Automated screening of bacterial meningitis by cytofluorimetric analysis of cerebrospinal fluid: preliminary results

Sabrina Buoro[1], Sara Apassiti Esposito[1], Cosimo Ottomano[2], Maria Grazia Alessio[1], Sabina Fumagalli[1], Alberta Caleffi[2], Claudio Farina[3], Annibale Raglio[3], Alberto Crippa[1], Giuseppe Lippi[2]

[1]Unità Operativa Laboratorio di Analisi Chimico Cliniche e [3]Unità Operativa di Microbiologia e Virologia, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo

[2]Unità Operativa di Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma

ABSTRACT

This study was planned to assess the diagnostic performance of the automated urine particle analyzer Sysmex UF-1000i for the rapid screening of cerebrospinal fluid (CSF) in patients with suspected meningitis. Cytometric analyses with either optical microscopy (OM) or UF-1000i, along with assessment of glucose and protein in CSF, were performed on 101 consecutive CSF of patients with suspected meningitis. In 50 out of 101 samples, cultural analysis was also performed with different culture media. Four different diagnostic combination were developed, with different mix of the tested parameters. A high correlation was found between OM and UF-1000i (r=0.99; mean bias, -4.9/μL). The diagnostic agreement was 0.90 in adults and 0.97 in children. The diagnostic agreement between CSF culture and bacterial count by UF-1000i was 0.98, with 1.00 sensitivity and 0.98 specificity. Results showed that the diagnostic combination based on CSF glucose and total proteins, cytometric analysis (leukocyte count ± neutrophilia) and bacterial count on UF1000i exhibited the best performance when compared with microbiological examination (area under ROC curve, 1.00). In conclusion, the results of this study show that the combination of two rapid clinical chemistry tests such as glucose and total proteins with UF-1000i analysis could represent a valid approach for supporting more complex analyses or even for replacing OM and CSF culture during stat examination and to achieve a quick detection of central nervous system infections.

INTRODUCTION

The analysis of cerebrospinal fluid (CSF) represents an essential part of diagnosis and follow-up of a several disorders of the central nervous systems (CNS), especially meningitis and bacterial, parasitic, viral and other forms of encephalitis (1, 2). The microbiological examination of CSF in case of pathology with suspected microbial etiology traditionally combines microscopic analysis (after Gram staining) with cultural exam. However, if the response time of microscopic analysis is relatively short, the cultural exam requires an incubation time not lower than 24-48 h that should be extended to 5 days in absence of bacterial growth to exclude bacterial meningitis. In addition, false negative results of cultural exam can be observed in case of previous or current antibiotic therapy. On the other hand, the possibility of identifying, even in the latter cases, the etiological agent with biomolecular techniques is not yet a universally available procedure (3-5).

Beside cultural analysis of CSF, the complete investigation of a patient with suspected CNS infection in the emergency setting includes macroscopic analysis of the CSF (i.e., visual observation of color and clarity), microscopic analysis of its sediment after staining with Gram reagent, measurement of total proteins and glucose (and calculation of their ratio with plasma or serum concentrations), along with cytometric analysis, entailing enumeration and classification of various cellular elements (1, 2, 6).

In patients with bacterial meningitis, the macroscopic analysis of whole CSF reveals the presence of various degree of turbidity, whereas the observation of a clear supernatant associated with a conspicuous sediment is common after centrifugation. Cytometric analysis of CSF often reveals an increased white blood cell (WBC) count, typically above the conventional diagnostic thresholds (i.e., >27 WBC/μL in newborns up to 2 months of age; >7 WBC/μL in children aged between 2 months and 7 years; >5 WBC/μL in adults), principally constituted by polymorphonuclear neuthrophils. The protein concentration of CSF is also frequently increased above

Correspondence to: Sabrina Buoro, Laboratorio Analisi Chimico Cliniche, Azienda Ospedaliera Papa Giovanni XXIII, Piazza OMS 1, 24128 Bergamo. Tel. 0352674550, Fax 0352674939, E-mail sbuoro@hpg23 .it

