RESEARCH ARTICLE

Use of the Sysmex XT-4000i hematology analyzer in the differentiation of cerebrospinal fluid cells in children

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Background: Routine analysis of pleocytosis and cellular composition of cerebrospinal fluid (CSF) is carried out with a phase-contrast microscope. The use of hematology analyzers seems to be an alternative to the manual method. The aim of the study was to assess the usefulness of the automated technique for counting and differentiating CSF cells in children.

Methods: The study group consisted of 59 children (28 girls and 31 boys) aged from 4 to 17 years suffering from viral and bacterial meningitis. Children were divided into three subgroups according to CSF cell count: 1st group had a pleocytosis of 6–50 cells/ μL , 2nd group—51–100 cells/ μL , and 3rd group— >100 cells/ μL . A reference group involved 32 children (17 girls and 15 boys) aged from 2 to 18 years with a normal range of 0–5 cells/ μL . Examination of CSF was performed in parallel by two different methods, manual and automated.

Results: The analysis of pleocytosis revealed that the values obtained by the manual method were statistically significantly lower in relation to the values obtained by automated technique in subgroups I and II. The number of mononuclear and polymorphonuclear cells in subgroups I, II, and III determined by both manual and automated methods was comparable.

Conclusion: We conclude that automated method cannot fully replace the previously used manual method and some of the dubious cases, such as samples with low pleocytosis rates or abnormal cells indicated by the analyzer, will still require microscopic examination.

KEYWORDS

automated method, cerebrospinal fluid, manual method, mononuclear cells, pleocytosis, polymorphonuclear cells

1 | INTRODUCTION

Cerebrospinal fluid (CSF) is a clear liquid that circulates through fluid-filled cavities of the central nervous system (CNS). It exhibits specific physical and biochemical properties, which can change in pathological conditions affecting the CNS. Evaluation of the chemical and cellular composition of CSF is one of the most

important examinations in the diagnostics of neuroinfections and CNS neoplasms.^{1,2}

Cytodiagnostic analysis of CSF is essential in the diagnosis of CNS infections. The number and type of cells present in the fluid can clearly indicate the type of infection; for example, in bacterial inflammatory diseases, granulocytic pleocytosis reaches 1000 cells/ μL , while viral meningitis is characterized by

a predominance of lymphocytes in CSF.³⁻⁵ Routine analysis of pleocytosis and cellular composition of CSF is carried out with a phase-contrast microscope. The standard procedure requires counting nucleated cells using the Fuchs-Rosenthal chamber and performing the May-Grunwald-Giemsa stain to assess cell morphology.⁶ The use of hematological analyzers seems to be an alternative to the manual method in the determination of CSF pleocytosis. The analyzers have a mode of operation specially adapted to determine the rate of CSF pleocytosis.^{3,7,8} It seems that the automated methods may have some advantage over the microscopic analysis because of their higher precision, lower time consumption and effort, and 24-hour availability. This is very important especially in pediatric patients who need rapid diagnosis and treatment.

Therefore, the aim of the study was to assess the usefulness of the body fluid mode of the Sysmex XT-4000i hematology analyzer for counting and differentiating CSF cells in children. The novelty of the current study is the involvement of the pediatric population with a broad age group divided into three subgroups according to pleocytosis.

2 | MATERIALS AND METHODS

2.1 | Study group

The study group consisted of 59 children (28 girls and 31 boys) aged from 4 to 17 years (mean age: 7.8 ± 6.5) suffering from viral and

bacterial meningitis who were diagnosed at Emergency Department of Children's Clinical Hospital in Bialystok. These patients had no history of cancer. Children were divided into three subgroups according to the CSF cell count. Detailed characteristics of patients are presented in Table 1.

A reference group involved 32 children (17 girls and 15 boys) aged from 2 to 18 years (mean age: 6.8 ± 7.3) with a normal range of 0-5 cells/ μ L. These children had no history of neurological disease or cancer.

The study was approved by the local research ethics committee for Medical University of Bialystok (R-I-002/106/2016). Written informed consent was obtained from parents or guardians after explanation of the nature of the study.

