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Recommendations for Cerebrospinal Fluid Cytology. A Review Article

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1. Abstract

The description of cytological findings in cerebrospinal fluid (CSF) is very inconsistent in the literature since no generally recognized uniform classification of these findings has been proposed to date. The need for developing such a classification system becomes quite obvious against the background of renaissance CSF cytology is currently experiencing in our country. A precondition sine qua non for developing a uniform classification system is its general applicability and recognition as well as a capacity to establish, using precisely formulated conclusions, the aetiological diagnosis, something quite impossible with today's terminology.

Our draft classification is that used by a team of physicians working previously in the CSF Laboratory of the Department of Neurology of Charles University School of Medicine I in Prague. The classification employed there is based on monitoring pathology in the cytological picture both according to the presence of the prevailing cellular population in CSF and to the

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presence of activation in elements of lymphocyte and monocyte lines. We were able to combine both criteria into a single viable system expressing the current status of cellular response in CSF. The presence of a pathological cytological finding provides the basis for defining individual cytological CSF syndromes closely related to the etiological diagnosis of the patient, which in the great majority of cases make it possible to formulate the diagnostic conclusion. The classification employed allows to establish the diagnosis in diseases manifesting themselves by at least a mild alteration of the cytological picture. In general, it is useful for classifying inflammatory, neoplastic diseases, inter-meningeal haemorrhage and morphological manifestations of CNS tissue destruction. A distinct advantage is the plausible classification of cytological findings in oligo-cellular CSF specimens which to date has been difficult to make due to the low numbers of cellular elements detected in samples.

In cytological examination of CSF, the parameters evaluated include, in addition to the number of elements, qualitative representation of individual cellular lines. (1-Monografie). When evaluating the monocyte-macrophage system and/or the reticuloendothelial system, attention is focused on the proportions of activated monocytes and, particularly, on the presence of macrophages showing a specific substrate of phagocytosis. It is according to this substrate that macrophages are further divided into erythron-phages, sidero-phages, lipo-phages, lympho-phages, leuko-phages or myco-phages, etc. To visualize a substrate, it is often necessary to use additional staining e.g., staining by Oil Red for lipids, Berlin Blue for iron, etc.

If inter-meningeal haemorrhage is suspected, monitoring of the phagocytosis of red blood cells and haematogenic pigments allows us to determine the approximate age and course of the bleeding. Monitoring of lipo-phagocytosis visualizing the scavenging response on CNS parenchymal damage also has a number of potential applications.

As the number of CSF examinations increases, proportionately increasing numbers of cells are being detected. This is true especially of diseases involving the presence of primary or secondary neoplastic processes right in the CNS or in the vicinity of CSF pathways. The currently employed cytological methods of CSF examination, whenever malignant elements were detected, have made it possible to establish the presence of a tumor disease in general only. For instance, monitoring of the functional status of nucleoli, PAS positivity, or the presence of adipose droplets in the cytoplasm suggest only indirectly an increased metabolic activity of the cells monitored. Other morphological markers of atypical cells (polymorphism of cells, nuclei, polynuclear elements, cytoplasm basophilia, atypical mitoses, etc.,) may only raise suspicion of the presence of a tumorous process, but not identify the cellular system the belong to. Another problem which by no means is negligible is the low number of cells detected.

As a result, we started to study the mode of reaction of atypical elements with certain monoclonal antibody binding to individual antigens, tumor markers specific for the respective cellular populations. Moreover, the method can be used to determine the degree of their maturity, presence of individual receptors, state of activation in the course of their cellular cycle.

2. Keywords: CSF Cytology; Classification of CSF Cytological Findings; Monocyte-macrophagic system; Monoclonal Antibodies; Immuno-cytology; Tumorous Cells; Lipo-phages; Inter-meningeal Haemmorrhage

3. Classification of Cytological CSF Findings

There is currently no generally recognized classification system of cytological CSF findings and descriptions of cytological findings appearing in the relevant literature show considerable inconsistence and mostly do not even allow a syndromological conclusion or establishing of the aetiological

diagnosis. A team of physicians working in the CSF Laboratory of the Department of Neurology of Prague's Charles University, School of Medicine I, employs a uniform classification scheme allowing an exact formulation of cytological findings by determining the cytological CSF syndrome which, in most cases, makes it possible to establish an exact diagnosis of patients examined.