Ricevuto: 03.09.2013 Revisionato: 10.10.2013 Accettato: 11.10.2013

200 mg/dL (7, 8), whereas the glucose concentration and its ratio with glycemia are both decreased, with the latter typically ≤0.4 (9, 10). Some exceptions to this typical presentation are represented by bacterial meningitis with limpid CSF, usually caused by *Mycobacterium tubercolosis*, *Listeria monocytogenes*, *Treponema pallidum*, *Leptospira interrogans* and by certain fungal etiologies such as that by *Cryptococcus neoformans*. In patients with these types of CNS infections, the increase of WBC in the CSF is often modest and is mainly sustained by lymphocytes. The level of protein concentrations in CSF is also modest and accompanied by a relative decrease of both CSF concentration and ratio of glucose (11).

Although the gold standard for enumeration and classification of cellular elements in CSF is still represented by optical microscopy (OM), this technique has some intrinsic limitations, such as high intra- and inter-observer imprecision, long turnaround time and need of specialized personnel, which should be specifically trained for identifying and differentiating the large array of cells that may be present in CSF, including choroid plexus epithelial cells, ependymal cells, endothelial cells, meningeal cells, leukocytes, metastatic and blast cells, as well as parasites (12, 13).

Several studies in recent years have shown that cytometric analysis of CSF may be suited to automation by means of a variety of laboratory instrumentation, including conventional cytofluorimeters, hemocytometers and automated urine particle analyzers (14-24). The Sysmex UF-1000i is a cytofluorimeter commercialized for urine particle analysis (25), which may also be reliably used for screening of CSF (24, 26). The leading strengths of UF-1000i, as compared with conventional hemocytometers, are represented by count and classification of different types of cells in various cytograms and histograms, combined with the possibility of enumeration of bacteria and mycetes, when these are present in body fluids (26, 27). Therefore, the aim of this study was to evaluate the performance of scoring systems for rapid screening of bacterial meningitis (especially in the stat setting) obtained by combining the results of UF-1000i with measurements of total proteins and glucose in the CSF.

MATERIALS AND METHODS

The study samples were 101 consecutive CSF of patients with suspected meningitis or encephalitis (38 aged <16 years and 63 aged ≥16) referred over one month to the laboratory of the general hospital of Bergamo. The specimens, collected in sterile tubes containing no additives, included 27 samples obtained from external ventricular drainage, 13 from reservoir and 61 from lumbar puncture.

Cytometric analysis of CSF was performed either using UF-1000i or by OM. Samples were analyzed on UF-1000i without any pretreatment. Cell counting in OM was performend using a Nageotte counting chamber (BlauBrand) after diluition of samples (1:2 or 1:20, depending on cellular concentration) with Turk's reagent (Carlo Erba Reagents) (6). WBC differentiation was performed by OM after cytocentrifugation of CSF native samples (100g for 3 min) on microscopic slides (Cytospin2 Thermo Scientific). Slides were stained with May-Grunwald-Giemsa reagent and examined by light microscopy at x400 magnification (6).

Data obtained on UF-1000i were compared with those obtained by OM by Person's correlation, Passing and Bablok regression, Bland-Altman plots and ROC curve analysis. In 50 out of 101 samples, cultural analysis was also performed with different culture media, including blood, chocolate, MacConkey and Columbia agar. CSF were also cultured in Brain Heart Infusion (BHI) enrichment broth. The concentrations of total proteins and glucose were measured on Advia 2400 (Siemens Healthcare Diagnostics).

The results of these tests were used to develop some diagnostic scores for rapid screening of suspected bacterial meningitis, as described in Table 1. Four different diagnostic combinations (DC) were used for the 50 samples for which cultural examination was available. Briefly, DC I was based on results of CSF glucose, CSF proteins and cytometric analysis by OM; DC II was based on cytometric analysis and bacterial count on UF-1000i; DC III was based on CSF glucose, CSF proteins, cytometric analysis and bacterial count on UF-1000i; DC IV was based on the result of DC III plus evaluation of neutrophilia (present or absent).

Statistical analysis was performed with Analyse-it (Analyse-it Software Ltd) and the study was performed in accord with the Declaration of Helsinki, under the terms of all relevant local legislation.