2.2 | CSF sampling

Cerebrospinal fluid specimens (2 mL) were taken via lumbar puncture and collected in the sterile tubes. Material that remained after routine analysis was used. Examination of CSF was performed immediately after sample collection, in parallel by two different methods, manual and automated. In manual technique, the native cells (pleocytosis) were counted unstained using a Fuchs-Rosenthal counting chamber (gold standard technique), and CSF smears were prepared by May-Grunwald Giemsa stain method (for differentiating WBCs into mononuclear cells: MNC and polymorphonuclear cells: PMCs). In automated procedure, the single platform Sysmex® XT-4000i (Sysmex Corporation, Kobe, Japan) with body fluid mode was used for counting as well as differentiating white blood cells in CSF.

TABLE 1 Characteristics of the study group

Group/subgroups	Pleocytosis (cells/ μ L)	Diagnosis No. (%) of cases	Etiology
Study whole (n = 59)	6-592	Meningitis: Viral n = 44 (75%) Bacterial n = 15 (25%)	<i>Enteroviral meningitis:</i> Coxsackie B5 Coxsackie B3 ECHO 9 Coxsackie B4 <i>Bacterial meningitis:</i> Streptococcus agalactiae Streptococcus pneumoniae Neisseria meningitidis
Subgroup I (n = 25)	6-50	Meningitis: Viral n = 19 (78%) Bacterial n = 6 (22%)	
Subgroup II (n = 14)	51-100	Meningitis: Viral n = 11 (80%) Bacterial n = 3 (20%)	
Subgroup III (n = 20)	>100	Meningitis: Viral n = 13 (66%) Bacterial n = 7 (34%)	

2.3 | Methods

2.3.1 | Determining the rate of pleocytosis by manual counting in a Fuchs-Rosenthal chamber and evaluation of CSF cells in May-Grunwald-Giemsa-stained samples

Pleocytosis, that is, the number of cells in 1 μL of CSF, was determined using the Fuchs-Rosenthal chamber. To this end, 100 μL of CSF was dispensed into test tubes and 10 μL of Samson's reagent was added in order to visualize the leukocyte nuclei. The samples were then mixed and allowed to stand for several minutes at room temperature. The liquid thus prepared was placed in a Fuchs-Rosenthal chamber and viewed in a phase-contrast microscope under 40 $\times$ magnification. Cells were counted on the entire surface of the mesh, and the achieved result was divided by 3, thereby obtaining the number of cells in 1 μL of CSF. In order to differentiate CSF cells, May-Grunwald-Giemsa-stained samples were prepared. The fluid was centrifuged in a cytocentrifuge for 8 minutes at 700 g . The cell preparations on the primary slides were allowed to dry. They were then stained with May-Grunwald's reagent for 3 minutes and then with Giemsa's reagent diluted 1:20 for 7 minutes. Dried slides were viewed under 100 $\times$ magnification using immersion oil. Each sample was viewed, following which cells were counted to 100 and divided into 4 groups: lymphocytes, granulocytes, monocytes, and macrophages. Microscopic examination of CSF did not show malignant cells.

2.3.2 | CSF pleocytosis and mononuclear and polymorphonuclear cell analysis using the Sysmex XT-4000i

Sysmex® XT-4000i analyzers for performing 5-Diff analysis utilize fluorescence flow cytometry. The analyzer enables automatic analysis of CSFs. It provides information on the number of white and red blood cells in CSF, as well as the total nucleated cell (TNC) count and polymorphonuclear (granulocyte) count. The TNC count may include macrophages, erythrophages, cancer cells, and plasma cells. WBCs are separated into individual groups based on the complexity of the cells (side scattered light and fluorescent signal). The sample is stained with a fluorescent dye that binds to intracellular DNA and RNA. The fluorescence signal strength is proportional to the RNA/DNA content of individual cells.

2.4 | Performance studies

The WBC within-run precision testing for the Sysmex XT-4000i analyzer was performed on six CSF samples that were analyzed 10 consecutive times. The analyses were carried out in normal conditions, by the same operator, in the same period of day and with the same reagent lots. Mean, standard deviation (SD), and coefficient of variation (CV) of the WBC count were calculated in each sample. The acceptable precision of the analyzer for CSF

counting is CV $\leq 30\%$ for WBC-BF count from $0.015 \times 10^3/\mu\text{L}$ to $0.030 \times 10^3/\mu\text{L}$.

