Original Article

Evaluation of body fluid mode of Sysmex XN-9000 for white blood cell counts in cerebrospinal fluid

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Background: This study was planned to evaluate the analytical performance of the novel and fully automated Sysmex XN-9000 analyzer for rapid analysis of cerebrospinal fluid (CSF) samples.

Methods: Forty-four CSF samples were used for method comparison studies between Sysmex XN-9000 body fluid mode and conventional optical microscopy. The bias between data obtained with the two methods was estimated with Bland-Altman plot analysis. The analytical evaluation also included the assessment of imprecision, linearity and carry-over.

Results: A good agreement was found between results obtained with Sysmex XN-9000 body fluid mode and optical microscopy. The mean bias was 1.6×10^6 cells/L for total white blood cells (95% CI: -21.8×10^6 to 25.1×10^6 cells/L), 1.3×10^6 cells/L for polymorphonuclear cells (95% CI: -13.9×10^6 to 16.5×10^6 cells/L) and -0.6×10^6 cells/L for mononuclear cells (95% CI: -21.5×10^6 to 20.3×10^6 cells/L). The carryover was found to be lower than 0.01% and the imprecision was lower than 5%. The XN-9000 body fluid mode was also characterized by excellent linearity in the range of values comprised between 85×10^6–$3{,}197\times10^6$ cells/L, with correlation coefficients (r) always equal to 1.00 ($P<0.001$).

Conclusions: The Sysmex XN-9000 body fluid mode displays excellent analytical performance in terms of imprecision, linearity, carry-over and comparability with conventional optical microscopy, so that it may be used as a first-line, screening technique for rapid analysis of CSF samples referred for both routine and, especially, for urgent testing.

Keywords: Cerebrospinal fluid (CSF); Sysmex XN-9000; optical microscopy; leukocytes

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Introduction

The infections of the central nervous system (CNS) are conventionally classified as meningitis or encephalitis. The analysis of white blood cells (WBC) in cerebrospinal fluid (CSF) is a key diagnostic element for the different diagnosis of a kaleidoscope of diseases, thus including inflammatory conditions and infectious or non-infectious disorders involving the CNS (1-3).

The CSF normally contains a very low number of WBC. In adults, the number of nucleated cells in the CSF is conventionally $<5.0\times10^6$ cells/L, whilst their count should remain below 7.0×10^6 cells/L in the CSF of children and below 30.0×10^6 cells/L in that of neonates, respectively (4). The presence of a high number of WBC may hence be suggestive, or even diagnostic, for an

infection, for diseases such as meningitis and encephalitis, or for other neurological disorders (1,5). The normal cellular components of CSF include mainly lymphocytes (i.e., approximately 70%, mostly T-cells) and monocytes (i.e., approximately 30%). Neutrophils are not normally present in CSF, and their occasional observation in non-pathological CSF samples can be attributed to a microscopic contamination during lumbar puncture, which is then magnified by cytocentrifugation. Therefore, occasional finding of rare neutrophils in CSF is not necessarily a hallmark of a pathological condition (4).

Optical microscopy is still regarded as the "gold standard" for identification and enumeration of WBC in CSF (4,6). Nevertheless, this technique has some well-known drawbacks, such as the relatively high inaccuracy, low imprecision, lengthy turnaround time (TAT) and the need to educate and train specialized personnel for this type of manual analysis (7). A new generation of fully-automatic hematologic analyzers has been developed and commercialized in recent years. In 2006, the Sysmex (Kobe, Japan) *in vitro* diagnostic (IVD) company has integrated a specific body fluid (BF) mode on its XE-5000 analyzer, which is hence mainly aimed to be used for analysis of biological fluids other than blood, so mainly including CSF, ascites, synovial, pericardial and pleural fluids. This mode not only exhibits acceptable analytical performance, but also displays many potential advantages for laboratory workout and organization, mostly attributable to the faster TAT and the more cost-effective management of specimens than using optical microscopy (8-10). Nevertheless, high imprecision in CSF samples with a number of WBC $\leq 20\times10^6$/L, probably due to overestimation of polymorphonuclear cell (PMN) counts, was an important shortcoming that has been highlighted in some previous investigations (11-13).

