The differential cytology of cerebrospinal fluids prepared by cytocentrifugation

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The total cell count and differential count are an essential part of the analysis of a specimen of cerebrospinal fluid (CSF). The accuracy of the differential count has been improved significantly by the use of such concentrating techniques as sedimentation, filtration, and centrifugation.[1-10] The cytocentrifuge (Cytospin, Shandor-Elliot), which we have used since 1974, concentrates the formed elements of the fluid directly onto a slide without significant alteration of morphology.

This paper reports our estimate of the normal differential count and the findings in spinal fluids examined over a 9-month period.

Material and methods

From January to September 1974 approximately 1,600 cerebrospinal fluids were examined. The appearance of the fluid was noted, and in all instances a cell count and quantitative protein measurement were made. Other tests (e.g., glucose, protein electrophoresis, tests for syphilis) were performed if ordered by the clinician. The cells were counted in a hemocytometer and if there was 1 cell/μl or less, a differential count could be waived at the discretion of the technologist. Slides were prepared from the

237

remaining fluids by centrifuging a mixture of 0.5 ml fluid and 2 drops 22% bovine albumin at 1,500 rpm for 2 minutes in a cytocentrifuge. The air-dried smears were stained with Wright's stain.

All smears were reviewed and those in which at least 25 leukocytes were present and well preserved were used in this study. This group comprised 571 fluids from 423 patients. Differential counts were tabulated without knowledge of the patients' clinical statuses.

Results

To establish the normal percentage of the various cell types for our laboratory, those fluids which were clear and colorless and had white cell counts of $5/\mu l$ or less, red cell counts of $0/\mu l$, and were derived from non-leukemic patients were examined (N = 81). Only slides with 25 or more cells were included in the study, but despite the paucity of cells the centrifuged preparation allowed a 100 cell differential count in 34 (42%) of the specimens.

Cells were characterized as lymphocytes, monocytoid cells, or segmented neutrophils. Normal lymphocytes were characterized by round or ovoid, dense, nonnucleolated nuclei and small or moderate amounts of light blue cytoplasm. Most were less than 10 μ in diameter. Undulations in the cytoplasmic membrane were present infrequently. A rare atypical lymphocyte was encountered but no other cell species was noted. Monocytoid cells included blood monocytes and tissue histiocytes. The nuclei were indented or lobulated, contained one or two nucleoli, and were larger than lymphocyte nuclei. The cytoplasm was pale

and abundant and often vacuolated; its membrane frequently was irregular with numerous projections. Segmented neutrophils occurred often enough in the normal fluids to be considered in the calculations. Eosinophils and basophils appeared only sporadically.

The relative percentages of lymphocytes, monocytoid cells, and segmented neutrophils were calculated independently; any value more than two standard deviations from the mean removed the entire specimen from the group of fluids used to establish the normal range. The normal ranges were then based on a total of 75 patients. *Table 1* summarizes the findings, and *Figure 1* shows the age distribution of this normal population. No significant difference was found between the cerebrospinal fluids of men and women. There was a slight tendency for young patients to have relative lymphocytosis.

Although this group of spinal fluids was objectively normal, these patients were ill. The findings were compared therefore with those of a group of 86 patients whose conditions (back strain, spondylosis, normal pressure hydrocephalus, idiopathic epilepsy, headache, pseudotumor cerebri, and anxiety state) would not be expected to affect the spinal fluid findings. Twenty-six of these patients' fluids were represented in both groups. In 40 of the fluids, minimal peripheral blood contamination was present (1 to 30 red cells/μl). The differential leukocyte count ranges in this group (lymphocytes, 72.1% ± 22.9; monocytoid cells, 27.6% ± 21.1; segmented neutrophils, 0.9% ± 1.8) did not differ significantly from that in the group of objectively normal fluids.

Table 1. Normal CSF differential counts

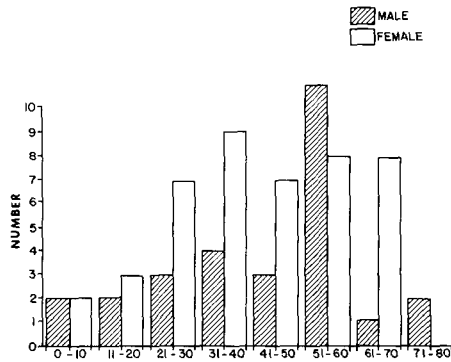|	
Lymphocytes	75.8% ± 10.0
Monocytoids	24.9% ± 10.7
Segmented neutrophils	0.4% ± 1.1

Fig. 1. Age and sex of patients whose cerebrospinal fluids determined normal leukocyte differential count.