The WBC carryover testing for the Sysmex XT-4000i analyzer was performed on six CSF samples and blank (CellPack Diluent; Sysmex Europe GmbH, Norderstedt, Germany). The CSF samples had different levels of WBC count. The samples were analyzed in triplicate (H1, H2, and H3), immediately followed by the analysis of the CellPack also in triplicate (L1, L2, and L3). Carryover values were calculated by the Broughton formula: carryover (%) = $(L1-L3) \times 100 / (H1-L3)$. The carryover value should not exceed that given by the manufacturer (WBC-BF up to 0.3% or $0.001 \times 10^3/\mu\text{L}$).

The WBC linearity testing for the Sysmex XT-4000i analyzer was performed on three CSF samples with a different number of cells. The samples were diluted in series with the CellPack diluent to obtain 10 levels of concentration. Each of the diluted samples was analyzed three consecutive times. Expected and measured cell counts were plotted. Bias (%) was calculated as mean obtained values minus expected values and divided by expected values and multiplied by 100. According to the manufacturer, the WBC-BF count of the analyzer is linear from 0 to $0.050 \times 10^3/\mu\text{L}$ (bias of approximately 10 cells) and from 0.050 to $10\,000 \times 10^3/\mu\text{L}$ (bias of $\pm 20\%$).

2.5 | Statistical analysis

All analyses were conducted in Statistica 12 PL (StatSoft Polska, Cracow, Poland) and free MedCalc for Windows trial (MedCalc software, version 18.6, MedCalc Software BVBA, Ostend, Belgium). To check the normality of distribution, the Shapiro-Wilk test was used. Because of variables were distributed not normally, nonparametric tests were applied in the analyses. Results were expressed as median and range. The differences between number of cells obtained by manual and automatic methods were evaluated with Wilcoxon signed-rank test for dependent samples. To check the agreement between the methods, the Passing-Bablok regression analysis and to show the bias, the Bland-Altman difference plots were used. Passing-Bablok test calculates a linear regression equation ($y = a + bx$), including 95% CIs for the intercept (a) and the slope (b). We tested the assumption of linearity by the cumulative sum linearity test (CUSUM test). In Bland-Altman analysis, absolute average differences (y -axis) and arithmetic averages (x -axis) of cell count by the two methods were plotted. The 95% confidence intervals (95% CI) were calculated to highlight significant differences between the methods. The correlations between number of cells obtained by two methods were analyzed using Spearman's correlation. The closer the values came to 1, the stronger the positive correlation was. The P -value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Within-run precision

The results of within-run precision for pleocytosis, MN, and PMN counts are presented in Table 2. The precision profile depends on the number of cells that are counted. The samples with low counts

Sample	Pleocytosis (Cells/ μ L)	CV (%)	MN (%)	CV (%)	PMN (%)	CV (%)
CSF 1	4	36.9	2	316	98	6.4
CSF 2	10	30.3	12	80.7	88	10.6
CSF 3	14	19.7	95	5.6	5	98.4
CSF 4	40	12.5	84	5.9	16	30.6
CSF 5	83	10.8	76	3.2	24	40.4
CSF 6	107	12.1	85.5	6.5	14.5	38.2

CSF, cerebrospinal fluid; CV, coefficient of variation; MN, mononuclear; PMN, polymorphonuclear.

TABLE 2 Results of precision expressed as CV of Sysmex XT-4000i for pleocytosis, MN, and PMN counts

of pleocytosis (≤ 10 cells/ μ L) showed CVs higher than those given by the manufacturer for WBC-BF count from 15 to 30.

3.2 | Carryover

The results of carryover are shown in Table 3. Carryover for pleocytosis, MN and PMN counts was 0.00% and did not exceed that specified by the manufacturer (WBC-BF up to 0.3% or $0.001 \times 10^3/\mu$ L).

3.3 | Linearity

Linearity is shown in Table 4 and Figure 1. Pleocytosis, MN, and PMN counts showed high coefficients of determination (r^2), demonstrating that the linear model resulting from the dilutions used was statistically reliable. Linearity for all types of cells was very good, although it was better in higher concentration ranges as compared to lower ranges. The mean bias was -4.0% for pleocytosis, $+0.5\%$ for MN, and -1.8% for PMN. The results of bias were in the acceptable limits of the analyzer.

