Automated Cerebrospinal Fluid Cytology

Limitations and Reasonable Applications

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OBJECTIVE: To evaluate the precision and clinical applicability of the Bayer ADVIA 120 cytometer (Bayer Healthcare, Fernwald, Germany) for cerebrospinal fluid (CSF) cell count and differentiation.
STUDY DESIGN: One hundred six analyses of CSF from 98 patients by the ADVIA 120 were compared with routine cell count and microscopic differentiation. Correlation coefficients were calculated.
RESULTS: In general, the total cell counts of both methods correlated well. The best correlations were seen at higher cell counts, ≥100 cells per microliter with <100 erythrocytes per microliter. The best correlations of cell differentiation were seen for lymphocytes and neutrophils, while the results for monocytes and eosinophils were less precise. In some cases, considerable differences between automated and microscopic cell counts and differentiation were seen that were relevant to clinical decision making. The detection of pathologic cell types, such as hemosiderophages, mitoses and neoplastic cells, was not provided by automated cytometry.
CONCLUSION: When experienced personnel are not available, a preliminary cell count and differentiation between neutrophilic and lymphocytic reactions by automated cytometry may be valuable in allowing initial therapeutic decision making. Since the detection of pathologic cell types is not provided and the precision at low cell counts is only moderate, a personal microscopic evaluation of each sample is still indispensable to avoid misdiagnoses. (Analyt Quant Cytol Histol 2005; 27:000–000)

Keywords: cerebrospinal fluid, flow cytometry, laboratory diagnosis.

Analysis of cerebrospinal fluid (CSF) is a key procedure for the diagnosis of infectious, hemorrhagic and neoplastic diseases of the nervous system. The analysis of CSF cells is important for the diagnosis of acute infections, CSF hemorrhage and neoplastic diseases. While automated quantification of blood cells is well established, CSF cells are still assessed predominantly with conventional microscopy. Automated cytometry is hampered by the low numbers of CSF cells, which are approximately 2,000

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times less numerous than blood cells. In the last few years, however, several cytometers (Abbott Cell Dyn 4000; Sysmex UF-100; ADVIA 120 [Bayer, Fernwald, Germany]) have been adapted and tested for CSF analysis.[1-5] All 3 analyzers are considered to be precise, reliable and suitable for automated assessment of CSF cells.

We evaluated the results achieved with the ADVIA 120 hematology analyzer for the concordance of microscopic and automated cell counts and differentiation, with special attention to the clinical impact of the results.

Materials and Methods

We evaluated 106 analyses of CSF samples from 98 consecutive patients analyzed routinely in our laboratory. Most of the samples were collected via lumbar puncture. Three samples were taken from ventricular catheters. Fifty-one samples had normal microscopic cell counts, and 47 had elevated counts. The indications for CSF examination were: inflammatory CNS disease (n = 43), CNS infections (23), psychiatric symptoms (12), neoplastic diseases (10), peripheral nervous system disease (4), epileptic seizures (1) and no information (3). The specimens were sent by the Departments of Neurology, Neurosurgery, Psychiatry and Hematology/Oncology.

Routine Examinations of CSF

In our laboratory, all CSF samples are examined microscopically to differentiate physiologic CSF cells and detect abnormalities. For this purpose, the CSF was centrifuged for 20 minutes at 1,000 rpm and the supernatant taken for chemical analyses. All centrifuged cells were resuspended with CSF medium (1 mL FCS + 9 mL CSF medium (50 mL Medium 199 [ICN Biomedicals Inc., Steinheim, Germany]; 444 mL distilled water; 5 mL penicillin/streptomycin; and 1 mL sodium bicarbonate, 7.5%). Cytospin probes were produced by centrifugation of 5,000–10,000 cells at 1,000 rpm for 10 minutes. The probes were stained with the standard May-Grünwald-Giemsa procedure. Approximately 100 cells were differentiated microscopically and classified as lymphocytes; monocytes; polymorphonuclear, eosinophilic or basophilic granulocytes; and plasma cells. Bone marrow cells, phagocytes, mitoses, cells lining the CSF space and bacteria were described separately. The time required for the cell count in a Fuchs-Rosenthal chamber was 3–5 minutes. The preparation and staining of the cytospin slides took 15–20 minutes for 1 or multiple slides. Leukocyte differentiation took 10–15 minutes per slide.