Therefore, the aim of study was to assess the analytical performance of the novel and fully automated Sysmex XN-9000 BF mode for identification and enumeration of WBC in CSF, as well as the evaluation of its basic analytical performance.

Methods

Analyzer description

The new automated haematology analyser Sysmex XN-9000 (Sysmex, Kobe, Japan) can operate either in "whole blood" mode, or with a second mode dedicated to BF analysis (i.e., BF mode). The analytical performance of the whole blood mode has been recently and exhaustively evaluated (14,15). The second mode, specifically dedicated to BF analysis, entails the use of an innovative software, which allows enumeration and classification of WBC, PMN, mononuclear (MN) and high fluorescent BF (HF-BF) cells in the "DIFF" channel according to their side scatter (so reflecting internal complexity) and fluorescence intensity (i.e., mirroring DNA/RNA content) (4,16,17). The analyzer only uses 88 µL for each BF sample analysis. A rinsing cycle is also activated before starting a new test, thus preventing spurious contamination from material of the previous sample.

Study design

The comparison study was carried out using 44 CSF samples referred to the local laboratory for routine analysis. All samples were tested within 1 h from arrival in the laboratory. CSF collection and analysis of cellular composition were performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) document H56-A (4). The results of total WBC count were directly compared with those obtained on the same CSF sample by optical microscopy on Nageotte chamber, after staining with Turk's solution. The slides for differential WBC count were prepared with cytospin (Shandon Cytospin 3 centrifuge; Thermo Shandon, Cergy Pontoise, France), starting from 200 µL of CSF. The slides were then stained whit May-Grunwald-Giemsa, using a Sysmex SP-10 slide-stainer unit (Sysmex, Etten-Leur, The Netherlands). Microscopic analysis was performed with light microscopy under oil immersion, at 50× magnification. Two independent experts performed all the microscopic analyses, and the individual results were then averaged to obtain the final count. The bias [and its 95% confidence interval (CI)] between data obtained with either optical microscopy or Sysmex XN-9000 BF mode was estimated with Bland-Altman plot analysis.

The within-run precision of the BF mode on XN-9000 was evaluated with 20 consecutive measurements of two CSF samples with low (mean value, 36×10^6 cells/L) and high (mean value, $1{,}346\times10^6$ cells/L) WBC counts. The imprecision was finally expressed in percentage, as coefficient of variation (CV), according to the CLSI document EP5-A2 (18).

Carry-over was assessed by measuring a CSF sample with high cell count in triplicate (H1, H2, H3), followed by

Table 1 Main results of comparison study using 44 cerebrospinal fluid (CSF) analyzed with Sysmex XN-9000 body fluid mode and optical microscopy (results are shown as $\times 10^6$ cells/L)

CSF parameter	Body fluid	Optical microscopy
WBC	Lowest value: 0	Lowest value: 0
	Highest value: 662	Highest value: 680
	Mean: 48.9	Mean: 47.3
	Standard deviation: 117.35	Standard deviation: 121.73
	Standard error of the mean: 17.69	Standard error of the mean: 18.35
MN	Lowest value: 0	Lowest value: 0
	Highest value: 317	Highest value: 360
	Mean: 25.4	Mean: 24.9
	Standard deviation: 63.3	Standard deviation:59.9
	Standard error of the mean: 9.03	Standard error of the mean: 9.54
PMN	Lowest value: 0	Lowest value: 0
	Highest value: 619	Highest value: 640
	Mean: 23.8	Mean: 22.5
	Standard deviation: 94.77	Standard deviation: 97.05
	Standard error of the mean: 14.29	Standard error of the mean: 14.63

WBC, white blood cells; MN, mononuclear cells; PMN; polymorphonuclear cells.

three consecutive measurements of blank solution (Cellpack; L1, L2, L3). The final result was calculated as follows: [(L1–L3)/(H3–L3)] ×100, as currently recommended by the International Council for Standardization in Haematology (ICSH) (19).