3.4 | Comparison between Sysmex XT-4000i and manual method

Statistical analysis showed that the median number of cells present in 1μ L of CSF determined by the manual method was statistically significantly lower in the whole study group in comparison with the median value obtained by the automated method (58 vs 63, $P < 0.008$; Table 5). By contrast, the number of MNC and PMNC determined by each method did not differ significantly in

the patients (80 vs 76, $P = 0.205$ and 20 vs 23, $P = 0.125$; Table 5). A statistically significant difference between the median pleocytosis determined by the traditional method and the median pleocytosis determined by the automated method, ($P < 0.001$) was observed in the control group.

The analysis of pleocytosis in terms of the number of cells in 1μ L of CSF revealed that the values obtained by the manual method were statistically significantly lower in relation to the values obtained by automated technique in subgroups I and II (18 vs 25, $P = 0.002$ and 64 vs 71.5, $P < 0.05$, respectively; Table 5). However, no statistically significant differences were observed in subgroup III (177.5 vs 160, $P = 0.751$; Table 5).

The number of MNC and PMNC in subgroup I determined by both manual and automated methods was comparable (81 vs 81 at $P = 0.407$ and 19 vs 19 at $P = 0.808$; Table 5). Similarly, in subgroups II and III there were no statistically significant differences: for MN 85.5 vs 77, $P = 0.080$ and 71 vs 57.5, $P = 0.240$, and for PMN 14.5 vs 23, $P = 0.081$ and 29 vs 43, $P = 0.279$ (Table 5). Analysis of the correlation between variations in the number of cells obtained by the manual and automated methods showed a significant positive correlation for each type of cells between the two methods, both in the whole study group and in subgroups (Table 5). The correlation between manual and automated methods was generally very strong or strong; however, wide dispersion of some results around the regression line was seen.

3.5 | Accuracy

Agreement between the methods was determined using Passing-Bablok regression analysis and Bland-Altman bias plots. The manual method was considered the reference procedure.

The Passing-Bablok regression analysis for the pleocytosis and the number of MN and PMN cells obtained by the two methods in the whole study group and subgroups are shown in Table 6 and Figure 2. In general, a CUSUM test for linearity showed no significant deviation from linearity (Table 6). There was a good agreement between manual pleocytosis and pleocytosis performed using Sysmex XT-4000i in the whole study group ($y = 3.58 + 1.03x$; CI slope: 0.96-1.12; CI intercept: -0.02 to 5.79 ; mean bias: $-5.4 \times 10^6/L$; Table 6, Figure 2, Figure 3). The Spearman's correlation between these methods was very strong, although the Wilcoxon signed-rank test showed a significant difference between the number of cells (Table 5). Further analysis using the

TABLE 3 Carryover for the Sysmex XT-4000i body fluid mode

Sample	Pleocytosis		Carryover (%)	
	Mean (Cells/ μ L)	Carryover (%)	MN	PMN
CSF 1	14	0.00	0.00	0.00
CSF 2	30	0.00	0.00	0.00
CSF 3	122	0.00	0.00	0.00
CSF 4	136	0.00	0.00	0.00
CSF 5	174	0.00	0.00	0.00
CSF 6	2675	0.00	0.00	0.00

CSF, cerebrospinal fluid; MN, mononuclear; PMN, polymorphonuclear.

TABLE 4 Results of linearity of Sysmex XT-4000i for pleocytosis, MN, and PMN counts

Parameter	Range tested ($\times 10^3/\mu\text{L}$)	r^2	Slope	Intercept	Bias (%)
Pleocytosis	0.005-0.066	0.9305	0.8176	5.9826	7.79
MN	0.002-0.039	0.9152	0.9003	3.2091	14.3
PMN	0.003-0.027	0.9074	0.7290	2.4953	0.18
Pleocytosis	0.001-0.451	0.9985	0.9832	-6.5556	-6.58
MN	0.001-0.204	0.9180	0.9851	2.1110	-9.76
PMN	0.001-0.247	0.9998	0.9692	-0.5858	-3.38
Pleocytosis	0.016-1.241	0.9892	0.9529	4.1053	-3.82
MN	0.001-0.114	0.9942	0.9942	-5.9429	3.25
PMN	0.016-1.127	0.9755	0.9547	12.6281	-1.45

MN, mononuclear; PMN, polymorphonuclear.