The classification is based on several criteria of the existing cellular alteration, which may be either pathological numerical prevalence of a certain cellular population, or signs of activation in the numerically prevalent line (or, possibly, in other lines). Another aspect is the number of elements in CSF where, up to 10/3 elements per chamber according to Fuchs-Rosenthal, one can speak of oligo-cellular CSF (oligo-cytosis of varying types) and, in the case of several CSFs, reference is made to pleiocytosis [1,7]. For a more detailed classification, a normal cytological finding must be mentioned with a prevalence of lymphocyte elements (65%-80%) and the remainder made up of elements of monocyte line, with both populations are in their prevalence represented by quiescent elements (Table 1-4).

Table 1: CSF Elements.

CSF Elements
Lymphocytes
Monocytes
Granulocytes
Macrophages
Epiteloid Lining Cells of CSF Pathways (Ependyma)
Erythrocytes
Atypical Cells
Tumour Cells
Leukemic Cells

Table 2: Lymphocytic Elements in CSF.

Lymphocytic Elements
Lymphocytes - Small (Naked-Nucleated)
Medium-Sized
Large - I.E., Lymphoid Cells
Lymfoplasmocytes
Plasma Cells

Oligo-cellular and pleiocytic CSF´s can be divided, by their cytological composition, into several groups, with individual cytological CSF syndromes defined within these. In the presence of pleiocytosis, CSF findings can be classified quite easily (Table 5).

Table 3: Monocytic Elements of CSF.

Monocytic Elements
1) Quiescent (Non-Activated) Monocytes
2) Activated Monocytes
3) Macrophages - Erythrophages -Recent Haemorrhages Siderophages - older haemorrhages
Leukophages - Mostly In Purulent Inflammations
Lymphophages - Serous Inflammations And Multiple Sclerosis
Lipophages - destruction of CNS tissue So-called "BACTERIOPHAGES" - phagocytosis of bacteria (predominantly, it is mediated by neutrophiles)
Mycophages - mycotic diseases of CNS
4) Degenerative Forms Of Monocytic Cells - ring-shaped cells

Table 4: Myeloid Elements - Granulocytes.

Myeloid Elements (Granulocytes)
1) Neutrophils
2) Eosinophils
3) Basophils

Table 5: Types of CSF Pleiocytosis.

Pleiocytosis
1) Granulocyte Pleiocytosis (i.e., polynuclear pleiocytosis)
Neutrophilic granulocytes - mostly in bacterial neuro-infections
Eosinophilic granulocytes - parasitic, mycotic, allergic and Auto-agressive diseases
2) Mononuclear Pleiocytosis
A) Lymfocytic P. - activated lymphocytes (serous neuro-infections)
+plasma cells - chronic neuro-infections, multiple sclerosis (lymphocytic oligocytosis is more frequent)
B) Monocytic P. - very complicated differential diagnosis: Compressive syndromes - disc herniations, tumours.
Systemic vasculitis affecting CNS Brain ischaemia Guillain-Barré síndrome Terminal phases of neuro-infections with scavenger reaction.
C. Tumorous P. - presence of malignant cells, accompanying celullar reaction differs highly, predominantly of monocytic type.

1) Granulocytic pleiocytosis with a prevailing representation of granulocytes, usually neutrophils and much, less often, eosinophils. Using this criterion, granulocyte pleiocytosis can be further divided into two more subgroups:

a) Granulocytic pleiocytosis with a prevalence of neutrophils (neutrophilic pleiocytosis); this is a typical picture of purulent inflammations in CSF;

hence, it occurs especially in bacterial meningitis.

b) Granulocytic pleiocytosis with a prevalence of eosinophils (eosinophilic pleiocytosis); a relatively rare picture of so called "eosinophilic meningitis" which, however, is not an inflammatory infective disease but a general severe allergic reaction of the body.