RESULTS

Agreement between OM and UF-1000i

The number of positive CSF, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (i.e., >7 WBC/μL in children aged between 2 months and 7 years, and >5 WBC/μL in adults) (6), was 33/63 (52%) in patients aged ≥16 and 11/38 (29%) in those younger than 16. One specimen was excluded from further analysis due to the extremely elevated number of cells (9936 WBC/μL at OM and 10,011 WBC/μL by UF-1000i), so that 100 CSF were finally used for assessing the agreement between OM and UF-1000i, with WBC count comprised between 0 and 902 cell/μL.

A high correlation was found between OM and UF-1000i, with Pearson's correlation coefficent (r) of 0.99, slope of 0.90 [95% confidence interval (CI), 0.85 to 0.96] and intercept of 0.02 (95% CI, -0.34 to 0.17) on Passing and Bablock regression, and a mean bias of -4.9/μL (95% CI, -9.7 to -0.1). When considering the 91 CSF with WBC <150/μL, r was 0.98, the slope was 0.92 (95% CI, 0.82 to 1.00) and the intercept 0.03 (95% CI, -0.30 to 0.20), with a mean bias of -1.5/μL (95% CI, -2.9 to -0.1).

In the subgroup of adult patients, the diagnostic agreement at the 5 WBC/μL threshold was 0.90, the area

Table 1
Description of parameters used for defining diagnostic scores for screening of bacterial meningitis

Score	Cytometric analysis by optical microscopy	Cytometric analysis by UF-1000i	Evaluation UF-1000i cytograms	CSF glucose	CSF total proteins
0	WBC <5/μL in adults (aged ≥16) and <7/μL in children (<16 years)	WBC <5/μL in adults and <7/μL in children	-	>40 mg/dL	≤0.45 g/L
0.5			Neutrophilia		
1	WBC ≥5/μL in adults and ≥7/μL in children	WBC ≥5/μL in adults and ≥7/μL in children	-	≤40 mg/dL	>0.45 g/L
2	WBC ≥5/μL in adults and ≥7/μL in children + neutrophilia on cytospin	WBC ≥5/μL in adults and ≥7/μL in children + bacteria ≥20/μL	-	-	≥2 g/L
	WBC ≥1000/μL	WBC ≥1000/μL			
3	WBC ≥1000/μL + neutrophilia on cytospin	WBC ≥1000/μL + bacteria ≥20/μL	-	-	-

CSF, cerebrospinal fluid; WBC, white blood cell.

Figure 1
Examples of UF-1000i white blood cell (WBC) cytograms. Left: cerebrospinal fluid (CSF) sample with a WBC total count of 8/μL. Optical microscopy (OM) revealed the presence of 92% lymphocytes (red circle), 6% monocytes and 2% neuthrophils. Right: CSF sample with a WBC total count of 22/μL. OM analysis revealed the presence of 42% lymphocytes (red circle), 33% neuthrophils (blue circle) and 20% monocytes.

under ROC curve (AUC) for UF-1000i was 0.98 (95% CI, 0.95 to 1.00), with 0.94 sensitivity and 0.87 specificity, respectively. In patients aged <16 years, the diagnostic agreement at the 7 WBC/μL threshold was 0.97, the AUC for UF-1000i was 1.00 (95% CI, 1.00 to 1.00), with 0.91 sensitivity and 1.00 specificity, respectively.

Agreement between CSF culture and UF-1000i

44 out of 50 samples for which cultural examination was available were negative for bacterial growth. The remaining 6 samples were found to be positive for coagulase-negative *Staphylococcus* (n=2), *Klebsiella pneumonie* (n=1), *Serratia marcescens* (n=1), *Staphilococcus aureus* (n=1) and *Enterococcus faecium* (n=1). Two of these samples (one positive for coagulase-negative *Staphylococcus* and the other for *Enterococcus faecium*) displayed a very low bacterial load, were negative at microscopic analysis after Gram staining, and being obtained from patients for whom a final diagnosis of CNS infection was clinically excluded, were considered as contaminated. The final number of negative and positive samples were hence 46 and 4, respectively. The diagnostic agreement between CSF culture and UF-1000i (cut-off, ≥20 bacteria/μL) was 0.98, with an AUC for UF-1000i of 0.99 (95% CI, 0.98 to 1.00), a sensitivity of 1.00 and a specificity of 0.98, respectively.