Among the abnormal fluids, the largest group, 36, was from patients with intervertebral disc disease. Small numbers of red cells were common (range 1 to $322/\mu l$), probably a result of contamination during the tap. The white cell population did not differ significantly from normal either in number or type.

Thirty-nine fluids from 12 patients who had bacterial meningitis were examined. Despite individual variation, a general pattern of large numbers of segmented neutrophils was seen initially. Shortly after treatment was started the counts began to fall and the differential count changed to a relative lymphocytosis; this change was prominent by the end of the first week. The appearance of macrophages, characterized by evidence of phagocytosis, was variable but usually occurred in 5 to 10 days. Plasma cells, activated lymphocytes, and leukophagocytosis were occasionally prominent.

Viral meningoencephalitis, diagnosed in four patients, was character-ized by an absolute lymphocytosis (75% to 94% of 52 to 570 white cells/ μl). The lymphocytes in two of the patients were atypical, having distinct nucleoli. The tight chromatin pattern, however, distinguished them from neoplastic lymphoblasts.

A diagnosis of multiple sclerosis or other demyelinating disorder was made or suspected in 36 patients. There was a mild leukocytosis in this group (2 to $58/\mu l$) and lymphocytes were significantly increased (86.9% ± 6.0: $p < 0.001$). Nine (25%) of these patients had atypical lymphocytes with appearance of nucleoli. In five, the lymphocytes were decidedly plasmacytoid and were occasionally coherent into small groups.

Fifteen patients were undergoing intrathecal therapy for acute leukemia. Blasts were identified in five (*Fig. 2*). Meningeal remission was obtained within the first 2 weeks of treatment in the three patients who responded, and the blast cells were replaced by a normal cell count and differential count in two. The third patient had a transient 10% to 30% segmented neutrophil fraction in three determinations through the fourth week, although his total white cell count never exceeded $2/\mu l$. The morphology of the blasts was quite similar to that of the patient's circulating cells and could be typed as indicating acute lymphoblastic or acute granulocytic leukemia in the same way.

In several cases subarachnoid bleeding was strongly suspected from the morphology when macrophages were encountered with erythrocyte inclusions or hemosiderin granules (*Fig. 3*). These granules appeared dark golden brown in the Wright stain preparation, but Prussian blue
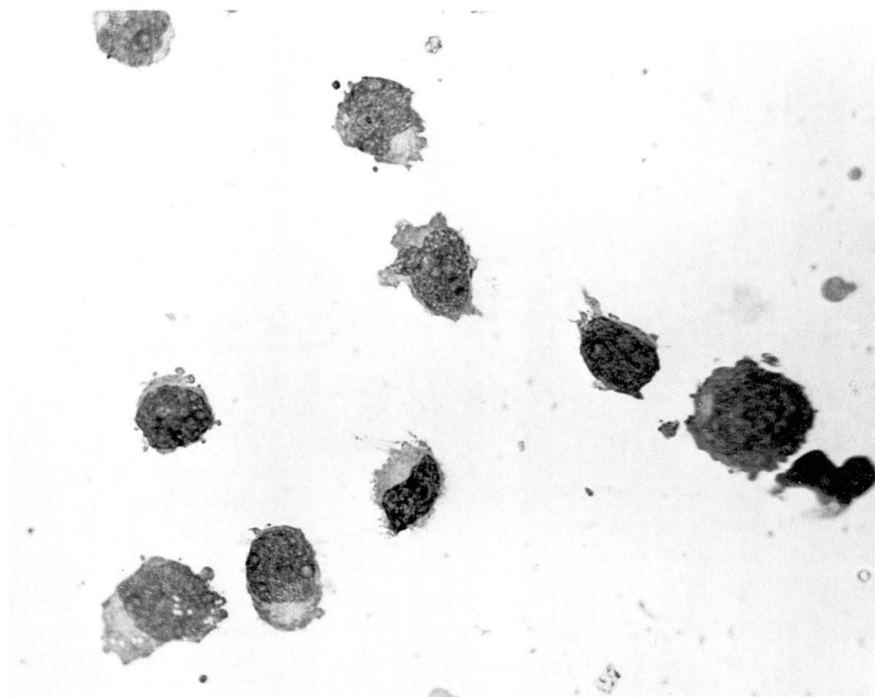
Fig. 2. Acute lymphocytic leukemia. The blasts are irregular, the nuclei have finely granular chromatin, and the nucleoli are multiple and bizarre (Wright's stain, ×640).