2) Lymphocytic pleiocytosis with a prevailing representation of lymphocyte line elements and a high representation of activated forms which, in the event of a chronic course of the lesion, evolve (in B-system elements) into plasma cells. This picture is quite typically associated with non-purulent inflammatory diseases (serous inflammation) whose pathogens in our conditions are especially viral agents; bacterial spirochaetal disease (borreliosis, leptospirosis and lues) may also be involved. The presence of other bacterial agents is suggested by purulent inflammation manifesting itself by granulocytic pleiocytosis.

3) Monocytic pleiocytosis with a prevailing representation of monocyte line elements; these elements usually show signs of activation, phagocytosis mediated by activated monocyte elements (macrophages) is quite frequent. Provided macrophagic elements are present, the aetiological diagnosis is usually easy to establish. Macrophages can be classified by the specific substrate of phagocytosis whose presence reflects individual pathological states. Erythro-phages appear in recent inter-meningeal haemorrhage (however, in these cases, the prevalent cellular response is only rarely monocytic), sidero-phages are usually present in inter-meningeal haemorrhage of an older age. Leuco-phages-macrophages phagocyting granulocytes, especially neutrophilic granulocytes are present in the terminal stages of purulent inflammations. Lympho-phages phagocyte lymphocyte-line elements and are typically present in the end stages of non-purulent inflammations. A characteristic feature of so called lipo-phages is the presence of adipose droplets in the cytoplasm, these elements phagocyte necrotic CNS tissue and can be seen as part of the monocytic cellular response in the cerebral ischemia and in degenerative disease.

4) Tumorous pleiocytosis, with the sample showing malignant elements as such; while the picture of the accompanying cellular response may be different, it is usually monocytic. Phagocytosis of malignant elements in CSF is quite frequent.

Evaluation of cytological findings in oligocytic CSF´s is fraught with a number of problems. The most serious problem is the low number of elements detected in the samples. The term pathological oligocytosis can be used to refer to the presence of a pathological cytological finding with an otherwise normal number of cellular elements in CSF. While some types of pathological oligocytosis can be regarded as smooth transition to pleiocytoses of the same types, other oligocytoses take on another functional relevance (Table 6):

Table 6: Types of Pathological Oligocytosis in CSF.

Pathological Oligocytosis
1) Lymphocyte Oligocytosis - activated forms of Lymphocytes + possible presence of plasma cells - chronic neuro-infections and M. S.
2) Monocyte Oligocytosis - prevalence of monocytes or marks of their activation present, phagocytosis also posible usually non-inflammatory diseases or terminal phases of inflammations.
3) Granulocytic Oligocytosis
A. Neutrophilic O - frequent in early stages of inflammations.
B. Eosinophilic O - rarer affection, some autoimmune diseases, chronic affections as a whole.
4) Tumorous Oligocytosis - presence of malignant cells, accompanying monocyte reaction is usual.

1) Granulocytic oligocytosis is associated with a prevalence of neutrophil granulocytes; granulocytic oligocytosis does not make a transition to granulocyte pleiocytosis and, unlike it, the former appears in the initial stages of non-purulent inflammations and in the early stage of cerebral ischemia.

2) Lymphocytic oligocytosis is characterized by the presence of a larger number of activated lymphocyte

elements and, in the case of a chronic course, even of plasma cells. It appears in association with multiple sclerosis and in some serous neurological infections. This type of oligocytosis is a smooth transition to lymphocyte pleiocytosis.

3) Monocytic oligocytosis is characterized by a numerical prevalence of monocyte line elements and signs of their activation or, at least, by one of the above phenomena. Cytological findings are very difficult to evaluate because of the generally low rate of detection of cellular elements and because of difficult evidence of phagocytes. However, if macrophagic elements are present at least occasionally, the aetiological classification of these findings is usually easier. Otherwise, monocytic oligocytosis occurs in the end stages of all neurological infections. A specific substrate of phagocytosis can often be demonstrated; if absent, the entity is called residual monocyte stage which may persist in the cytological picture for quite a long time after neurological infections. Monocytic oligocytosis is also quite a frequent cytological finding in Guillain-Barré polyradiculoneuritis (polyneuritis). Along with the accompanying lipophagocyte reaction, it can be also fairly often seen in CNS destruction.