Analysis of cytograms

The analysis of the UF-WBC cytogram (fluorescent light intensity on horizontal axis and scattered light intensity on vertical axis) revealed highly suggestive patterns, which allowed to gather suitable morphological information about the cells present in the CSF (Figure 1).

Analysis of diagnostic combinations

The ROC curve analysis of diagnostic performances (AUC) of the 4 DC (as compared with microbiological examination) were as follows: 0.98 (95% CI, 0.96 to 1.00)

for DC I, 0.99 (95% CI, 0.98 to 1.00) for DC II and III, 1.00 (95% CI, 1.00 to 1.00) for DC IV. No significant differences were found when comparing the 4 AUC.

DISCUSSION

The current gold standards for enumeration/classification of WBC in CSF and for the identification of bacterial infection of CNS are still represented by OM analysis and cultural examination. Both techniques are however cumbersome, require dedicated instrumentations and trained personnel, are relatively expensive and characterized by long turnaround time and high imprecision. The automated analysis of CSF thus represents a valuable approach to overcome most – if not all – these shortcomings. The results of this study, along with those already published (24, 26), confirm that Sysmex UF-1000i, an automated instrument for urine particles analysis, exhibits suitable performance for rapid examination of CSF in patients with suspected CNS infections. The overall correlation for the WBC count on UF-1000i as compared with OM found in this study (r=0.99) was much better than that earlier reported by Nanos et al. (r=0.734) (24) and substantially identical to that previously observed in our preliminary evaluation of the analyzer (26). The reliability of UF-1000i for WBC count in CSF was further confirmed by the very limited bias when compared with OM (-4.9/μL in all samples and -1.5/μL in those with WBC <150/μL). Importantly, the diagnostic agreement with OM was high in CSF of patients aged ≥16 and even perfect in those younger than 16.

The high agreement of the bacterial count obtained with the UF-1000i and the result of CSF culture is an additional strength of this instrument, which may hence represent a viable alternative to more complex and time consuming microbiologic analyses.

The evaluation of four different test combinations for rapid CSF screening of patients with suspected CNS infection is the innovative finding of this study. Preliminary data on the four systems provided good diagnostic screening performance, although DC IV (i.e., the combination of CSF glucose, CSF total proteins, WBC count, analysis of neutrophilia and bacterial count on UF-1000i) was characterized by the best performance. The combination of two rapid clinical chemistry tests, such as glucose and total proteins, with UF-1000i analysis would represent a valid approach for supporting more complex analyses or even for replacing OM and CSF culture during emergency examination of CSF, to achieve a faster and efficient detection of CNS infections. These preliminary results need, however, to be confirmed on a larger number of samples collected in a multicenter study.

CONFLICTS OF INTEREST

No authors declared any potential conflicts of interest.

