSF volume is used with precentrifugation, with cell numbers >100 per µl (M/L) it is omitted. For bloody CSF samples (>100 erythrocytes per µL (M/L)) larger cytochambers are used.

Coating of slides: Purified slides were coated with polydimethyldiallylammonium chloride (PDDA, 1 g/L). The efficacy of PDDA slides was tested by an adherence assay using erythrocytes as indicator cells. Efficacy of coating was limited to maximal 3 weeks.

For immunocytochemical analysis of CSF cells the staining equipment "Immunette" (ProMedeus Immun Technique) has been approved. It allows 10 preparations for parallel processing. No cell loss is seen on PDDA-coated slides when conditions of suction, incubation and washing solutions are standardized.

The sedimentation chamber procedure TS according to Sayk 1960, was used with the modification Rostock with 1 mL native CSF and ≈30 min sedimentation time.

Results: Cell recovery by TH is higher (>45%) than with TS (<10%) yielding higher cell numbers for sufficiently statistical evaluation. Values of cell differentiation and normal ranges differ between both techniques because higher proportions of lymphocytes (77-92%) were obtained with TH compared to TS (36-65%) yielding up to 25% of non-differentiated cells.

Usage of PDDA-coated slides increases cell recovery also with the TS (to about 10%); the values of TH preparations are not reached. Detection rates of diagnostic relevant cells are different: Cell structures appear to be more dispersed and cells are larger in TS preparations.

Conclusion: Cytocentrifugation with the Hettich cytocentrifuge (TH) for which standard conditions for CSF are presented, supplies cell-free supernatant and serveral CSF sediments of high cell yield. This is not the case with the sedimentation chamber procedure TS yielding no supernatant and only one CSF sediment showing substantially distored cell distributions.

Keywords: Hettich Cytocentrifugation; Sayk Sedimentation Chamber; Cerebrospinal Fluid; Cell Yields; Immunocytological Diagnostics.

Detection of Asialo-Transferrin (a-Tf) and Quantification of β-Trace Protein (β-TP) in Mixtures of Cerebrospinal Fluid (CSF) and Blood Serum as Models of Rhinorrhea and Otorrhea Diagnosis

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Objective: Sensitive methods are needed to detect traces of CSF with CNS specific constituents in secretions of various origin. ß-trace protein (ß-TP), identified as prostaglandin D synthase, and asialo-Tf (a-TF), respectively, $ß_2$-transferrin ($ß_2$-Tf; "tau-fraction") are recommended as CNS-specific parameters to detect CSF in rhinorrhea or otorrhea.

Study design: 30 different mixtures of a human CSF pool with a human serum pool containing 0.5% to 30% CSF and 0.5 to 59.9 g/L total protein, were analyzed for ß-TP and a-TF to evaluate optimum conditions of CSF detection in secretions. The mixtures were absorbed with cotton patties and gauze swabs (commercially available) and extracted by centrifugation at 2500xg in salivettes (Sarstedt) to evaluate sample collection procedures.

ß-TP was measured with N-latex-ß-TP research assay for Behring nephelometer systems using 0.035 mL sample (dilution 1 : 100) in a total volume of 0.250 mL; 12 min assay time, measuring range 0.25 to 15.8 mg/L, CV of imprecision was 4 to 5% [1].

a-TF and other TF isoforms were separated with isoelectric focusing (IEF) using PhastSystem™ (Pharmacia-LKB), 1 µl sample (iron saturated) on in-house made polyacrylamide microgels, pH 5-6, and immunofixation with anti-TF antibodies [1].

Results: Sample collection with cotton patties and gauze swabs causes some concentration of secretions as well as some binding of ß-TP; their consecutive extraction showed some Tf denaturation even at 4°C.

Cut off value of ß-TP in secretions depends upon ß-TP content in blood serum. Therefore, secretion/serum ratios of ß-TP contents have to be determined; ratios > 2 are indicative of CSF contamination with ≥5% volume CSF as it is confirmed with the a-Tf band IEF assay.

Conclusion: N-latex-ß-TP research assay and a-TF band IEF assay detect CSF contaminations ≥5% (v/v) in model secretions. However, high quantity of sialo-Tf in secretions interferes with the IEF assay when secretion/serum ratios of total Tf contents are >0.1.

Reference

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Keywords: Rhinorrhea and Otorrhea Diagnosis; ß-Trace Protein; Asialo-Transferrin; Model Mixtures of Cerebrospinal Fluid (CSF) and Blood Serum; Cut offs.

