Comparison of Conventional Manual Methods with the ADVIA 120 Automated Method for Counting of Red and White Blood Cells in Cerebrospinal Fluid

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ABSTRACT

Objective: To evaluate the correlation and agreement of erythrocyte and leukocyte count in cerebrospinal fluid (CSF) among two different manual methods and automated method.

Methods: We evaluated the correlations and agreements of the CSF RBC counts, WBC counts and WBC differential counts between two manual methods and automated method by using the ADVIA 120 CSF assay.

Results: We studied 83 CSF specimens in all methods. Absolute cell counts showed a high correlation and agreement between methods, with correlation coefficient (r) for all absolute counts of more than 0.89 and intraclass correlation (ICC) more than 0.9. The correlation and agreement of WBC differential counts from CSF specimens which had more than 20 WBCs/μL were also evaluated, which revealed good results only for polymorphonuclear cells, neutrophils and lymphocytes (r_s = 0.796, 0.835 and 0.779, respectively and ICC = 0.954, 0.899 and 0.907, respectively). When WBC counts more than 5 cells/μL in automated method were used as a cut-off point, the sensitivity is 100% but specificity is very low (60.87%). The cut-off point of 5 WBCs/μL for manual method and 11 WBCs/μL for automated method gave the highest agreement (Kappa 0.874, sensitivity 91.43% and specificity 95.65%).

Conclusion: The ADVIA 120 CSF assay provide a useful and efficient method for excluding the normal CSF specimens at cut-off 5 WBCs/μL.

Keywords: Automated cell count; ADVIA 120; CSF WBC count; CSF RBC count; cerebrospinal fluid

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Counting red blood cells and white blood cells in the cerebrospinal fluid (CSF) can provide clinicians with valuable diagnostic information, because abnormal numbers of WBCS in a CSF sample (a mononuclear cell count of >5/μl in adults or > 30/μl in newborns) can indicate one of several serious medical conditions such as meningitis, encephalitis, neurologic disorders and leukemic CSF infiltrations. CSF WBC counts also can be used to monitor the effectiveness of therapy for patients with leukemia or lymphoma, and unusually elevated RBC counts can indicate cerebral hemorrhage or can be used to correct CSF WBC count or CSF protein determination in cases with traumatic tap.[1]

Hematologic Analysis of CSF has been performed in laboratories using manual cell counting and differentiation methods. However, this analysis is imprecise, time consuming and labour-intensive, and has wide interoperator variability. Conversely, automated methods has reduced interoperator variability and improved turnaround time and seem to be a solution to improve both accuracy and precision of CSF analysis,[2] even though these methods are often hindered by electronic background noise, which might falsely elevate cell counts, especially in cytopenic specimens.

In our hospital, manual methods of CSF cell counts are performed by both Fuchs-Rosenthal chamber or Neubauer hemacytometer. The Fuchs-Rosenthal method requires a higher CSF volume (1.8 μL) than the Neubauer hemacytometer (0.9 μL) and has a higher diagnostic accuracy.

The ADVIA 120 CSF assay provides an automated analysis of CSF samples by counting and distinguishing different cell types. After mixing the CSF sample with ADVIA 120 CSF reagent, the cells are sphered and fixed. The incubation period before aspiration is between 4 min (minimum) and 4 h (maximum). As the prepared sample is aspirated, the cells are detected and enumerated based

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on light scatter and absorbance measurements. A scatter vs scatter and scatter vs absorbance cytogram are displayed with the thresholds and results are automatically calculated for each sample. Reportable parameters are WBC and RBC counts along with absolute and percentage counts for neutrophils, lymphocytes, monocytes, eosinophiles, PMN (polymorphonuclear cells) and MN (mononuclear cells).[3]

Although the Neubauer chamber method has been compared with the ADVIA 120 CSF assay in previous studies,[3-5] a comparison of this automated method with the Fuchs-Rosenthal chamber has been thus far not reported.

The objective of this study was to evaluate the correlation and agreement of cell count and differentiation among Fuchs-Rosenthal chamber, Neubauer chamber and automated method using the ADVIA 120 CSF assay.

MATERIALS AND METHODS

Cerebrospinal fluid

Cerebrospinal fluid samples were obtained from specimens submitted to the Neurology Division, Department of Medicine, Siriraj Hospital, Mahidol University in Thailand for CSF analysis during a 5-month period in 2005-2006. The CSF samples included were from pediatric, oncologic, medical, and surgical patients. We used only the samples which had excessive volume from the routine procedure (approximately more than 1.5 ml).