The linearity was assessed measuring two CSF samples with different cell counts (sample A: WBC, $3{,}197\times10^6$ cells/L; sample B: WBC, 85×10^6 cells/L). Each sample was serially diluted with Cellpack to obtain scalar values, which were then measured three consecutive times each. Results were then compared with expected values, as for indications of CLSI document EP06-A (20).

Statistical analysis

The statistical analysis was performed using MedCalc statistical package (version 17.6, MedCalc Software, Mariakerke, Belgium).

Results

The main results of this study are shown in *Table 1*. The overall bias between the Sysmex XN-9000 BF mode and optical microscopy was always clinically meaningless. In particular, the Bland-Altman plot analysis allowed to estimate a mean bias with optical microscopy of 1.6×10^6 cells/L (95% CI: -21.8×10^6 to 25.1×10^6 cells/L) for total WBC, 1.3×10^6 cells/L (95% CI: -13.9×10^6 to 16.5×10^6 cells/L) for PMN, and -0.6×10^6 cells/L (95% CI: -21.5×10^6 to 20.3×10^6 cells/L) for MN, respectively (*Figure 1*).

The XN-9000 boy fluid mode also displayed excellent linearity throughout a broad range of clinically significant values (i.e., between 85×10^6–$3{,}197\times10^6$ cells/L), with all correlation coefficients for WBC, PMN and MN equal to 1.00 ($P<0.001$). The carryover was negligible (i.e., <0.01%), and the imprecision excellent, with CVs <5% and <4% for CSF samples with mean values of 36×10^6 WBC/L and $1{,}346\times10^6$ WBC/L, respectively.

Discussion

The accurate identification and enumeration of cells in CSF are essential needs for rapid diagnosis and appropriate therapeutic treatment of patients with CSF disorders. Optical microscopy, using the so-called "counting chambers" (i.e., Fuchs Rosenthal, Burker, Neubauer or