Comparison of Two Analytical Systems for the Detection of β_2-Transferrin and ß-Trace Protein in Corresponding Cerebrospinal Fluid (CSF) / Serum Pairs

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Introduction: β_2-transferrin, the asialotrans-ferrin produced by neuraminidase activity in brain, and ß-trace protein, the abundant in the brain synthesized by prostaglandin D synthetase, show both a high CSF specificity. Therefore these proteins are ideal tools for the diagnostics of rhino – and otoliquorrhea.

Study design: Two different assay systems are commercially available: (I) an immunological staining procedure of β_2-transferrin with peroxidase labelled antibodies after electrophoretic separation of the secretion fluids (Sebia GmbH), (II) a nephelometric assay with latex-bound anti ß-trace protein polyclonal antibodies (Dade Behring).

Results: The recovery of β_2- transferrin and ß-trace protein was investigated in an up to 1:128 dilution of a CSF pool. In a second step 20 CSF/ serum pairs were analyzed in parallel with both systems.

Whereas the nephelometric method allows a clear discrimination of ß-trace protein from the background in the 1:128 dilution, with the electrophoretic system a clear identification of β_2-transferrin was only possible in the 1:32 dilution of the same pool. The analysis was further complicated by the overlapping localization of application area of the secretion fluids and the detection point of β_2-transferrin.

In the 20 serum/CSF pairs with the electrophoretic method we detected no β_2-transferrin in serum. In contrast, with the nephelometric sytems ß-trace protein was detectable in serum in concentrations between 0.295 and 1.030 mg/L (median 0.73 mg/L). Concentrations in CSF varied between 11.3 and 38.9 mg/L (median 16.3 mg/L).

Conclusion: In our hands the nephelometric system has two advantages: (I) the detection limit is two dilution steps lower and (II) single determinations are possible. But further investigation of nose and inner ear fluid are required to provide evidence for the missing detection of ß-trace protein in these secretion fluids or in the case of detection of small amounts of ß-trace protein to define cut off values for the safe diagnosis of liquorrhea.

Keywords: ß-Trace Protein; β_2-Transferrin; Liquorrhea.

Cerebrospinal Fluid Proteins as Inflammatory Markers in Patients with Neuroborreliosis and Multiple Sclerosis

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Evaluation of cerebrospinal fluid proteins, involved levels of acute phase reactants (CRP, orosomucoid, haptoglobin, transferrin, prealbumin), immunoglobulins (IgA, IgG, IgM), compressive markers (albumin, fibrinogen), markers of tissue destruction (Apo A-I, A-II, Apo B), components of complement (C3, C4) and proteinase inhibitors (antithrombin III, α_1- antitrypsin) was performed on 62 samples of cerebrospinal fluid (CSF) in patients with neuroborreliosis and on 55 samples of CSF in patients with *multiple sclerosis*.

The most significant abnormalities in the group of patients with *neuroborreliosis* were manifested especially in acute phase proteins and further in C3 complement compound (altogether elevated in 79%). The increase of the level of immunoglobulins was recorded in up to 77% of CSF samples (mostly IgA), intrathecal synthesis according to Reiber´s formula in 53% (most frequent in IgM class).

In samples of cerebrospinal fluid (CSF) from patients with *multiple sclerosis* was observed the elevation of immunoglobulins in 73% with intrathecal synthesis according to Reiber´s formula in 66%. Acute phase reactants showed abnormal levels in 62%.

According to our results, we conclude that specific CSF proteins can serve as very sensitive markers of possible in-

flammatory changes in the compartment of central nervous system.

Keywords: Cerebrospinal Fluid Proteins; Neuroborreliosis; Multiple Sclerosis.

Cytology of Cerebrospinal Fluid (CSF) on CD ROM

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The examination of CSF is considerably important in the diagnosis of neurological, infectious and other diseases. It consists of biochemical and cytological examinations and analyses.

While biochemical assays and examinations of CSF are carried out in field centers, the cytological examination is frequently limited to the determination of cell count only. Although a correct and timely cytological examination of CSF may provide essential diagnostic information, in many centers it is either not carried out at all or is examined only to a limited extent - especially because the professional community of physicians lacks more detailed information in this area. In response to the need for more information, this publication presents not only basic information but also detailed data on cytological examination of CSF.

We believe it is very important to supplement this publication on CD ROM with an overview of preparation and staining techniques and methods, which are suitable for practical use. The pictures in the appendix facilitate orientation in the topics of cytology of CSF, which may be of a rather complex nature. The appendix contains the description of typical elements of CSF that occur under physiological conditions as well as under various pathological situations.

Keywords: Cytological Examination of CSF; CD ROM; Staining Techniques of CSF.