Samples were collected in sterile glass tubes and examined by manual methods within 1 hour. The left specimens were mixed with the ADVIA 120 CSF reagent within 1 hour and subsequently examined with the ADVIA 120 CSF assay.

We excluded the specimens that had improper sample handling, i.e., two incomplete sample aspiration specimens on the ADVIA 120. Moreover, we excluded the specimens which contain budding yeast because budding yeast forms may overlay both CSF RBC and CSF WBC counting areas and cause falsely elevated CSF RBC and WBC counts.

The WBC differential counts were analyzed from specimens which had more than 20 WBCs/μL. We calculated polymononuclear cells from neutrophils and eosinophils, and mononuclear cells from lymphocytes and monocytes in manual methods to compare with automated method.

Manual methods

Manual WBC & RBC counting was performed by both Fuchs-Rosenthal and Neubauer methods by trained medical laboratory technicians according to the standard procedure of our laboratory. The presence of numerous RBCs in specimens were lysed by using glacial acetic acid. The 100-cell WBC differential was performed after cytocentrifugation of the samples, followed by May Grunwald Giemsa stain. The CSF protein and sugar were measured by using TCA agglutination and glucose oxidase method, respectively.

ADVIA 120 CSF assay

The ADVIA 120 CSF Assay, on the ADVIA 120 hematology analyzer, is an automated method that uses direct cytometry to enumerate RBCs, WBCs and WBC differential on CSF patient samples. Reportable parameters for the WBC differential include absolute and proportional counts for neutrophils, lymphocytes, and monocytes and for a research-use-only eosinophil count.

The ADVIA 120 CSF Assay procedure is composed of mixing 300 μl of the CSF specimen with ADVIA 120 CSF Reagent in a 1:1 ratio to sphere and fix the cells. According to the previous studies,[3,4] the incubation time before aspiration ranges between a minimum of 4 min and a maximum of 4 h. Previous study[4] suggest that laboratories that frequently receive hypochromic/microcytic samples should consider increasing the incubation time of prepared CSF samples from the standard 4 minutes to 10 minutes as this condition might result in falsely decrease in CSF RBC count and falsely elevate CSF WBC count and lymphocyte count. Since the presence of diseases with hypochromic/microcytic RBC is common in Thailand, we extended the incubation time of all specimens to 10 minutes.

Due to low cell numbers typically seen in CSF samples, it is necessary to eliminate potential background interference. Before using CSF assay, the operator will verify the total number of events displayed in the CSF scatter cytogram, including the noise region, is less than 10 and that the background count for all cell counting areas is zero. If counts fall outside these parameters, the operator must perform a manufacturer-defined cleaning procedure.

After aspirated into the system, the cells are then detected and enumerated based on 2 different light scatter angles and 1 absorption measurement. Results are calculated automatically.

Statistical analysis

A 0.01 one-sided Fisher's z test of the null hypothesis that the Pearson correlation coefficient $\rho = 0.5$, will have 90% power to detect a ρ of 0.75 when the sample size is 76. (nQuery Advisor)

Correlation and Agreement between manual method using Fuchs-Rosenthal chamber and Automated method, manual method using Fuchs-Rosenthal and Neubauer chamber, and manual method using Neubauer chamber and Automated method were evaluated by Spearman correlation analysis and Intraclass correlation coefficients two-way mixed effects model (Absolute Agreement Definition), comparing samples for absolute RBC and WBC count in all cases, and WBC differential counts in samples with CSF WBC counts greater than 20 cells/μl.

RESULTS

From eighty-three specimens collected from patients, two were excluded because of incomplete specimen aspiration. The mean age of patients was 41 ± 23 years (range 1 month-86 years, 44% are male). The mean CSF protein and sugar were 60 ± 73 mg/dl (range 4-403 mg/dl) and 65 ± 24 mg/dl (range 3-142 mg/dl), respectively.