4) Tumorous oligocytosis making a fully smooth transition to tumorous pleiocytosis. The criterion of importance in classification is the detection of malignant elements. An accompanying cellular reaction may often be of quite a different type; however, it is usually a monocytic cellular reaction. More rarely, the presence of phagocytosis of malignant elements can be observed.

The classification proposed has been employed by our team as a binding one. In addition to internal use within the departments, it is used for describing cytological conclusions to other departments which have been mushrooming lately. A syndromological cytological conclusions and a diagnostic analysis can be regarded as an integral part of every investigation of CSF; it would be especially helpful to use a uniform and standard classification in each centre.

4. Diagnostic Use of Macrophagic Elements in CSF

In addition to determining the total number of elements, cytological evaluation of CSF involves the qualitative representation of individual cellular lines. Cells of the monocyte-macrophagic (or, possibly, reticuloendothelial) system are represented both by quiescent elements but, also, by activated cells whose number should physiologically exceed 10 % of the total number of cells of the particular line. The finding of macrophages, that is, monocytes with a clear phagocytosis substrate, is a pathological finding (Table 3) [8]. In the course of activation, mostly metabolic changes take place involving, e.g., the spectrum of enzymes in the cytoplasm, the number of receptors on the membrane, the antigenic structure, or secretion of some substances. We can observe morphological manifestations of activation including the rounding of roll-shaped nuclei, expansion of the cytoplasm volume, formation of pseudopodia and so on but, also, the onset of phagocytosis of a specific substrate and its changes in the course of digestion. Depending on the character of the substrate, macrophages are further classified into erythron-phages, sidero-phages, lipo-phages, lympho-phages, leuko-phages, myco-phages, etc.

Activation of the monocytic line is a sign of primary non-inflammatory processes. It is just in oligo-cellular CSF´s that this evaluation may be of principal importance for establishing a correct diagnosis.

Monocyte-line activation occurs in the course of infectious, both serous and purulent, diseases of the CNS. The macrophages present (or, alternatively, lympho-phages and leuko-phages) are indicative of an advanced stage of the disease. In this case, lipo-phagocytosis is usually associated with a focal finding in the objective neurological examination and, hence, suspicion of meningoencephalitis.

Of essential importance is evaluation of macrophages on suspicion of inter-meningeal haemorrhage (Table

7) [9].

Table 7: Cytological Picture of Inter-meningeal Haemorrhage.

Cytological Picture of Intermeningeal Haemorrhage
(Adhesion of Erythrocytes - also detectable "in vitro")
1) Fagocytosis of Erythrocytes - recent haemorrhage, presence of macrophages called erythrophages.
2) Digestion of Fagocytosed Erythrocytes - decoloration and destruction of erythrocytes appears, optically empty vacuoles.
3) Hemosiderin - an iron containing haematogenous pigment, present in so-called siderophages.
4) Hematoidin - haematogenous pigment forming rhombic crystals in macrophages, then present extracelullary after decline of macrophages).

Here, the specific substrate are red blood cells whose phagocytosis by so-called erythron-phages (Figure 1) occurs in the early stage not earlier than 4 to 6 hours after the start of bleeding.

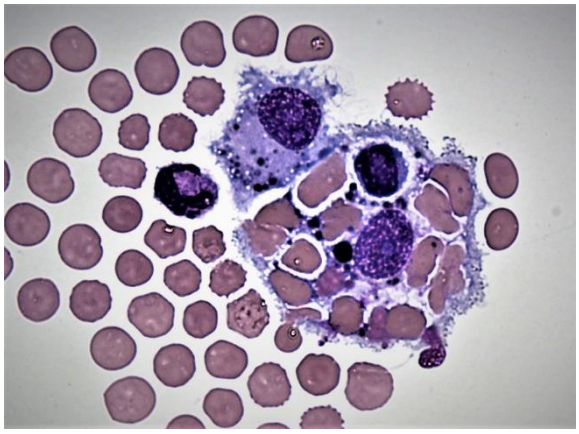

Figure 1: Erythro-sidero-phage. Basic Stain.