Processing of CSF for Automated Cytometry

During the study period, 300 µL of uncentrifuged CSF that was not needed for routine analysis was diluted 1:1 with ADVIA 120 working solution (Bayer Healthcare) and analyzed after 10 minutes to 6 hours in the cytometer.

The ADVIA System

The ADVIA 120 CSF assay is a specific CSF program available for the ADVIA 120 hematology analyzer. With direct flow cytometry, CSF cells and erythrocytes are differentiated according to their size and granularity, measured as forward and side scatter. The gating to distinguish the different cell populations is preadjusted. Total count and differentiation of CSF leukocytes is reported, as is the amount of erythrocytes.

To prepare CSF analyses with the ADVIA 120, a washing procedure of approximately 6 minutes was performed. CSF was mixed 1:1 with 300 µL of ADVIA 120 CSF reagent (Bayer Healthcare), which consists of formaldehyde and glutaraldehyde and ensures fixation of the cells. After an incubation period of 10 minutes, 150 µL of the solution was aspirated by the analyzer. The cytometer provided absolute counts of red and white cells and absolute and relative numbers of the differential white cell count. Reported cell types were lymphocytes, monocytes, neutrophils and eosinophils. Additionally, the percentage of mononuclear and polymorphonuclear cells was shown. Detection of cells atypical in size or shape is not provided by the system. In addition to the statistical results, scatter cytograms were generated to demonstrate the analyzed cell populations.

Statistical Analyses

Microscopic cell counts and leukocyte differentiation were compared with automated cytometry. Correlation coefficients were calculated for total red and white cell counts and for each white cell population reported with the Passing-Bablok test. Additionally, sample cases were analyzed for the clinical relevance of the results.

Results

After the washing procedure, which lasted 3–10 minutes, the ADVIA 120 provided the results within a few seconds. When the washing procedure and incubation with the CSF reagent were done in par-

Table I *Coefficients r for the Correlation (Passing-Bablock) Between Microscopic and Automated Cell Count and Differentiation: Correlation for Total Number of Leukocytes*

Cell type	Correlation *r*
Leukocytes	0.984
Erythrocytes	0.367
Lymphocytes	0.761
Monocytes	0.367
Neutrophils	0.913
Eosinophils	0.015

Table II *Coefficients r for the Correlation Between Automated and Microscopic Cell Counts: Cytometric Accuracy with Low Erythrocyte and High Leukocyte Counts*

Total cell count	n	Correlation *r* for leukocytes
Leukocytes < 25	58	0.619
Leukocytes 25–99	19	0.797
Leukocytes ≥ 100	21	0.980
Erythrocytes < 100	81	0.9183
Erythrocytes ≥ 100	17	0.8725

allel, results could be achieved after approximately 10 minutes. No major problems were experienced with the CSF analysis program during the evaluation period. For routine examinations, it was feasible to collect all samples from that day to be analyzed together after the hematologic program.

Total White Blood Count

In general, the correlation between the conventional microscopic count and the automated method was good, with a coefficient *r* of 0.984 (Table I). The precision of the counts depended on the number of CSF cells. The best correlation was found with cell counts ≥ 100, with an *r* of 0.9286 (Table II, Figure 1). In samples with microscopic cell counts of 25–99 cells per microliter, *r* was 0.797 (Figure 2). Only a moderate correlation was seen with counts < 25 cells per microliter, with an *r* of 0.619 (Figure 3). In 4 cases with normal microscopic counts, the cytometer assessed elevated cell counts of 8–25 cells (4% false positive results). In only 1 case with an el-