TABLE 1. Median, minimum, maximum and percentile of the CSF RBCs and WBCs (n=81)

		RBC (cells/μL)			WBC (cells/μL)		
		F	N	A	F	N	A
Median		20	16	31	4	4	8
Minimum		0	0	0	1	0	0
Maximum		9750	9300	9869	1950	2140	2900
Percentiles	25	3	3	11.5	1	1	3.5
	75	470	486.5	514	15	13	20.5

F=Fuchs-Rosenthal chamber, N=Neubauer chamber, A=Automated method by the ADVIA 120 CSF assay

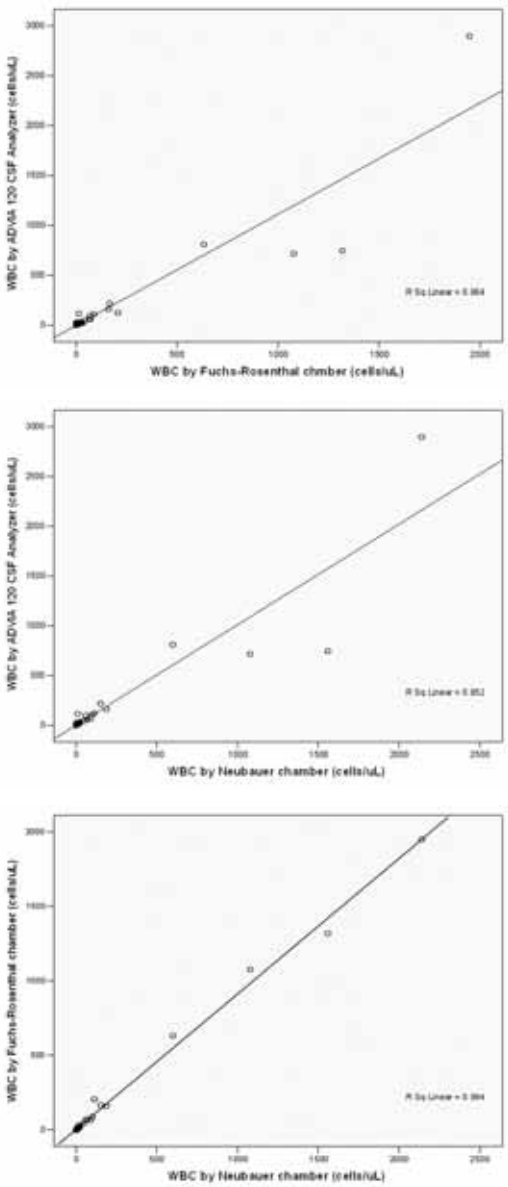

Fig 1. Graphic representation comparing the automated and manual methods (Fuchs-Rosenthal chamber and Neubauer chamber) for absolute WBC counts.

The CSF RBCs, WBCs, and WBC differential counts showed a non-normal distribution. The median, minimum, maximum and percentile of the CSF RBCs and WBCs from each method is shown in Table 1 and Fig 1.

The median, minimum and maximum of WBC differential results from manual method using May Grunwald Giemsa stain and automated method using the ADVIA 120 CSF assay with WBC counts greater than 20/μL are demonstrated in Table 2. There were four specimens which had other cells besides neutrophils, lymphocytes, monocytes and eosinophils. These cells were malignant or blast cells.

Correlations and agreements of the CSF RBC and WBC counts

The correlations between manual method using Fuchs-Rosenthal chamber and Neubauer chamber, manual method using Fuchs-Rosenthal chamber and the ADVIA 120 CSF assay, and manual method using Neubauer chamber and the ADVIA 120 CSF assay for RBC and WBC counts were determined as shown in Table 3. All comparisons had well to excellent relationship, with correlation coefficient of >0.75. (Colton 1974) Absolute agreement between each pair of different method also had good agreement, with intraclass correlation coefficients (ICC) of more than 0.8.

Correlations and agreement of the CSF WBC differential counts

Sixteen specimens which had more than 20 WBC counts were analyzed to compare correlations and agreement between manual method and automated method. Polymorphonuclear cells, neutrophils and lymphocytes showed good to excellent correlation (r>0.75), but the mononuclear cells displayed only a moderate degree of relationship (r_s=0.25-0.49), while monocytes and eosinophils show no correlation (r_s <0.24). In addition, the agreement between the ADVIA 120 CSF assay and manual method were good in polymorphonuclear cells, neutrophils and lymphocytes, but mononuclear cells, eosinophils and monocytes did not have a good agreement between automated method and manual method (Table 4).

Cut-off point

When we use WBC counts of more than 5 cells/μL as the cut-off point, the sensitivity of automated method is high (100%) but specificity is very low (60.87%) when compared with manual method using Fuchs-Rosenthal chamber as gold standard which is shown in (Table 5). The agreement of cut-off point between 5 WBCs/μL for manual method and 11 WBCs/μL for automated method are highest which have Kappa of 0.874.