in a ferricyanide reaction. The clinical history was either compatible with a diagnosis of subarachnoid hemorrhage or included a recent neurosurgical procedure or traumatic tap. In some cases of massive intracranial bleeding, amorphous, thready deposits with amphophilic staining characteristics were suggestive of fibrin formation. Erythrocyte crenation was no aid in distinguishing central nervous system bleeding from traumatic lumbar punctures.

A solid tumor metastasizing to the leptomeninges and shedding recognizable cells into the CSF was detected only once among 18 patients with widely disseminated lesions; the tumor was a carcinoma whose primary origin was not ascertained. Among nine patients with poorly differentiated primary central nervous system malignancies, one malignancy (retinoblastoma) was noted in retrospective review. Sixteen of these 27 patients had simultaneous examinations using the Papanicolaou technique. The one case reported as compatible with malignancy was the same one in which carcinoma cells were revealed with the cytocentrifuge technique. However, based upon comparative experience with other body fluids, cytologic examination for malignancy of fixed cells was superior with better demonstration of nuclear chromatin detail and nucleolar atypia.

Occasionally, the technique showed single or grouped large cells with round, homogenous, eccentric nuclei and pale, foamy cytoplasm identified by Woodruff[4] as pia-arachnoid cells. Their appearance was sporadic

and suggested no group of diseases. Rarely, phagocytic cells lacking nuclear or cytoplasmic characteristics of macrophages were seen; they were thought to be phagocytic pia-arachnoid cells (*Fig. 4*). When looked at from the point of view of predominant cell type, 49 patients had a lymphocytosis greater than 90%, which was absolute in 35 instances. Within this group were eight patients with multiple sclerosis and six with disseminated carcinoma. Other diseases occurring more than once were viral meningitis, resolving bacterial meningitis, chronic lymphocytic leukemia, spondylosis, tabes dorsalis, and berry aneurysm.

The fluids from 29 patients showed an increase in monocytoid cells greater than 50%; in 15 the increase was absolute. The most frequent di-agnoses in this group were intervertebral disc disease (five patients), recent cranial surgery (six patients), and acute leukemia under therapy (eight patients).