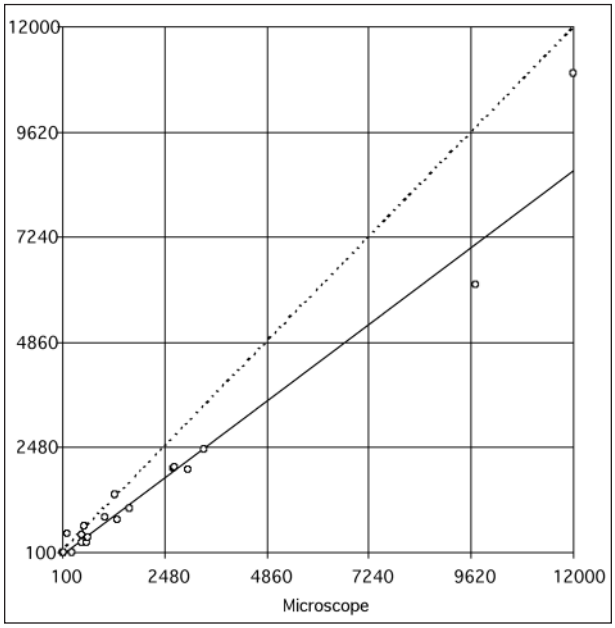

Figure 1 Linear regression (continuous line) for the correlation between microscopic (x-axis) and automated (y-axis) cell count as assessed with the Passing-Bablock test. The best correlation was seen with a total cell count of ≥ 100 (1); the correlation was less reliable with counts of 25–99 cells (2) and with > 25 cells (3). No correlation was seen for lymphocyte counts (4). The correlation was relatively good for neutrophil counts (5) and for low contents of erythrocytes (6).

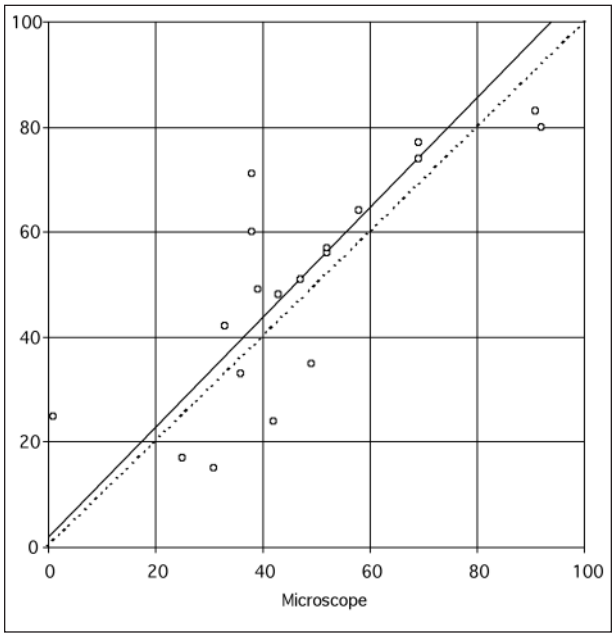

Figure 2 Linear regression (continuous line) for the correlation between microscopic (x-axis) and automated (y-axis) cell count as assessed with the Passing-Bablock test. The best correlation was seen with a total cell count of ≥ 100 (1); the correlation was less reliable with counts of 25–99 cells (2) and with > 25 cells (3). No correlation was seen for lymphocyte counts (4). The correlation was relatively good for neutrophil counts (5) and for low contents of erythrocytes (6).