TABLE 2. Median, minimum, maximum and percentile of the CSF WBC differential (n=16)

		Manual method		Automated method	
		Mononuclear	Polymononuclear	Mononuclear	Polymononuclear
Median		74	12	89.05	10.95
Minimum		1	0	15.3	0.7
Maximum		100	99	99.3	84.7
Percentiles	25	16.25	2.75	63.13	5.28
	75	92.75	59.25	94.73	36.88

		Manual method					Automated method			
		N	L	E	M	Other	N	L	E	M
Median		12	70	0	1	0	7.3	68.9	2.3	11.25
Minimum		0	1	0	0	0	0.5	8.1	0	1.1
Maximum		99	97	3	6	93	77.6	98.2	19.5	53
Percentiles	25	2.5	15.75	0	0	0	5.15	45.13	0.28	5.1
	75	59.25	91.5	0	2	0	25.65	81.45	6.18	20.3

N=neutrophils, L=lymphocytes, E=eosinophils, M=monocytes, Other=other cells e.g. malignant or blast cells

DISCUSSION

The correlations of red and white blood cell counting method between Fuchs-Rosenthal chamber and Neubauer chamber are good to excellent, which have r_s = 0.978, 0.947 respectively. The absolute agreement between two chamber methods are also high (ICC of RBC and WBC are 0.992 and 0.997 respectively). Therefore, it can be

TABLE 3. Correlations(r_s) and Agreements (ICC) of the CSF RBC and WBC counts among two manual methods and automated method

Comparison between	Correlations (Spearman)		Agreement	
	r_s	p-value	ICC	95%CI
RBC(F) vs RBC(A)	0.933	<0.001	0.989	0.983-0.993
RBC(A) vs RBC(N)	0.946	<0.001	0.994	0.991-0.996
RBC(F) vs RBC(N)	0.978	<0.001	0.992	0.988-0.995
WBC(F) vs WBC(A)	0.892	<0.001	0.956	0.931-0.971
WBC(A) vs WBC(N)	0.897	<0.001	0.959	0.936-0.973
WBC(F) vs WBC(N)	0.947	<0.001	0.997	0.995-0.998

F=Fuchs-Rosenthal chamber, N=Neubauer chamber, A=Automated method by the ADVIA 120 CSF assay

assumed that the two methods can be used interchangeably.

Previous studies[3,4] comparing the ADVIA CSF 120 assay with Neubauer chamber which demonstrated a high correlation of greater than 98% correlation for absolute WBC and RBC counts. Our study compare the ADVIA 120 CSF assay with both Fuchs-Rosenthal chamber and Neubauer chamber which showed good to excellent correlation and good agreement in red and white blood cell counting. (r_s = 0.933, 0.946 for RBC and r_s = 0.892, 0.897 for WBC).

Additionally, correlation and agreement between manual and automated method in WBC differential counts are also good for neutrophils, lymphocytes and PMNs. In contrast, eosinophils, monocytes and MNs are not well correlated. This discrepancy may be the result from many reasons such as eosinophil data are for research purposes only and are nonreportable from its manual. Furthermore, few specimens have eosinophils or monocytes or have low number of these cells and blast or malignant cells are reported as monocytes or lymphocyte which will affect MN count. Our study included four specimens which had blast or malignant cells.

The reportable range for CSF RBC count is 0 to 2,880 cells/μL. However, our study does not exclude four specimens which have RBC count more than 2,880 cells/μL.

The difference of cut-off point from automated method will result in various sensitivity and specificity when compared with manual method using Fuchs-Rosenthal chamber as gold standard. By using 5 WBCs/μL as cut-off point, the sensitivity is very high (100%); however, the specificity will be low (60.87%). Conversely, using 11 WBCs/μL as cut-off point, the agreement is highest (kappa = 0.874) with lower sensitivity and higher specificity. In clinical practice, missing the positive cases will cause morbidity and mortality; therefore, using 5 WBCs/μL as cut-off point will be more appropriate.

In this study, prevalence of abnormal CSF WBCs is 43.2% (35/81) when using WBCs more than 5 cell/μL from Fuchs-Rosenthal counting chamber as gold standard. Negative predictive value and positive predictive value were calculated, which were 100% and 66%, respectively. Thus, due to its high NPV, we can apply the automated instrument to exclude the normal CSF specimens which have WBC counts fewer than cut-off point. Then, if the WBC differential count has high proportion of monocytes or MNs, technician should perform slide manually and examine carefully for malignant cells.