FIGURE 1 Linearity of XT-4000i counts in cerebrospinal fluid for pleocytosis, mononuclear cells, and polymorphonuclear cells counts. The identity line is drawn

Bland-Altman plots revealed that the mean bias ranged from -8.0 to $-10.2 \times 10^6/\text{L}$ in subgroups I and II, which means that at low and medium pleocytosis, the results obtained by the automated method could be overestimated (Figure 2, Figure 3). In addition, the distribution of results showed that they were within 95% limits of the agreement range, but most of them were situated above the

average difference line (Figure 3). At higher pleocytosis (subgroup III), the mean bias was lower than that at low and medium and the results obtained by the two methods were more comparable; however, the Spearman's correlation was only moderate.

Good agreement between the manual and automated methods was also observed for the number of MN cells in the whole study group

TABLE 5 Comparison and correlation of manual and automated white blood cell counts in cerebrospinal fluid

Groups	Cell counts	Manual method	Automated method	P-value	Correlation		
					R	P-value	Power
Study whole (n = 59)	Pleocytosis/ μ L	58 6-592	63 5-620	0.008 [*]	0.890	<0.001 ^{**}	Very strong
	MN (%)	80 9-100	76 7.8-100	0.205	0.801	<0.001 ^{**}	Very strong
	PMN (%)	20 0-91	23 4-92.2	0.125	0.793	<0.001 ^{**}	Strong
Subgroup I Pleocytosis 6-50/ μ L (n = 25)	Pleocytosis/ μ L	18 6-48	25 5-85	0.002 [*]	0.800	<0.001 ^{**}	Very strong
	MN (%)	81 21-100	81 20-100	0.407	0.757	<0.001 ^{**}	Strong
	PMN (%)	19 0-75	19 4-80	0.808	0.722	0.001 ^{**}	Strong
Subgroup II Pleocytosis 51-100/ μ L (n = 14)	Pleocytosis/ μ L	64 52-97	71.5 48-166	<0.05 [*]	0.862	<0.001 ^{**}	Very strong
	MN (%)	85.5 12-100	77 7.8-96	0.080	0.764	<0.001 ^{**}	Strong
	PMN (%)	14.5 0-88	23 4-92.2	0.081	0.764	0.001 ^{**}	Strong
Subgroup III Pleocytosis >100/ μ L (n = 20)	Pleocytosis/ μ L	177.5 108-592	160 37-620	0.751	0.621	0.003 ^{**}	Moderate
	MN (%)	71 9-100	57.5 11-96	0.240	0.898	<0.001 ^{**}	Very strong
	PMN (%)	29 0-91	43 4-89	0.279	0.907	<0.001 ^{**}	Very strong
Control Pleocytosis $\leq$ 5/ μ L (n = 32)	Pleocytosis/ μ L	1 0-5	2 0-7	<0.001 [*]	0.336	0.060	Weak
	MN (%)	-	75.5 11-100	-	-	-	-
	PMN (%)	-	72 7-100	-	-	-	-

All data are expressed as medians and ranges.

MN, mononuclear; PMN, polymorphonuclear.

^{*}Significant difference when compared to the automated method ($P < 0.05$; calculated by Wilcoxon signed-rank test).

^{**}Significant correlation ($P < 0.05$; calculated by Spearman's rank correlation coefficient).

($y = 3.93 + 0.93x$; CI slope: 0.81-1.05; CI intercept: -4.92 to 15.07; mean bias: 3%; Table 6, Figures 2 and 3); however, some of the MN results showed poorer agreement at a higher concentration range (Figure 2). In this range, the number of the MN cells obtained by the automated method will be reduced. A slope of 0.93 confirms the very strong correlation as calculated in Spearman's correlation and linear regression. In general, there were no significant differences between the number of MN cells obtained using both methods. Positive mean bias was seen primarily in higher pleocytosis ranges (subgroups II and III), and the number of MN cells obtained by the automated method could be underestimated (Table 5, Figure 3). The two methods are the most comparable at a low pleocytosis range (subgroup I), where the mean bias is reduced to a minimum and the differences between MN count obtained by the manual and automated techniques are close to zero (Table 6, Figure 3).

The PMN count also showed good agreement between the methods in the whole study group ($y = 2.44 + 0.95x$; CI slope: 0.82-1.07; CI intercept: -0.31 to 4.51; mean bias: -3.6%; Table 6, Figures 2 and 3). A slope of 0.95 confirms the strong Spearman's correlation and linear regression, although many of the results showed poorer agreement at low and medium concentration ranges (Tables 5 and 6, Figure 2). In these ranges, some of the PMN results obtained by the automated method will be overstated. The agreement between the two methods in all subgroups was also good (Tables 5 and 6, Figure 2). The Bland-Altman plots in Figure 3 showed that the negative mean bias was the lowest in subgroup I and the highest in subgroup II.