This is followed, two to three days later, by their digestion manifesting itself by the formation of a halo a bright circle around phagocyted red blood cells and their progressive discoloration until empty vacuoles are left in the macrophage. In the ensuing stage, hematogenic pigments hemosiderin and hematoidin crystals start to be scavenged. Hemosiderin, because of its contents of trivalent iron, is readily visualized with Berlin Blue enhancing its diffuse and granular nature; it cannot be seen until 4 to 5 days later. As a result, it is a reliable sign of a previous inter-meningeal haemorrhage. Hematoidin which no longer contains Fe^{3+} and presents in the form of yellow-ochre crystals in macrophagic cytoplasm appears still later, on about day 13. Later, it can also be noted extracellularly as late as six months after haemorrhage. The presence of several of these stages at a time enables us to detect protracted or repeated inter-meningeal haemorrhage.

An important part is evaluation of lipo-phagocytosis [2,4-6] (Figure 2).

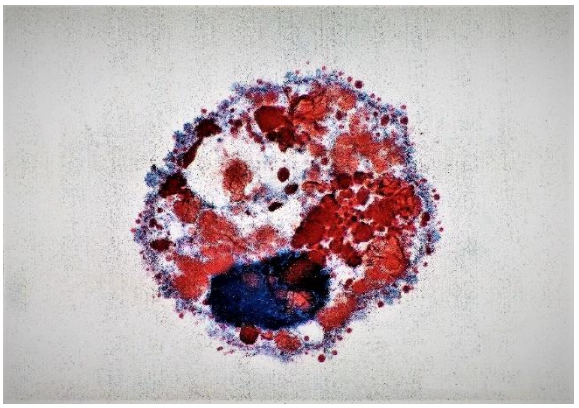

Figure 2: Lipo-phage. Oil Red O + Gill Haematoxylin.

With basic staining, the non-specifically looking foamy cytoplasm would have escaped attention. Oil Red O staining (or Sudan Black B or Scarlet R) of lipids provides for excellent visualization, which is why Oil Red is the second most often used stain besides basic stain with May-Grünwald Giemsa-Romanowski in our laboratories. Lipo-phagocytosis occurs as a scavenging response of the monocytic system on damage to and breakup of cerebral parenchyma for a number of causes. It is, consequently, a parameter with a wide area of applications.

A typical finding is that of monocyte oligocytosis or pleiocytosis in cerebral ischemia. The degree of pleiocytosis, which is a frequent finding in cerebral ischemia, cannot be regarded as a measure of parenchymal damage since the distance of the ischemic focus to CSF pathways space makes a difference. A diagnosis with lipo-phagosis also being a regular finding and allowing us to assess the activity of the disease, is vasculitis with CNS damage.

5. Use of Monoclonal Antibodies in CSF Cytology

A finding which continues to be frequent is that of

tumour cells in CSF (Table 8) (Figure 3-5) [3,10-18].

Table 8: Malignant Cells in CSF.

Malignant Cells in CSF
1) Tumourous Cells - problematically distinguishable in chamber acc. to Fuchs-Rosenthal.
2) Leukemic Cells - resemble to mononuclear cells in FR chamber.
Criteria of Malignancy
1) Polymorphism of cells
2) Polymorphism of nuclei
3) Numerous and activated nucleoli
4) Giant cells
5) Multinucleated cells
6) Increased nucleus/cytoplasm ratio
7) Increased stainability
8) Numerous mitoses
9) Atypical mitoses
10) Basophilia of cytoplasm
11) Formation of syncytia
12) Polychromasia