Table III *Results of 8 Sample Double Examinations of the Same CSF Samples*

Case (no.)	*Leuko (no.)*	Leuko (no.)	*Ery (no.)*	Ery (no.)	*Lympho (%)*	Lympho (%)	*Mono (%)*	Mono (%)	*Neutro (%)*	Neutro (%)	*Eo (%)*	Eo (%)
1	*0*	1	*0*	0	*95*	100	*5*	0	*0*	0	*0*	0
		0		0		100		0		0		0
2	*1*	1	*Few*	83	*70*	75	*23*	25	*7*	0	*Few*	0
		1		73		100		0		0		0
13	*3*	3	*0*	0	*86*	84.6	*14*	15.4	*0*	0	*0*	0
		6		0		100		0		0		0
15	*1*	3	*0*	0	*91*	30	*9*	70	*0*	0	*0*	0
		0		0		100		0		0		0
18	*1*	3	*0*	2	*76*	83.3	*24*	8.3	*0*	8.3	*0*	0
		2		2		50		50		0		0
29	*1*	1	*0*	0	*85*	50	*7*	50	*0*	0	*0*	0
		1		1		100		0		0		0
46	*1*	1	*45*	39	*86*	83.3	*14*	16.7	*0*	0	*0*	0
		3		42		91.7		0		8.3		0
50	*4*	3	*0*	2	*76*	64.3	*24*	35.7	*0*	0	*0*	0
		4		0		56.3		37.5		6.3		0

Columns in italics represent the microscopic evaluation.
Leuko = leukocytes, Ery = erythrocytes, Lympho = lymphocytes, Mono = monocytes, Neutro = neutrophils, Eo = eosinophils.

evated microscopic count of 11 cells did the cytometer count zero cells (1% false negative results).

The precision depended also on contamination with blood (Table II). The correlation coefficient *r*

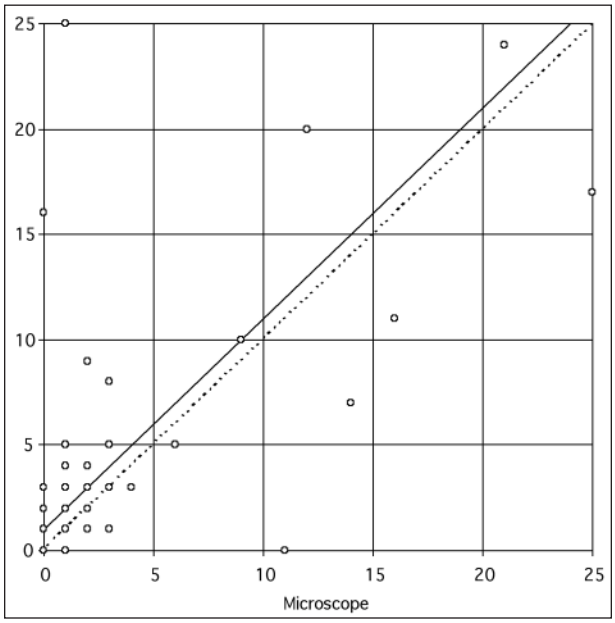

Figure 3 Linear regression (continuous line) for the correlation between microscopic (x-axis) and automated (y-axis) cell count as assessed with the Passing-Bablock test. The best correlation was seen with a total cell count of ≥100 (1); the correlation was less reliable with counts of 25–99 cells (2) and with >25 cells (3). No correlation was seen for lymphocyte counts (4). The correlation was relatively good for neutrophil counts (5) and for low contents of erythrocytes (6).

