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4. CELLULAR DIAGNOSTICS OF CSF

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4.1 Aim of CSF Diagnostics:

What are the diagnostic questions of the neuropathologist/ neurologist to be answered by investigation of CSF?

· Inflammatory meningitis (bacterial, viral, fungal)

· Neoplastic meningitis

· Autoimmune diseases (Multiple Sclerosis, Guillain Barre….)

· Degenerative diseases (Alzheimer's Disease, Creutzfeld-Jacob Disease)

· Secondary reaction to abscesses

4.2 CSF General Characteristics:

· Colourless fluid, specific gravity (1.004-1.007) slightly greater than water.

· Total volume: 80-150 ml

· normal pressure:

· Compared to blood plasma: less protein, lower pH, lower concentration of glucose, potassium, calcium, bicarbonate and amino acids higher concentration of sodium, chloride, magnesium

Table 1. Comparison of CSF and serum analytes:

	CSF	SERUM
Sodium	135-157mmol/l	135-150 mmol/l
Potassium	2.6-3.7 mmol/l	3.5-5.5 mmol/l
Chloride	116-132 mmol/l	95-110 mmol/l
Glucose	(50-60% of serum) 2.5-4.2 mmol/l	5-8 mmol/l
Lactate	1.2-2.1 mmol/l	0.5-2.2 mmol/l
Protein	200-400 mg/l	60-80g/l
Albumin	-340 mg/l	35-52g/l
IgG	-40 mg/l	7.0-16 g/l
IgA	-6mg/l	0.7-4 g/l
IgM	-1mg/l	0.4-2.3 g/l
Beta 2 microglobuline	-0.9 µg/l	-3mg/l

· Only few cells (12/3 cells or 4cells per µl): lymphocytes, monocytes (mesenchymal)

· CSF is formed by the choroids plexus: 500 ml per day, total volume is replaced 2-3 times per day

4.3 Morphological diagnosis of the CSF:

How to interpret the cell picture?

We do CSF cytology on a cytospun specimen stained according May Giemsa Grünwald.

· Inflammatory meningitis: the first common reaction invasion of the CSF by a microorganism is granulocytic pleocytosis. It mainly consists of neurotrophilic granulocytes and some monocytes (some of them are activated). But in viral infections the cell picture very quickly switches (within some hours) to a monocytic/ lymphocytic pleocytosis. Very similar changes are observed in Lyme disease with CSF involvement. TBC and fungal meningitis reveal a mixed-cell picture composed of granulocytes monocytes and lymphocytes with activation. Cell count is very high in bacterial infections (> 1000-10.000/3 cells). Viral infections resemble lower cell counts ranging from 300-1000/3 cells similar to fungal meningitis, whereas neuroborreliosis very often shows cell counts from 200-500/3cells.

· Neoplastic meningitis: In CSF with carcinomatosis and also lymphoproliferative involvement, clusters of pleomorphic cells can sometimes be observed. The ratio between nucleus and cytoplasm is switched. Cytoplasm can be very dark blue. Some nuclei resemble an increased number of nucleoli. CSF cells show a reactive change. But not all cases are easily to diagnose as neoplastic involvement of the CSF. Carcinoma cells may be rare and not very pleomorphic. Lymphoproliferative CSF involvement sometimes resembles only a small rather monomorphic lymphocytic cell population with some reactive cells added. In such cases morphological diagnosis needs to be completed by some more accurate diagnostic tool.

· Autoimmune diseases: Morphological changes in autoimmune diseases may be rather unspecific. A lympho-monocytic pleocytosis with a maximum of 100/3 cells, activation with activated

lymphocytes and plasma cells is evident, characteristic and macrophages may vary. Oligoclonal bands are an important part of CSF diagnoses in such conditions.

· Degenerative Diseases: The cell picture and cell count in these diseases is not specific. The cell count may be slightly elevated and activation of the CSF cells may be present. As CSF is neighboured to the brain some products of cell degradation will be found -(Tau protein, TNF-alpha, TGF-beta,14-3-3 protein) in CSF.

· Secondary reaction to abscesses: Sometimes a CSF neighboured inflammatory process may cause a reactive granulocytic / monotypic CSF pleocytosis with macrophages. In such cases microbiological examinations will fail to demonstrate an underlying microorganism, although the cell count of CSF may be up to 1000/3 cells.