The acceptable accuracy for CSF cell counts and differential count by an automatic analyzer are $r = \geq 0.90$, ≥ 0.70 , ≥ 0.70 (WBC, MN%, PMN%, respectively, and the slopes are 1 ± 0.30 for WBC count and 1 ± 0.50 for MN and PMN differential count. In our

TABLE 6 Comparison of white blood cell counts in cerebrospinal fluid between manual and automated methods using Passing-Bablok regression and Bland-Altman analysis

Groups	Cell counts	Passing-Bablok regression analysis					Bland-Altman analysis	
		Intercept	95% CI	Slope	95% CI	Regression conformity	Bias	95% limits of agreement
Study whole (n = 59)	Pleocytosis/ μ L	3.58	-0.02 to 5.79	1.03	0.96 to 1.12	Yes	-5.4	-107 to 96.2
	MN (%)	3.93	-4.92 to 15.07	0.93	0.81 to 1.05	Yes	3.0	-25.5 to 31.5
	PMN (%)	2.44	-0.31 to 4.51	0.95	0.82 to 1.07	Yes	-3.6	-30.0 to 22.8
Subgroup I Pleocytosis 6-50/ μ L (n = 25)	Pleocytosis/ μ L	-2.10	-10.23 to 5.55	1.35	0.96 to 1.85	Yes	-8.0	-31.9 to 15.9
	MN (%)	12.5	-3.44 to 29.67	0.87	0.67 to 1.05	Yes	-0.7	-29.6 to 28.2
	PMN (%)	-0.71	-7.33 to 5.0	0.97	0.70 to 1.33	Yes	-1.0	-25.8 to 23.9
Subgroup II Pleocytosis 51-100/ μ L (n = 14)	Pleocytosis/ μ L	-17.21	-82.5 to 18.67	1.36	0.83 to 2.38	Yes	-10.2	-47.7 to 27.3
	MN (%)	-6.24	-156 to 24.15	1.03	0.65 to 2.60	Yes	8.2	-23.6 to 40.1
	PMN (%)	3.60	-4.00 to 10.85	1.03	0.65 to 2.60	Yes	-8.2	-40.1 to 23.6
Subgroup III Pleocytosis >100/ μ L (n = 20)	Pleocytosis/ μ L	-59.84	-247 to -7.88	1.30	1.02 to 2.24	Yes	1.1	-170.7 to 173
	MN (%)	2.27	-19.8 to 11.06	0.95	0.79 to 1.23	Yes	3.9	-20.4 to 28.2
	PMN (%)	2.79	-2.52 to 11.07	0.95	0.77 to 1.18	Yes	-3.5	-27.1 to 20.2
Control Pleocytosis $\leq 5/\mu$ L (n = 32)	Pleocytosis/ μ L	0	-	2.0	-	Yes	-1.1	-3.6 to 1.4
	MN (%)	-	-	-	-	-	-	-
	PMN (%)	-	-	-	-	-	-	-

MN, mononuclear; PMN, polymorphonuclear.

FIGURE 2 Passing-Bablok regression analysis showing the comparison of pleocytosis, mononuclear cells, and polymorphonuclear cells obtained with manual and automated methods in the study whole group and subgroups. The regression equation is given in bottom right corner. The solid line represents the fitted regression line. The dotted line represents the line of identity. The dashed lines represent the regression line confidence interval (95% CI for the regression line)

FIGURE 3 Bland-Altman difference plots for pleocytosis, mononuclear cells, and polymorphonuclear cells obtained with manual and automated methods in the study whole group and subgroups. The solid line shows average of the differences obtained and corresponds to the bias between cells results obtained with two methods. The dashed lines correspond to the lower and upper limits of the confidence interval at 95% (mean $\pm$ 1.96 SD) of the difference between results obtained with two methods

study, the accuracy of the Sysmex XT-4000i was in the acceptable limits.