Discussion

Among the various techniques available for concentrating the cells in CSF, sedimentation, cytocentrifugation, and filtration have been the most successful. Cellular morphology is better preserved by sedimentation and centrifugation. The former takes approximately 30 minutes longer to perform, and any added time may allow degenerative changes to occur in the cells. Our experience with cytocentrifugation indicates that this is an excellent technique for concentrating cells while maintaining their morphology. The technique is
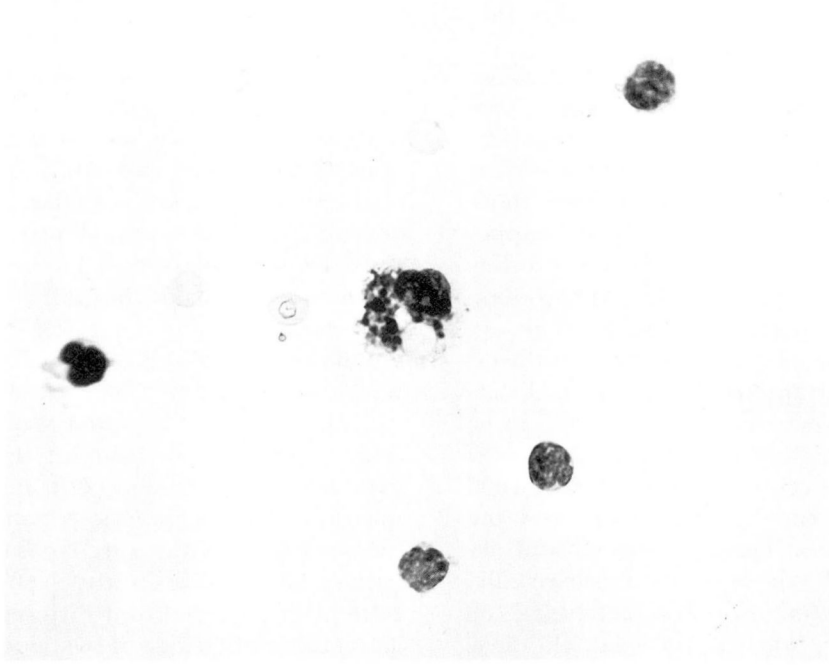
Fig. 3. Macrophage with ingested erythrocyte and hemosiderin-containing granules. The patient had a recent subarachnoid hemorrhage (Prussian blue counterstained with Wright's stain, ×640).
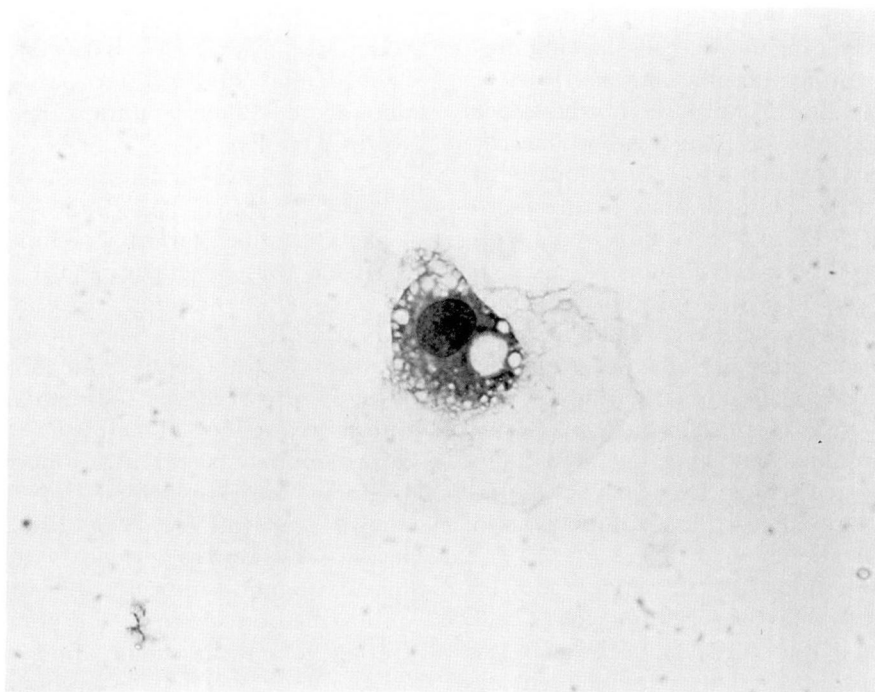
Fig. 4. Macrophage with ingested melanin granules. Although the patient had secondary meningeal melanomatosis, the bland nucleus with normal nucleoli and the vacuolated cytoplasm distinguish this cell from a malignant melanocyte (Wright's stain, ×640).

equally applicable to other body fluids which normally contain few cells. A possible bias may be introduced because larger mononuclear cells have been shown to stick more readily to glass than small lymphocytes. This may affect both the differential count itself and the distribution of various cell types on the slide.

The concentrating ability of the cytocentrifuge usually allows a differential count of at least 25 cells to be made on fluids containing 1 to 5 cells/μl. As a result, more accurate normal ranges can be established, and the distinction between normal and abnormal can be made more readily. The definition of a normal total cell count is still controversial; the level of 5 or less cells/μl is widely accepted,[11-13] although other investi-

gators have suggested an upper limit of 2/μl.[5, 14] In all probability the definition of normal should include both a total number and a differential count since, for instance, the presence of five cells may be abnormal if they are all neutrophil leukocytes, but may be normal if they are almost all lymphocytes.