REFERENCES

1. De Luca GC, Bartleson JD. When and how investigate the patient with headache. Seminaries in Neurology 2010;30:131-44.
2. Fitch MT, Abrahamian FM, Moran GJ, et al. Emergency department management of meningitis and encephalitis. Infect Dis Clin North Am 2008;22:33-52.
3. Bonadio WA. The cerebrospinal fluid; physiologic aspects and alteration associated with bacterial meningitis. Pediatr Infect Dis J 1992;11:423-31.
4. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. Pediatrics 2001;108:1169-74.
5. Sáez-Llorens X, McCracken GH. Bacterial meningitis in children. Lancet 2003;361:2139-48.
6. Szamosi DI, Bautista JM, Cornbleet J, et al. Body fluid analysis for cellular composition: approved guidelines. CLSI document H56-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2006;26:1-99.
7. Genton B, Berger JP. Cerebrospinal fluid lactate in 78 cases of adult meningitis Intensive Care Med 1990;16:196-200.
8. Bonadio WA. The cerebrospinal fluid; physiologic aspects and alteration associated with bacterial meningitis. Pediatr Infect Dis J 1992;11:423-31.
9. Donald PR, Malan C, van der Walt A. Simultaneous determination of cerebrospinal fluid glucose and blood glucose concentrations in the diagnosis of bacterial meningitis. J Pediatr 1983;103:413-5.
10. Andreani P, Bianco I, Bottan F, et al. Vademecum per l'esame del liquor cerebrospinale in urgenza. Milano: Mediamed, 2009:1-19.
11. Schoeman JF, Donald PR. Tubercolous meningitis. Handb Clin Neurol 2013;112:1135-8.
12. Mahieu S, Vertessment F, Van Der Planken M. Evaluation of ADVIA 120 CSF assay (Bayer) vs. chamber counting of cerebrospinal fluid specimens. Clin Lab Haem 2004;26:195-9.
13. Aune MW, Becker JL, Brugnara C, et al. Automated flow cytometric analysis of blood cells in cerebrospinal fluid. Am J Clin Path 2004;121:690-700.
14. Zur B, Eichhorn L, Albers E, et al. Evaluation of 2 hematology analyzers in body fluid mode versus flow cytometry immunophenotyping of mainly neurosurgical cerebrospinal fluid samples. J Neurol Surg A Cent Eur Neurosurg 2012;73:93-8.
15. Zimmermann M, Ruprecht K, Kainzinger F, et al. Automated vs. manual cerebrospinal fluid cell counts: a work and cost analysis comparing the Sysmex XE-5000 and Fuchs-Rosenthal manual counting chamber. Int J Lab Hematol 2011;33:629-37.
16. Fleming C, Brouwer R, Lindemans J, et al. Improved software on the Sysmex XE-5000 BF mode for counting leukocytes in cerebrospinal fluid. Clin Chem Lab Med 2013;51:61-3.
17. De Jonge R, Brouwer R, de Graaf T, et al. Evaluation of new body fluid mode on the Sysmex XE-5000 for counting leukocytes and erythrocytes in cerebrospinal fluid and other body fluids. Clin Chem Lab Med 2010;48:665-75.
18. Paris A, Nhan T, Cornet E, et al. Performance evaluation of body fluid mode on the platform Sysmex XE-5000 series automated hematology analyzer. Int J Lab Hematol 2010;32:539-47.
19. Perné A, Hainfellner JA, Womastek I, et al. Performance evaluation of the Sysmex XE-5000 hematology analyzer

for white blood cell analysis in cerebrospinal fluid. Arch Pathol Lab Med 2012;136:194-8.
20. Kleine TO, Nebe CT, Löwer C, et al. Cell analysis in cerebrospinal fluid (CSF) using Sysmex® hematology analyzers XT-4000i and XE-5000: evaluation with CSF controls of the Joint German Society for Clinical Chemistry and Laboratory Medicine (DGKL). Cytometry A 2012;81:255-64.
21. Boer K, Deufel T, Reinhoefer M. Evaluation of the XE-5000 for the automated analysis of blood cells in cerebrospinal fluid. Clin Biochem 2009;42:684-91.
22. Walker TJ, Nelson LD, Dunphy BW, et al. Comparative evaluation of the Iris iQ200 body fluid module with manual hemacytometer count. Am J Clin Pathol 2009;131:333-8.
23. Glasser L, Murphy CA, Machan JT. The clinical reliability of automated cerebrospinal fluid cell counts on the Beckman-Coulter LH750 and Iris iQ200. Am J Clin Pathol 2009;131:58-63.
24. Nanos NE, Delanghe JR. Valuation of Sysmex UF-1000i for use in cerebrospinal fluid analysis. Clin Chim Acta 2008;392:30-3.
25. Broeren MA, Bahçeci S, Vader HL, et al. A. Screening for urinary tract infection with the Sysmex UF1000i urine flow cytometer screening. J Clin Microbiol 2011;49:1025-6.
26. Buoro S, Ottomano C, Appassiti Esposito S, et al. Analytical and clinical evaluation of Sysmex UF1000i for automated screening of cerebrospinal fluids. J Med Biochem 2014;33:191-6.
27. Sandhaus LM, Ciarlini P, Kidric D, et al. Automated cerebrospinal fluid cell counts using the Sysmex XE-5000 is it time for new reference ranges? Am J Clin Pathol 2010;134:734-8.