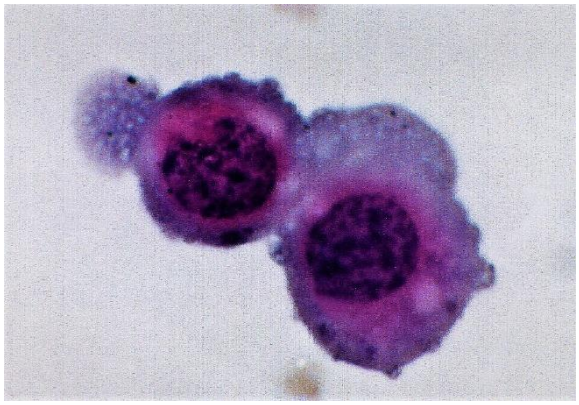

Figure 3: Tumorous Cells – Malignant Melanoma. PAP Stain.

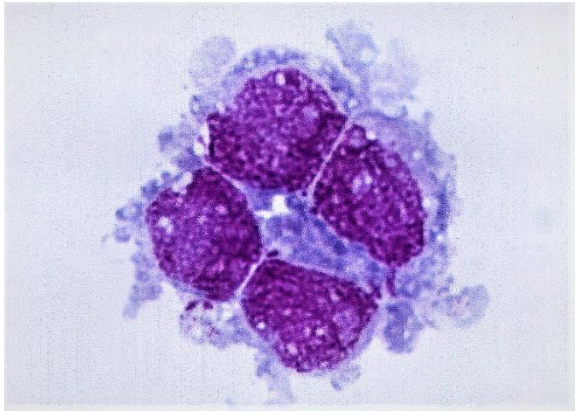

Figure 4: Breast Carcinoma. Basic Stain.

Recently, there has even been an increase in the rate of detection of tumour elements, e.g., in haematological malignancies, where cytology is a frequent indication with respect to the possible leukemic meningeal infiltration. In other cases, malignant elements appear in CSF in the presence of metastases into the brain, the spinal canal and in vertebral body destruction by the malignant process.

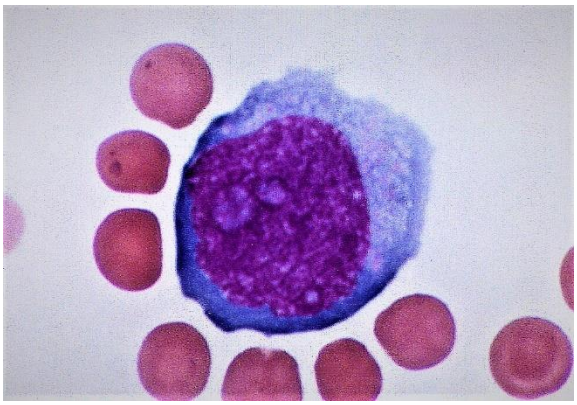

Figure 5: Leukemic cell - Acute lymphatic leukaemia. Basic Stain.

A less frequent finding is that of malignant elements in CSF in primary tumour processes involving the CNS. In some forms of carcinomas, tumour cells can occasionally be detected even without the presence of metastases. On meningeal infiltration, if the tumour is in the vicinity of CSF pathways or intraventricularly, the rate of detection of malignant elements is 40-50% as a maximum. Provided no malignant elements have been detected in the cytological preparation, the presence of a tumour process can be indirectly suggested by the finding of monocytic pleiocytosis or monocyte oligocytosis.

In some cases, it may be difficult to distinguish malignant cells from normal cells. For instance, when counting elements per chamber according to Fuchs-Rosenthal, it is not easy to distinguish cells from CSF pathway lining or common mononuclears. Some potential for misidentification in the cytological picture with neurological infection exists especially with so called leukemic meningeal infiltrations. It is therefore reasonable to assess, in the cytological preparation, the functional status of nucleoli stained with Toluidine Blue (staining according to Smetana), PAS positivity, or the presence of adipose droplets in the cytoplasm as markers of increased metabolic activity. Other usual criteria of malignancy include cellular polymorphy, nuclear polymorphy, multiple and activated nuclears, giant cells, polynuclear elements, considerable size of nuclei vs cytoplasmic

volume, increased tincture properties, frequent mitoses, non-typically dividing elements, cytoplasmic basophilia, syncycial formation or polychromasia.