4 | DISCUSSION

Cerebrospinal fluid is one of the biological materials routinely used in laboratory diagnostics. Analysis of CSF cell composition enables recognition of many diseases of the CNS.^{4,5,9-11} Current methods of CSF cytodiagnosics are based mainly on microscopic analysis. Pleocytosis is determined using the Fuchs-Rosenthal chamber, whereas cell differentiation requires stained preparations.^{3,7,12}

A promising alternative to manual tests may be the use of hematological analyzers. These devices must meet certain requirements, including the detection limit of <6 cells/ μ L and the ability to differentiate cells into mono- and multinucleated cells. In addition, the required volume of the test sample should be as small as possible, <100 μ L. Some analyzers, such as the Sysmex® XT-4000i and XE-5000, are already equipped with a special mode of operation allowing the analysis of body cavity fluids and CSF. They use the flow cytometry method to determine pleocytosis and fluorescent flow cytometry to differentiate cells, dividing them into mononuclear, multinucleated and high fluorescent cells (macrophages, epithelia and cancer cells).^{3,7,8,13,14}

Our research aimed to check the applicability of the Sysmex® XT-4000i analyzer in the assessment of pleocytosis and

differentiation of CSF cells. Statistical analysis showed compatibility of the automated and manual methods. In the entire study group, both for pleocytosis and the number of mononuclear and polymorphonuclear cells, the correlation was very high (0.890 vs 0.801 vs 0.793, respectively, at $P < 0.001$). A similar result was obtained in subgroups I and II (R for pleocytosis was 0.800 vs 0.862, respectively, at $P < 0.001$; for MN cells, R was 0.757 vs 0.764, respectively, while for PMN cells, R was 0.722 vs 0.764 at $P < 0.001$). A slightly lower correlation occurred in subgroup III in the pleocytosis analysis (0.621 at $P = 0.003$). Mononuclear and polymorphonuclear cells had a very high positive correlation in this group (0.898 vs 0.907 at $P < 0.001$). In the reference group, the correlation was weak (0.336 at $P = 0.060$). Agreement between automated and manual methods was determined using Passing-Bablok regression analysis and Bland-Altman bias plots. Passing-Bablok regression analysis showed a good agreement between pleocytosis, MN and PMN percentage in the whole group and three subgroups. In general, a CUSUM test for linearity presented no significant deviation from linearity, although the Wilcoxon signed-rank test showed statistically significant differences between pleocytosis in the whole group and some subgroups. Some of the MN cells displayed poorer agreement at a higher concentration range. Therefore, in this range the number of the MN cells obtained by the automated method will be lowered. Taking into account the PMN cells, many of the results demonstrated poorer agreement at low and medium ranges, which means that some of the results obtained by the automated method will be overstated.

Further analysis by the Bland-Altman plots revealed a bias ranging from -10.2 to $8.2 \times 10^6/L$. Results of low and medium pleocytosis obtained by the analyzer could be overestimated because of negative bias. Positive mean bias for MN cells was seen in higher pleocytosis, and the number of these cells from the analyzer could be underestimated, while for PMN cells the mean bias was negative.

Similar studies have been conducted by other authors, who confirmed the effectiveness of the body fluid and CSF module on hematological analyzers. Bottini et al³ discovered a very high correlation of the automatic method with the manual one, both for pleocytosis ($R \geq 0.95$) and for mononuclear and polymorphonuclear cells ($R = 0.95$ and 0.98 , respectively, at $P < 0.05$). Boer et al conducted CSF analysis using the Sysmex®XE-5000 analyzer.⁷ They showed that cytosis was slightly higher when obtained by the automatic method than when obtained with a Fuchs-Rosenthal chamber. These differences, however, proved to be irrelevant from a diagnostic point of view. Cell division into mono- and polynuclear was comparable in both methods. In general, the studies showed good agreement between the two methods and greater precision of the automatic method.⁷