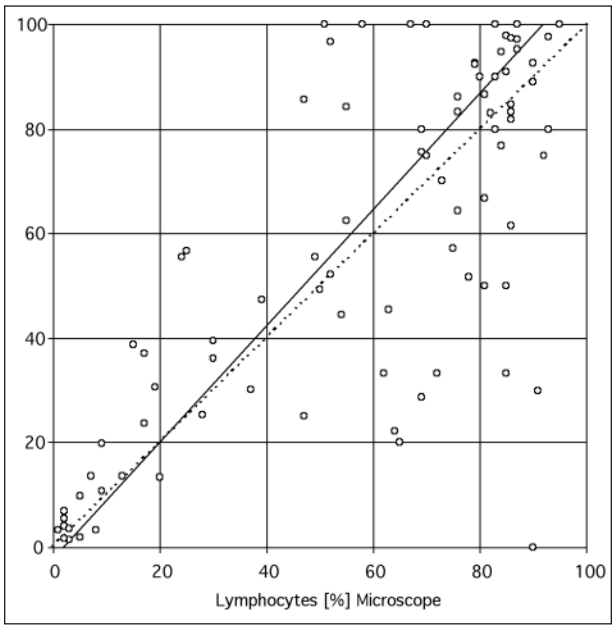

Figure 4 Linear regression (continuous line) for the correlation between microscopic (x-axis) and automated (y-axis) cell count as assessed with the Passing-Bablock test. The best correlation was seen with a total cell count of ≥100 (1); the correlation was less reliable with counts of 25–99 cells (2) and with >25 cells (3). No correlation was seen for lymphocyte counts (4). The correlation was relatively good for neutrophil counts (5) and for low contents of erythrocytes (6).

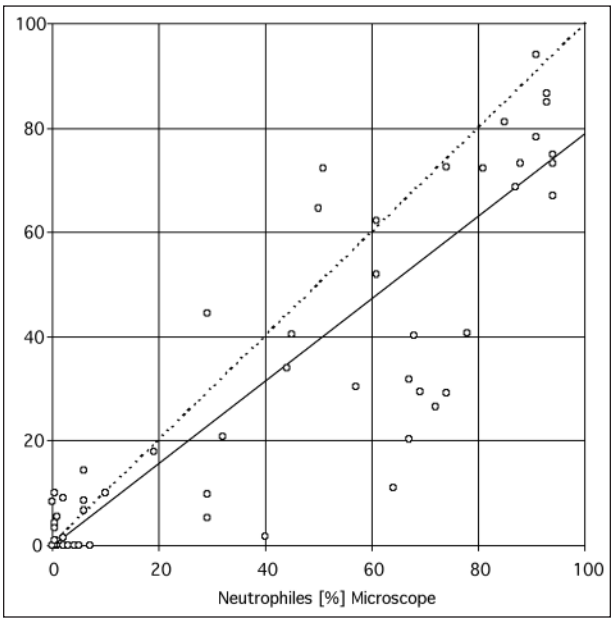

Figure 5 Linear regression (continuous line) for the correlation between microscopic (x-axis) and automated (y-axis) cell count as assessed with the Passing-Bablock test. The best correlation was seen with a total cell count of ≥ 100 (1); the correlation was less reliable with counts of 25–99 cells (2) and with > 25 cells (3). No correlation was seen for lymphocyte counts (4). The correlation was relatively good for neutrophil counts (5) and for low contents of erythrocytes (6).

was better in samples with < 100 red blood cells (r = 0.9183, n = 81) than in those with ≥ 100 erythrocytes (r = 0.8725, n = 17). All double examinations were done on samples with normal cell counts. Differences of > 1 cell per microliter between the 2 automated counts were seen in 3 of 8 samples (Table III). In 1 of these samples (case 13), the first count gave normal and the second, elevated cell count.

Cell Differentiation

In cases of neoplastic infiltration or bone marrow aspirate, a quantitative microscopic cell differentiation was not done, in accordance with the rules in our laboratory. Therefore, the total number of differentiation analyses was lower than for the total cell counts.

Lymphocytes

The correlation between the methods for the lymphocyte count was worse than with the total cell count, with r of 0.761 (Figure 4). In double examinations, 4 of 8 repeated counts differed by > 20% from the first value.

Monocytes

No correlation was found for the monocyte counts, with r of 0.0144. The cytometric values differed in double examinations in 4 of 8 samples by > 20%.

Granulocytes

A relatively good correlation was found for neutrophil counts, with r of 0.913 (Figure 5). By contrast, the determination of eosinophils by the ADVIA 120 did not correlate with the microscopic method (r = 0.015). Consistent with the microscopic evaluation, no eosinophils were counted in any of the double examinations.