Essentially, classification of malignant elements in CSF is extremely difficult because of the low number of cells detected in this manner and, also, because of the considerable morphological changes occurring in these cells on crossing into CSF and their presence therein. These changes include, mainly, loss of typical morphological markers and the rounding up of malignant cells.

If a low number of suspicious cells in the cytological picture is available, mostly in oligocellular CSFs, it is possible to multiply malignant or controversial cellular elements using the methods of tissue culture and their further identification.

In such cases, a more exact diagnosis is possible only when using specific monoclonal antibodies against specific tumour markers - antigens (Table 9) (Figure 6-8).

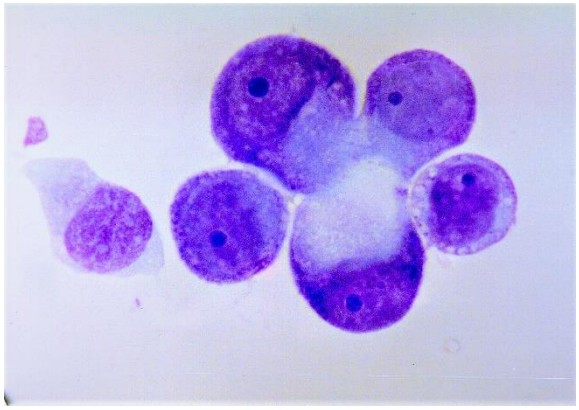

Figure 6: Myco-phage. Cryptococcal Meningitis.

Individual cells or whole cellular populations can thus be assigned, using monoclonal antibody, to the respective cellular systems they belong to. The degree of their maturity, presence of some receptors or products of their secretion, the status of activation or the degree during their cellular cycle can be determined.

Cytological findings are thus divided into several groups. The first group embraces tumours with no signs of invasive growth. In these cases, evidence of tumour elements is rare on account of the primarily "benign" nature of tumour growing in this manner. The presence of these cells in meningiomas or neurinomas is a very rare finding.

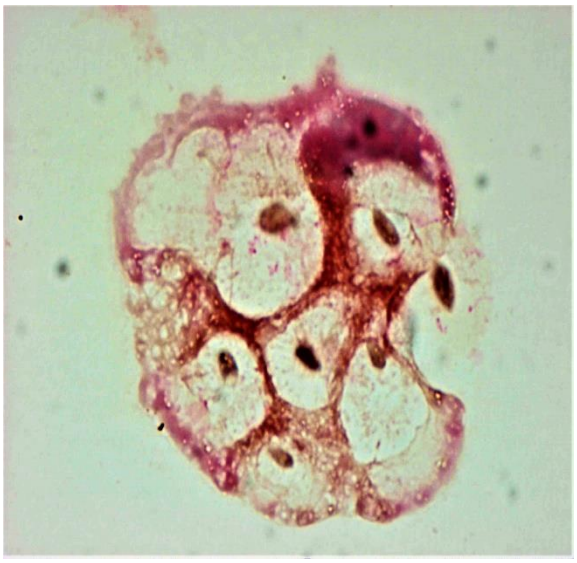

Figure 7: Lung Carcinoma – a tripolar amitosis. Toluidine Blue Stain.

Table 9: Significant Tumorous Markers Detectable in CSF Elements.