Our study did not include specimens which had budding yeast because of falsely elevated CSF RBC and WBC counts. Nevertheless, the presence of budding yeast in CSF can be discernible in slide preparation performed by the technicians.

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TABLE 4. Correlations(r_s) and Agreement(ICC) of the CSF WBC differential counts between manual method and automated method

Manual vs Automated method	Correlations (Spearman)		Agreement	
	r_s	p-value	ICC	95%CI
Polymorphonuclear cells	0.796	<0.001	0.954	0.836-0.985
Mononuclear cells	0.362	0.169	0.690	0.063-0.894
Neutrophils	0.835	<0.001	0.899	0.595-0.968
Lymphocytes	0.779	<0.001	0.907	0.737-0.967
Eosinophils	0.169	0.531	-0.003	-0.871-0.569
Monocytes	0.056	0.836	-0.026	-0.565-0.483

TABLE 5. Agreement, sensitivity and specificity for different cut-off point

Automated method cut-off point	Manual method: cut-off point > 5 WBCs/μL		
	Kappa	Sensitivity	Specificity
> 5 WBCs/μL	.573	100%	60.87%
> 6 WBCs/μL	0.616	97.14%	67.39%
> 7 WBCs/μL	0.732	97.14%	78.26%
> 8 WBCs/μL	0.801	94.29%	86.96%
> 9 WBCs/μL	0.850	94.29%	91.30%
> 10 WBCs/μL	0.825	91.43%	91.30%
> 11 WBCs/μL	0.874	91.43%	95.65%
> 12 WBCs/μL	0.796	82.86%	95.65%

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บทคัดย่อ

เปรียบเทียบการนับเม็ดเลือดแดงและเม็ดเลือดขาวในน้ำไขสันหลังโดยวิธีดั้งเดิมกับการนับโดยเครื่องอัตโนมัติชนิด ADVIA 120

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วัตถุประสงค์: เพื่อประเมินความสัมพันธ์ของการนับเม็ดเลือดแดงและเม็ดเลือดขาวในน้ำไขสันหลังโดยวิธีดั้งเดิม 2 วิธี กับการนับโดยใช้เครื่องอัตโนมัติ

วิธีการ: ศึกษาความสัมพันธ์ และ agreement ของจำนวนเม็ดเลือดแดง, เม็ดเลือดขาว และการนับแยกชนิดเม็ดเลือดขาว ระหว่างวิธีดั้งเดิม 2 วิธี และการนับเซลล์โดยเครื่องอัตโนมัติชนิด ADVIA 120

ผลการศึกษา: น้ำไขสันหลังจากผู้ป่วย 83 รายได้รับการตรวจทั้งสามวิธี พบว่าจำนวนเซลล์ในแต่ละวิธีมีความสัมพันธ์และ agreement กันอย่างมาก โดยมีค่าความสัมพันธ์ (r_s) มากกว่า 0.89 และ intraclass correlation มากกว่า 0.9 การนับแยกชนิดของเม็ดเลือดขาวในน้ำไขสันหลังที่มีเม็ดเลือดขาวมากกว่า 20 เซลล์/μL พบว่ามีความสัมพันธ์และ agreement ดีเฉพาะเซลล์โพลีมอร์โฟนิวเคลียร์, นิวโตรฟิลล์ และลิมโฟซัยต์ (r_s = 0.796, 0.835 และ 0.779 ตามลำดับ และ ICC =0.954, 0.899 และ 0.907 ตามลำดับ) เมื่อใช้ค่าเม็ดเลือดขาวที่มากกว่า 5 เซลล์/μL ในวิธีอัตโนมัติเป็น cut-off point พบว่ามีความไว 100% แต่มีความจำเพาะต่ำมาก (60.87%) หากใช้ค่า cut-off point ของเม็ดเลือดขาว 5 เซลล์/μL ในวิธีดั้งเดิมเทียบกับค่า cut-off point ของเม็ดเลือดขาว 11 เซลล์/μL ในเครื่องอัตโนมัติพบว่ามี agreement สูงที่สุด (Kappa 0.874, ความไว 91.43% และความจำเพาะ 95.65%)

สรุป: เครื่องอัตโนมัติชนิด ADVIA 120 CSF assay เป็นวิธีที่มีประโยชน์และมีประสิทธิภาพในการนับเซลล์ในน้ำไขสันหลัง ช่วยแยกน้ำไขสันหลังที่ปกติออกไปได้ โดยใช้ค่า cut-off point ของเม็ดเลือดขาวที่ 5 เซลล์/μL