In their studies, De Jonge et al⁸ indicated good agreement in both the rate of pleocytosis and the number of MN cells. However, the number of PMN cells counted with a hematology analyzer was higher. According to the authors, this was caused by the different execution times. Manual cell counting takes a longer time, and thus, some cells, especially neutrophils, may break down, causing lower CSF cell count. The researchers also pointed out the presence of high fluorescent cell fractions in scattergrams. Similarly to pursuing an abnormal complete blood count, the detection of abnormal cells may be the basis for their evaluation by manual or cytometric methods.⁸ Other authors who used the Sysmex® XE-5000 analyzer in their research also confirmed the effectiveness of automated tests in CSF testing. Paris et al¹⁵ observed a very high positive correlation ($R = 0.9$) for pleocytosis and a high correlation for MN and PMN (0.734 and 0.581 , respectively). However, they indicated that some of the test samples contained blast cells, which may have been incorrectly classified by the analyzer, hence a lower value in the cell division. The researchers also recommend that the CSF of oncological patients should be additionally evaluated by manual method.¹⁵ Williams et al¹⁶ concluded that the automatic method is characterized by good precision and a high correlation with the previously used procedure. Zimmermann et al¹⁴ support this thesis, arguing in their studies that the Sysmex® XE-5000 analyzer may exhibit approximately 100% sensitivity and specificity in the detection of abnormal samples (at pleocytosis >20 cells/ μL). The latest research published in 2014 by Lehto et al¹³ shows that the Sysmex® XT-4000i analyzer has a high positive correlation for pleocytosis >20 cells/ μL . In addition, the automated method has very high sensitivity (91.7%) and specificity (82.1%).¹³

Despite the general agreement among the authors as to the suitability of the automated method in the determination of CSF cytosis, in some cases it should be compared to the manual method. The main problem seems to be the determination of

pleocytosis rate at its low levels. In our research, no such correlation was noted. However, many researchers point out that hematological analyzers often overestimate the number of leukocytes in CSF tests with pleocytosis ranging from 6 to 20 cells/ μL . Boer et al observed a similar correlation in their studies.⁷ This tendency was noted particularly in relation to mononuclear cells. The authors observed the imprecision of the method for samples with pleocytosis <20 cells/ μL , as the tests performed with the analyzer resulted in some healthy people being wrongly classified as unhealthy.⁷ Similarly, De Jonge et al⁸ noticed that Sysmex®XE-5000 overestimated the number of CSF leukocytes in people with low pleocytosis. Lehto et al¹³ showed that in the tests correlation of methods was poor ($R = 0.29$ $P = 0.056$) in samples with pleocytosis <20 cells/ μL . Sandhaus et al¹⁷ conducted a study in which low pleocytosis samples were analyzed. They divided the study group into subgroups according to the rate of pleocytosis—0-5, 0-10, and 0-30 cells/ μL . It was concluded that the lower the range of pleocytosis, the lower the correlation of the method used. For the 0-30 cells/ μL range, ρ amounted to 0.65, for the 0-10 cells/ μL range— $\rho = 0.47$, while for 0-5 cells/ μL — ρ amounted to 0.31. The solution to this problem could be designating other ranges for the automated method. The authors suggested that the range of pleocytosis from 0 to 5 cells/ μL should be replaced with a range of 0-23 cells/ μL .¹⁷ Perne et al¹⁸ presented yet different results, dividing patients into subgroups, those with normal pleocytosis (0-5 cells/ μL) and those whose pleocytosis was very high (>200 cells/ μL). The study findings indicated comparability of both methods, although the hematology analyzer correctly assigned only 47% of the samples in the 5-10 cells/ μL subgroup. The researchers suggested combining the groups to form one subgroup with 0-10 cells/ μL pleocytosis rate. In this way, they observed almost 100% compliance and recognized the automated method as a suitable alternative to the manual method.¹⁸ Kleine et al¹² conducted their research using two Sysmex® analyzers: XT-4000i and XE-5000. Based on the results obtained, they concluded that the method is highly imprecise ($CV = 40\%$), especially in low pleocytosis cases. Moreover, they concluded that cell differentiation is unreliable, as the analyzer significantly underestimates the MN results while overestimating the PMN results. This may be due to the diversity of nuclei and cell shapes as well as the duration of the analysis process.¹²

Despite the discrepancies in the rate of pleocytosis determined by the manual and the automated methods, the Sysmex XT-4000i hematological analyzer may in the future become the primary tool in the cytological analysis of CSF. The differentiation of CSF cells into populations of mononuclear and polymorphonuclear cells by the Sysmex XT-4000i hematology analyzer provides a reliable clinical picture of CNS disorders and makes giving an accurate diagnosis in a timely manner possible. The automated method cannot fully replace the previously used manual method. Some of the dubious cases, such as samples with low pleocytosis rates or abnormal cells indicated by the analyzer, will still require microscopic examination.

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