Significant Tumour Markers
GFAP (Glial fibrillary acidic protein) - majority of glial tumours
HMB-45 (Human melano-blastoma) - malignant melanomas
CEA - carcinoembryonic antigen - mostly in tumours of GIT
Alfa1-phaetoprotein - expressed in seminomas penetrating to CNS
Vimentin - mesenchymal tumours
C-erbB-2 Oncoprotein - non-specific marker, more frequent in breast carcinoma
L-26 (i. e. CD 26) - B-lymphomas
BLA-36 (i. e. HDLM-3) - Hodgkin lymphoma
PCNA (Proliferating cell nuclear antigen) - breast carcinoma, also in other epithelial tumours
UCHL (=IL-2 dependent T-cell line) - T-lymphomas
CD 43 - T-lymphomas
Ki-1 (i. e. CD 30) - lymphomas as a whole
CD 14 a CD 68 (=KP1) – histio-monocyte malignancies
CD 71 - proliferating cells as a whole (marker is a transferrin receptor)
MLA (Mucosa lymphocyte antigen) - Hairy cell leukaemia
LCA (Leukocyte common antigen) (= CD 45 RB) - all lymphomas
OPD 4 (Helper/Inducer phenotype) - T-lymphomas
Cytokeratin - epithelial tumours

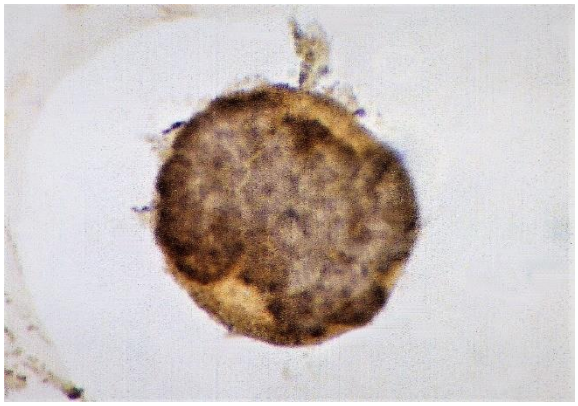

Figure 8: Leukaemic Cell. Monoclonal Antibody CD 11c (HRP) + Harris Haematoxylin.

A more frequent finding is that of completely benign tumour cells in ependymomas and papillomas of the choroid plexus mainly because of their presence in the vicinity of CSF pathways. The other group comprises tumours with signs of invasive growth. These include especially malignant gliomas and metastatic tumours. In this group, malignant cells are a more frequent finding, phagocytosis of tumour cells is more frequent and activation of the lymphatic line or pleiocytosis can be seen more often (neutrophilic pleiocytosis is less often and eosinophilic pleiocytosis is very rare). The picture of phagocytosis partly covers also the contact mechanism between macrophages and lymphocytes.

The actual response of monoclonal antibodies with individual cells is usually visualized either directly, typically using fluorescent stains, or indirectly, using the reaction of antibody labelled with horse radish peroxidase (HRP) (or other enzyme) with diaminobenzidine (DAB) (or other substrate while using other enzymes) under the microscope or in the flow cytometer; the latter, however, is not employed routinely in CSF immuno-cytology, while it is used with advantage in clinical haematology.

The HRP method consists in 1) specimen fixation with aceton-methanol, 2) inhibition of the existing enzymatic activities of CSF cells with sodium azide, 3) incubation with primary (murine) monoclonal antibody, 4) application of secondary (porcine) HRP-labelled polyclonal antibody, 5) incubation with tertiary (rabbit) polyclonal antibody, again labelled with HRP, 6) visualization of conjugated monoclonal antibody using colour reaction mediated by peroxidase with DAB, 7) additional colouring of nuclei in Harris haematoxylin, 8) mounting of the preparation into Entellan or into Aquatex providing for a longer life.

The main advantage of evaluating the cytological preparation under the microscope is that it allows better assessment of cellular morphology also in oligocellular CSF´s. A limitation of immuno-typing is especially the relatively high cost of the procedure. In haematologic indications, 5% of malignant cells must be present as a minimum. Another major obstacle is the difficulty in distinguishing of reactive granulocytosis with a shift to the left and reactive monocytosis from neoplastic states with small well differentiated cells, such as in chronic myeloid leukaemia.

Still, we do believe that widespread use of the method of monoclonal antibody in CSF immune-cytology in tumour disease as part of the arsenal of routine techniques of examination will help improve markedly the prognosis of patients, thanks to the possibility of establishing of diagnosis early and, consequently, prompt initiation of aimed therapy.

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