In cases of low cell numbers, 1–3 per microliter, the ADVIA 120 calculated percentages of lymphocytes or monocytes of exactly 33, 50, 66 or 100. Obviously, these percentages were not calculated from the total number of counted cells (most often > 100) but referred to the number of cells per microliter (e.g., 2 cells per microliter: 50% lymphocytes, 50%

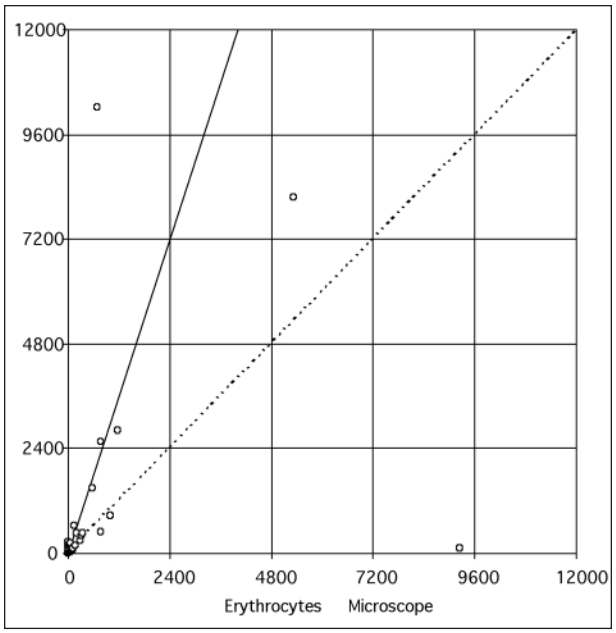

Figure 6 Linear regression (continuous line) for the correlation between microscopic (x-axis) and automated (y-axis) cell count as assessed with the Passing-Bablock test. The best correlation was seen with a total cell count of ≥ 100 (1); the correlation was less reliable with counts of 25–99 cells (2) and with > 25 cells (3). No correlation was seen for lymphocyte counts (4). The correlation was relatively good for neutrophil counts (5) and for low contents of erythrocytes (6).

monocytes).

Red Blood Count

The determination of red blood count correlated well with the microscopic method at low levels of CSF blood contamination. The overall *r* was 0.7406 in the whole group (Table I). A considerable discrepancy was seen in some cases with >500 erythrocytes counted microscopically (Figure 6). In 1 case with >9,000 red blood cells counted microscopically, only 121 erythrocytes were counted by flow cytometry.

Other Cell Types and Contents

The detection of cell types other than physiologic CSF cells is not integrated into the CSF analysis program. Therefore, the diagnosis of pathologic cell types, such as hemosiderophages, bone marrow aspirate, mitoses or tumor cells, is not possible with the cytometer.

Case Reports

One patient with CSF involvement by a primary central nervous system lymphoma was analyzed during the study. The total cell automated count was correctly assessed at 1 cell per microliter by flow cytometry. The percentage of lymphocytes and monocytes was 50 each at automatic cell count (microscope: 85% lymphocytes, 7% monocytes). The scattergram showed no abnormalities. The microscopic evaluation, however, revealed 8% malignant cells with abnormalities of size and shape, enhanced nuclear/cytoplasmic ratio, nucleoli and several mitotic figures.

In 1 female patient with a known plasmacytoma, lumbar puncture was performed to exclude meningitis and malignant cells. The total cell counts correlated well, with 0 cells counted microscopically and 1 cell per microliter assessed by machine. The cytometer counted 33% monocytes and 67% neutrophilic granulocytes. Microscopic analysis showed numerous bone marrow cells that were not differentiated as CSF cells, in accordance with the rules.

An immunocompromised patient presented in our emergency room with fever and neck stiffness. The ADVIA counted 24 leukocytes, and differentiation showed 39% lymphocytes, 18% monocytes, 20% neutrophils and 23% eosinophils. The microscopic evaluation revealed 42 cells, with 67% neutrophils, 18% monocytes, 15% lymphocytes and abundant numbers of bacteria intracellularly and extracellularly. The microbiologic workup of the sample revealed a pneumococcal infection.