Before establishing a normal differential count for CSF, the fluid must be normal in all other respects (e.g., color, protein content). However, a problem still exists since normal fluids include specimens containing no cells and other specimens contain so few cells, even after concentration, that a significant differential count cannot be made. Thus, our criterion of at least 25 recognizable cells automatically injects a bias into the

study. Nevertheless, the normal ranges we report should represent the upper limits of normal if not the "absolute" normal.

The normal ranges obtained in this study correlate with other published results and with the findings in a group of patients whose CSF would be expected to be normal (*Table 2*). Marks and Marrack[12] reported a distribution of 10% to 30% lymphocytes with approximately 70% mononuclear cells. This probably represents a difference in morphologic definition, although it may reflect a difference in cell distribution in the preparation used for the counts. The finding of neutrophil leukocytes in a normal CSF probably reflects a difference and perhaps an improvement in technique.[7]

The variety of terms used by authors reflects a lack of uniform opinion concerning the nature of the cells. In this study we did not subclassify lymphocytes in normal fluids because some distortion and variation in staining are inherent in the method; lymphocytes near the center of the slide are always smaller and denser than those at the periphery. The distinction between monocytes and reticulomonocytes represents two extremes in a morphologic spectrum. Since both are normal cells and

since diagnostic advantages have not been demonstrated in the distinction, we chose not to separate the species. In general, the cells appeared larger than those in blood smears as pointed out by Woodruff.[4]

The presence of macrophages appears to be diagnostically important,[1, 6, 15] and these cells should be separated in a differential count. When erythrophagocytosis is noted in a fresh specimen, it is indicative of bleeding into the CSF. This can often be striking. If the macrophages contain hemosiderin, bleeding started at least 18 hours before.[6] Lipomacrophages are said to be seen in traumatic or ischemic liquefaction necrosis,[1] and are therefore of use in distinguishing intracranial hemorrhages from infarcts. In the six cases of infarction in this series, no lipomacrophages were noted, perhaps because the vascular disturbances were too recent for gitter cell formation to occur. Lipomacrophages may also be seen after Pantopaque myelography and in Tay-Sachs disease.[14] The recognition of macrophages is also important because their pleomorphic nature may lead to a false positive cytologic diagnosis of malignancy.[12] The origin of these macrophages is in doubt. Rebuck et al[16] in skin window preparations observed transforma-

Table 2. Previously reported normal CSF differential counts

Tourtellotte et al, 1968[14]	Large lymphocytes	63% ± 18
	Small lymphocytes	17% ± 15
	Monocytes	16% ± 10
Sörnäs, 1972[13]	Lymphocytes	86.5% (99-63)
	Monocytoids	10.5% (28-0)
	Macrophages	2.0% (9-0)
Dyken, 1975[6]	Small lymphocytes	18% ± 14
	Large lymphocytes	45% ± 18
	Plain monocytes	6% ± 6
	Reticulomonocytes	31% ± 13
	Granulocytes	1% ± 1

tion of lymphocytes to macrophages. Sörnäs,[15] however, with phase contrast microscopy of living spinal fluid cells demonstrated a similar morphologic transformation of the monocytoid cell. Moreover, these cells show a greater propensity to transform in any inflammatory conditions characterized by CSF granulocytosis.

The identification of pia-arachnoid mesothelial cells is of interest because similar cells have been identified as ependymal or choroid plexus cells, and the shedding of such cells indicates a pathologic process within the craniospinal cavity.[1] This appeared to be true of the 10 patients in this series with such cells. The pia-arachnoid cells usually occurred singly, but were seen in increased numbers in those conditions marked by macrophage infiltration. Some pia-arach-

noid cells were phagocytic, and some clumps of macrophages had a bubbly cytoplasm reminiscent of that seen in the pia-arachnoid cell, making the distinction difficult at times. Wertlake et al[3] recognized that pia-arachnoid cells are normally phagocytic, and Sörnäs[15] speculated that macrophages may be derived from totipotential leptomeningeal stem cells. The recognition of ependymal cells depends on finding cuboidal to low columnar, sometimes ciliated cells arranged with polarity[3] (Fig. 5). Choroid plexus cells are similar but form larger groups and may suggest a papillary appearance.