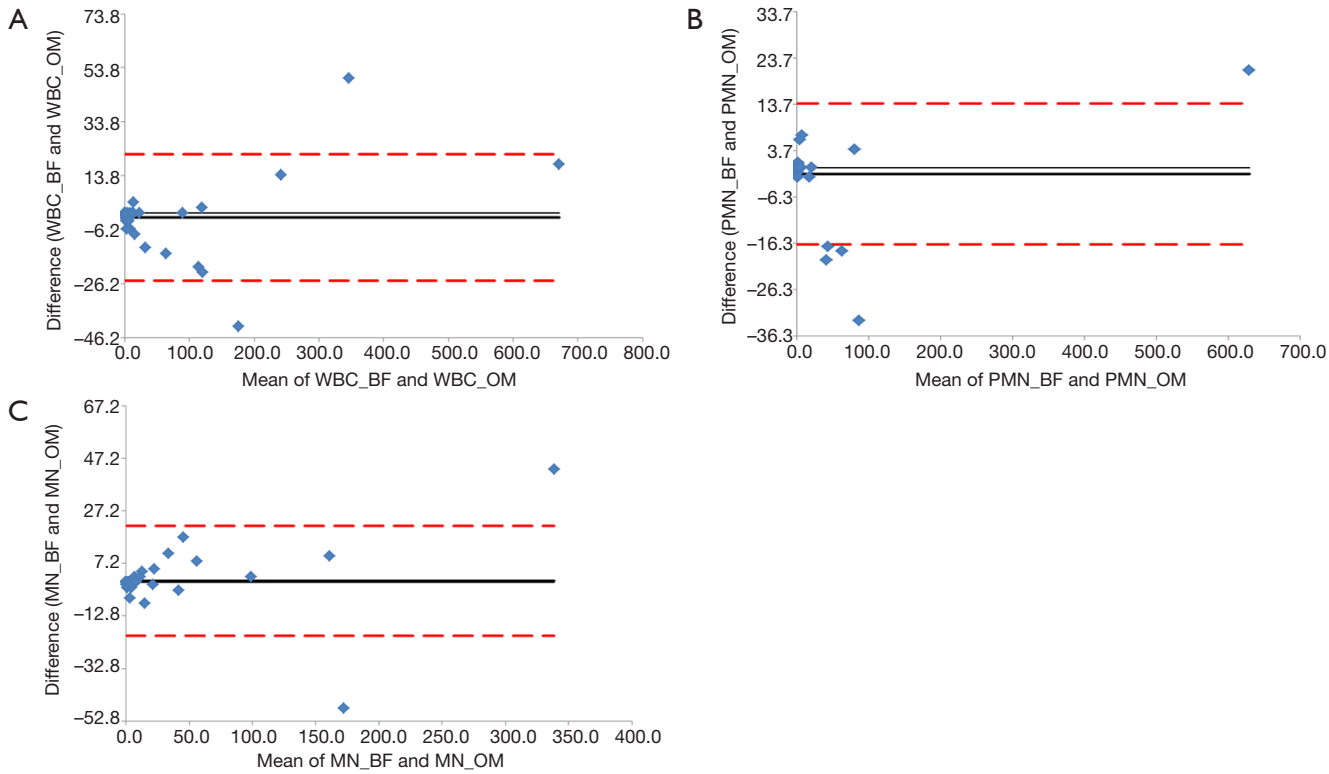

Figure 1 Bland and Altman plot analysis for total white blood cells (WBC) (A), polymorphonuclear cells (PMN) (B) and mononuclear cells (MN) (C) in 44 cerebrospinal fluid (CSF) samples analyzed with Sysmex XN-9000 body fluid mode (BF) and optical microscopy (OM) (results are shown as $\times 10^6$ cells/L).

Nageotte), remains "gold standard" for total WBC and erythrocyte counts in this BF, whilst stained cytocentrifuged slides are typically used for differentiating WBC into MN and PMN (8,13).

The considerable technological advancements occurred in recent years have allowed to introduce a new generation of automated hematological analyzers in routine clinical laboratories, which are now increasingly used for cell identification and counting in samples other than whole blood. For certain types of BF, automated cell count has hence allowed to achieve a high degree of accuracy and precision, concomitantly reducing both inter-observer variability and TAT (21,22), even if some doubts remain about their reliability in BF samples with low cellularity (9,10,23). In a previous evaluation of the Sysmex XE-5000 BF mode, Fleming *et al.* found a good agreement with optical microscopy for WBC (r=0.97) and MN (r=0.93) counts, whereas a less satisfactory concordance was observed for the PMN count (r=0.87) (5). Paris *et al.* (6) also showed a good correlation for total WBC (r=0.96) and MN counts (r=098) between XE-5000 BF mode and optical microscopy using 81 CSF samples, which were obtained from oncology, pediatric and adult hematological patients. Notably, the poor correlation between XE-5000 BF mode and optical microscopy was confirmed in their study (r=0.58) (6), whilst it was also highlighted that automated testing was not efficient enough to identify blast cells, since the XE-5000 BF mode overlaps blast cells with MN, so that optical microscopy would remain mandatory in these cases (6).

Conclusions

Our study was mainly aimed to assess the analytical performance of the new BF mode on the Sysmex XN-9000 using CSF samples, and comparing data with those obtained with the reference technique (i.e., optical microscopy). Taken together, our results suggest that this BF mode displays excellent analytical performance in terms of imprecision, linearity and carry-over throughout

a broad range of cellularity in CSF samples. This data, combined with the excellent correlation with optical microscopy observed from the analysis of 44 routine CSF samples displaying heterogeneous cellularity, suggests that the Sysmex XN-9000 BF mode may be used as a first-line, screening technique for rapid analysis of CSF samples either referred for routine or, especially, for urgent testing.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2018.02.01). Giuseppe Lippi serves as the unpaid Editor-in-Chief of *Journal of Laboratory and Precision Medicine* from November 2016 to October 2021. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The investigation was entirely based on the use of routine samples refereed to the local laboratory for CSF analysis, all samples were anonymized before testing, test results did not impact the clinical management of patients and, therefore, ethical approval or patient's permission to use the samples for this study were cleared by the local institutional review board. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and under the terms of all relevant local legislations.

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