4.4 FACS investigation of CSF

In our lab we use FACS analysis for its quick and accurate results. For the differentiation of clonal and monoclonal lymphocytic cell populations the usual approach of immunphenotyping by means of fluorescence labelled monoclonal antibodies is used. Thus it is possible to distinguish between inflammatory lymphocytes and lymphoproliferative neoplastic populations. This approach can be used also for the follow up to demonstrate therapy success.

In cases of suspected neoplastic CSF involvement aneuploidy measurement are helpful to evaluate malignant cell populations. For the discrimination between mesenchymal cells and carcinoma cells staining and gaiting with a specific antibody towards cytokeratine that stains for carcinoma cells is helpful.

Both techniques will be presented in detail.

4.5 FISH analysis on CSF

Additionally fluorescence in situ hybridisation on a cytospun specimen is used for detecting carcinoma cells and lymphoma cells in CSF. Numerical chromosomal aberration can be detected on single cell level using e.g. a probe for chromosome 1q12. Studies proved that the cells in leptomeningeal metastases very often resemble the same numerical content of chromosomes 1,7 and 10 as the corresponding malignant cells in the periphery. This method is also used for follow up examinations during specific therapeutic regimen.

4.6 Personal communications (abstracts)

FACS ANALYSIS-A NEW AND ACCURATE TOOL IN THE DIAGNOSIS OF LYMPHOMA IN THE CEREBROSPINAL FLUID.

Urbanits S, Griesmacher A, Hopfinger G, Stockhammer G, Karimi A, Muller MM, Pittermann E, Grisold W.

BACKGROUND: Fluorescence activated cell scanning (FACS) is a useful tool for identifying malignant cell clones of lymphoma cells in cerebrospinal fluid (CSF) by immunological phenotype.

METHODS: We used FACS analysis for demonstrating it to be a quick and reliable technology that is available in most haematological laboratories. In this study, we demonstrate the clinical application of FACS analysis within a series of 15 lymphoma patients with suspected CSF involvement. CSF from three patients with another diagnosis than lymphoma serves as negative control. RESULTS AND

CONCLUSION: A malignant cell clone cannot only be identified in CSF phenotypically, but also classified according to the immunological surface profile. As this method improves the diagnostic sensitivity and specificity, it should be implemented into routine diagnosis.

METHOD: In FACS analysis, cells are analysed via a FACS scanner. Cells are stained for several surface markers via one run. When they are analysed and activated with light the fluorochrome connected antibodies emit light of a given wavelength. These data are collected and a surface profile of the analysed cells is evaluated. According to the forward scatter also data on granularity and cell size are available.

MEASUREMENT OF DNA-ANEUPLOIDY AND VEGF (VASCULAR ENDOTHELIAL GROWTH FACTOR) IN THE CEREBROSPINAL FLUID OF A PATIENT WITH MENINGEAL CARCINOMATOSIS UNDER CONTINUOUS CHEMOTHERAPY.

Urbanits S (1;2), Heinschink A (2), Stockhammer G (3), Karimi A (2), Oberndorfer S (1), Lahrmann H (1), Grisold W (1), Müller MM (2).

INTRODUCTION: The evaluation of the therapeutic success in a patient with meningeal carcinomatosis under chemotherapy allows a more precise and better management of the patient. As the identification of malignant cells in CSF from a cytospun specimen has its difficulties, also the immmunocytochemical identification of carcinoma cells with cytokeratine may be very tricky.

In the following case report we identify malignant cells in CSF according to their aneuploidy (hyperploidy) examined by FACS. Additionally vascular endothelial growth factor (VEGF) was measured by ELISA technique in CSF and serum. The VEGF isoform 165 is known to be produced by tumor cells.

These data are compared with the results of the morphological and immmunocytochemical analysis of the cytospin specimen.

SUMMARY: The evaluation of aneuploidy and VEGF in the CSF correlated well with the results of the morphological analysis from cytospin specimen and with the cell count. This demonstrated monitoring model might improve the quality of the diagnosis and the follow up of patients undergoing chemotherapy because of meningeal carcinomatosis. As this is only a case report many more patients have to be evaluated according to this procedure for introducing such a diagnostic system into the routine.

METHOD: DNA is analyzed for aneuploidy with probidium iodid with a Becton Dickinson Scan (FACS Calibur). Cell activity is characterized as follows: G0/G1 Phase, S-Phase and G2-M-Phase, additionally DNA-indices were evaluated. Tumour cells were phenotypically characterized using an anti-pan-cytokeratine antibody.

The VEGF ELISA assay (Quantikine Kit, R&D Systems, Minneapolis, MN) detects the isoform 121 and 165.

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