We observed that stimulated lymphocytes and plasma cells were most commonly observed in multiple sclerosis and in various meningitides. This is in agreement with the find-
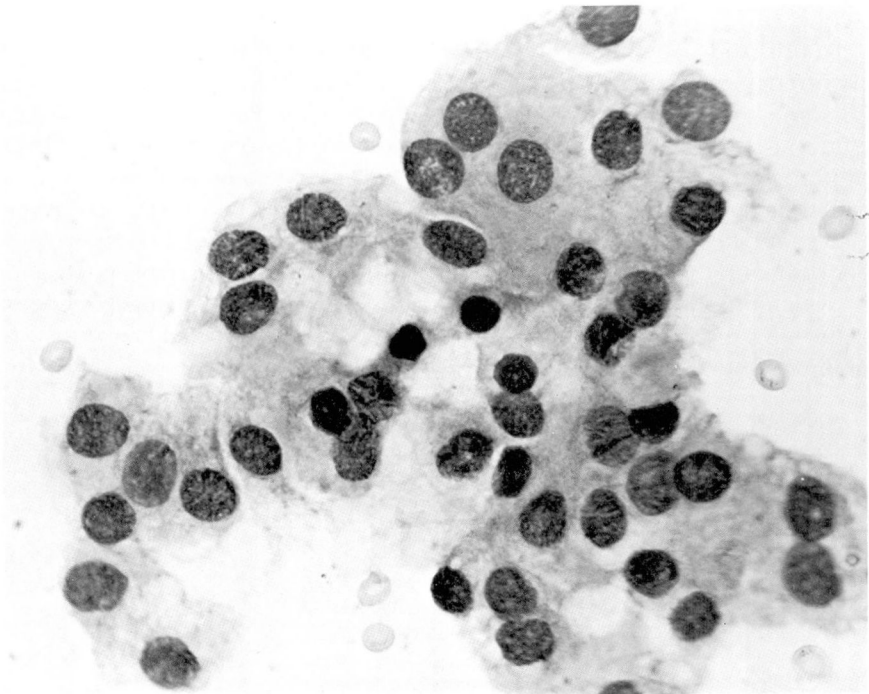

Fig. 5. Benign columnar cells from a 6-week-old infant representing normal ependyma (Wright's stain, ×640).

ings of Tourtelotte el al[14] who found similar plasmacytoid cells in the spinal fluids of 20% of patients with multiple sclerosis and Kolar and Zeman[1] who found similar cells to be evidence of subacute or chronic inflammation or demyelinating disease. We think that the somewhat vague term "atypical cell" is to be avoided in favor of "atypical lymphocyte" or "plasmacytoid lymphocyte" when describing these cells in a differential count; this prevents confusion with exfoliated or leukemic neoplastic cells.

The cytologic diagnosis of malignancies, other than lymphoma or leukemia, is more easily made on fixed specimens stained by Papanicolaou technique than on air-dried specimens.[3, 17–19] However, the slide prepared by centrifugation can be used with either staining method and has the advantage of using small amounts of fluid.

There is no doubt that a differential cell count is an essential part of the examination of CSF. The use of the cytocentrifuge permits the rapid preparation of well-preserved slides, and the morphology of the cells when stained with Wright's stain is quite comparable to that of peripheral blood smears. The slides may be stored for future reference.

Summary

The use of the cytocentrifuge to concentrate cells from CSF was studied. A total of 571 specimens were selected, in which at least 25 recognizable nucleated cells were found. The technique preserves the cellular morphology, and when as few as 1 or 2 cells/μl are present a reliable differential count can be performed. Seventy-five specimens were used to establish the normal leukocyte differential count of 75% ± 10 lymphocytes and 25% ± 12 monocytoid cells. Occasional segmented neutrophil leukocytes (0.4% ± 1.1) were found in normal fluids. The figure correlates well with published data. Clumps of pia-arachnoid mesothelial cells, ependymal cells, and macrophage transformation from monocytoid cells were seen only in abnormal fluids. In addition to the well-documented changes in inflammatory meningeal conditions, the following changes were noted among the abnormal fluids: lymphocytes may assume an activated or plasmacytoid appearance in multiple sclerosis; meningeal leukemia is reliably detected and solid tumors may exfoliate identifiable cells, but air-dried preparations are not the best for cytologic detection of such cells.

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