Discussion

The ADVIA 120 cytometer is the only analyzer reported in the literature that provides differentiation into lymphocytes, monocytes, neutrophils and eosinophils.[3-5] In the study described here, automated cytometry with the ADVIA 120 provided acceptable results for counting total cell content, lymphocytes and neutrophils. Monocyte counts were less precise, and the assessment of eosinophils did not correlate with the microscopic evaluation. The detection of pathologic cell types or conditions, such as hemosiderophages, mitoses and neoplastic cells, is not provided by any of the cytometers that are designed for CSF cell analysis.[1,3-5]

In contrast to other studies, in which a considerable number of samples (19%) was completely excluded[5] or cell differentiation was performed only with cell counts >5 per microliter[3] or 10 per microliter,[4] we analyzed all consecutive samples for cell count and differentiation. This may be the major reason why the correlation with microscopic counting was better in previous reports than in our study. The best results of both total count and differentiation in our study were achieved with leukocyte counts ≥100 per microliter and erythrocyte counts <100 per microliter. For clinical decision making, however, the highest accuracy is required around the cutoff of 4 cells per microliter to determine if the cell count is normal or pathologic. Although correlation coefficients and p values of automated cytometry were acceptable at lower cell counts, also, we observed 4% false positive and 1% false negative results for the differentiation between normal and pathologic cell counts. In 1 case of meningitis with an anergic reaction of only 42 cells per microliter but clear neutrophilic predominance, the cytometer underestimated both total cell count and content of neutrophils. In our view, the precision of the automated cell count in cytopenic samples is not yet sufficient to rely upon. For microscopic analysis, a maximum of 15 cells has to be counted in the Fuchs-Rosenthal chamber (volume, 3 µL) to quantify normal counts of up to 5 cells per microliter. No significant imprecision of microscopic counting can be expected at these cell counts.

None of the analyzers reported to date was designed to detect pathologic cell types or conditions.[1,3-5] Only the CellDyn 4000 (Abbott) has been reported to detect unknown but also classifies

leukemic cells as lymphocytes and not as atypical.[4] Also in our study, 1 case of lymphomatous meningeosis showed no distinct cluster in the cytogram. In order to avoid misdiagnoses, microscopic cell differentiation cannot be replaced by a cytometer. Also, restriction of cytologic examination to cases of elevated cell counts or suspected neoplastic diseases is not feasible. Over the last 2 years, microscopic differentiation in our laboratory revealed neoplastic cells in 12 samples with normal cell counts. In 1 of these patients, a neoplastic disease had not been expected and would have been overlooked with such a restrictive strategy.

The study presented here was designed to evaluate the cytometer as a ready-to-use method without specific analysis of the underlying technique. One point of consideration, however, was the use of the analysis reagent, which is a mixture of formaldehyde and glutaraldehyde. A cytospin preparation of cells fixed with this solution showed marked shrinkage of the cells, which diminished the differences in size of the distinct cell types (not shown), comparable to the known alterations of cells fixed with formaldehyde. A method that differentiates cells by analyzing size (forward scatter) and granularity (side scatter) may be altered by such a shrinkage of cells. Another concern was that the ADVIA obviously calculated the percentages of the differential cell count from the number of leukocytes per microliter instead from all evaluated cells (usually >100). In that way the imprecision of the leukocyte differentiation appeared to be enhanced artificially. Finally, eosinophils were not detected reliably. In our view, a cumulative assessment of all types of granulocytes might be easier to achieve by automated cytometry and would be sufficient for clinical purposes.

In summary, although statistical evaluation for overall cell counts showed good results, the imprecision at lower cell counts and lack of detection of pathologic cell types and conditions prevents replacement with the ADVIA microscopic CSF evaluation even with normal cell counts. The cytometer, however, can achieve quicker results than the microscopic method, which takes about 30 minutes in total. When experienced personnel are not available or a rapid therapeutic decision is needed, a preliminary cytometric analysis of cell count and differentiation can be valuable.

We conclude that automated cytometry provides acceptable precision for a preliminary count of red and white blood cells and differentiation of leukocytes in the CSF, especially with elevated cell counts. Imprecision at lower cell counts and the lack of detection of pathologic cells still make microscopic evaluation of each CSF sample indispensable to avoid misdiagnoses.

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