Clinical Biochemistry 47 (2014) 25–30

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Automated cerebrospinal fluid cell count — New reference ranges and evaluation of its clinical use in central nervous system infections ☆

Daniel Bremell [a,*], Niklas Mattsson [b], Fredrik Wallin [c], John Henriksson [c], Mariann Wall [b], Kaj Blennow [b], Henrik Zetterberg [b,d], Lars Hagberg [a]

[a] Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, 416 45 Gothenburg, Sweden
[b] Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, 413 45 Gothenburg, Sweden
[c] Department of Anaesthesia and Intensive Care, Sahlgrenska University Hospital, Gothenburg 413 45, Sweden
[d] UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form 4 November 2013
Accepted 13 November 2013
Available online 22 November 2013

Keywords:
Lyme neuroborreliosis
Cell count
Cerebrospinal fluid
Reference ranges
Viral meningitis
Viral encephalitis

ABSTRACT

Objectives: The purposes of this study were to establish new reference ranges for leukocytes in the CSF and to examine if the separation of mononuclear cells into lymphocytes and monocytes could be used to differentiate between various CNS infections that present with a similar picture in manual CSF cell counts.

Design and methods: The automated cell counter Siemens ADVIA 2120i was used. For the reference range section, we analyzed CSF from 80 neurologically healthy volunteers. For the differential diagnosis section we analyzed cell counts and hospital records from 175 patients with CSF mononuclear pleocytosis.

Results: Correlation was good between automated and manual leukocyte counts for samples with erythrocyte counts <250 cells/μL. For the neurologically healthy volunteers studied in the reference range section, the 95th percentile was 3.0 cells/μL for lymphocytes, 1.0 cell/μL for monocytes and 1.0 cell/μL for granulocytes. In the differential diagnosis section, comparisons were done between the groups Lyme neuroborreliosis and viral CNS infection. There were no significant differences between these two groups regarding cell counts; neither for lymphocytes, median 58 cells/μL vs. 72 cells/μL (P = n.s.); nor for monocytes, median 13 cells/μL vs. 16 cells/μL (P = n.s.); nor for granulocytes, median 1 cell/μL vs. 2 cells/μL (P = n.s.)

Conclusions: We suggest new CSF cell count reference ranges of <4 cells/μL for lymphocytes, <3 cells/μL for monocytes and <3 cells/μL for granulocytes. The separation of mononuclear cells into lymphocytes and monocytes did not facilitate the discrimination between Lyme neuroborreliosis and viral CNS infection.

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Introduction

Cerebrospinal fluid (CSF) cell count is an important analysis in the investigation of central nervous system (CNS) infections. The results of the analysis form the basis for initial decisions on therapy and further examinations [1]. Traditionally, the analysis has been manually performed with microscope and cell counting chamber. This technique has several drawbacks. It is time-consuming, requires trained laboratory personnel on duty 24 h a day, and studies have shown high inter- and intra-operator variability even among trained staff [2,3]. Recently, automated CSF cell count systems have been introduced. Several studies have shown equal performance of the newest automated systems to that of manual counts [2,4–6]. The use of automated systems can lead to reduced turn-around-time for samples and lower costs. Zimmerman et al. describe a reduced turn-around-time from 635 s to 85 s and a reduced cost from 6.74 EUR to 1.22 EUR for automated instead of manual CSF cell counts [7]. Furthermore, some of the automated systems give a more detailed cell differentiation than manual analysis. Manual analysis usually separates the cells into erythrocytes, granulocytes and mononuclear cells, whereas certain automated systems further separate mononuclear cells into lymphocytes and monocytes [8].

The new technique raises questions, such as what reference ranges to use, and if the additional information on lymphocytes and monocytes is of clinical use. Reference ranges for manual CSF cell counts have been universally used for decades. Yet, their origin is largely unclear. When the method of analysis changes significantly, it is common practice to establish new reference ranges [9]. Therefore, one of the objectives of

☆ Sources of support: The study was supported by grants from the Swedish State Support for Clinical Research (ALFGBG 144431, ALFGBG 74100), the Swedish Research Council and Yngve Land's Memory Fund. The funding sources were not involved in the design or the implementation of the study.

* Corresponding author at: Infektionskliniken, Sahlgrenska Universitetssjukhuset, 416 85 Göteborg, Sweden.
E-mail addresses: daniel.bremell@infect.gu.se (D. Bremell), niklas.mattsson@neuro.gu.se (N. Mattsson), fredrik.wallin@vgregion.se (F. Wallin), john.henriksson@vgregion.se (J. Henriksson), mariann.wall@vgregion.se (M. Wall), kaj.blennow@neuro.gu.se (K. Blennow), henrik.zetterberg@clinchem.gu.se (H. Zetterberg), lars.hagberg@medfak.gu.se (L. Hagberg).

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http://dx.doi.org/10.1016/j.clinbiochem.2013.11.010

this study was to determine reference ranges for granulocytes, lymphocytes and monocytes in the CSF by using the automated analyzer Siemens ADVIA 2120i.

The second objective was to investigate if the more detailed results produced by automated analyzers could be used to differentiate between CNS infections that had hitherto presented with a similar picture in manual CSF cell counts. There are several CNS infections that present with low levels of granulocytes but elevated levels of mononuclear cells, moderately elevated protein and no glucose consumption, e.g. Lyme neuroborreliosis, neurosyphilis, viral meningitis and viral encephalitis [10]. As the symptoms of these infections partly overlap, diagnosis in the acute stage may be difficult [11,12]. In this retrospective study we examined if the separation of mononuclear cells into lymphocytes and monocytes could be of clinical use.

Material and methods

Study participants

For the initial evaluation of the performance of the automated analyzer Siemens ADVIA 2120i, both manual and automatic cell counts were performed on all CSF samples sent to the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital during a two-month period.

For the reference range section of the study, neurologically healthy volunteers were recruited among patients undergoing orthopedic surgery where spinal anesthesia was used. Inclusion criteria were absence of neurological disease and neurological symptoms. The spinal needle was inserted in the L2/3, L3/4 or L4/5 interspace. Before administering the anesthetic agent, 5 mL of CSF was aspirated and immediately transported to the laboratory for analysis. Written informed consent was obtained from all patients.

For the differential diagnosis section of the study, we reviewed hospital records for all patients that had undergone lumbar puncture for CSF sampling at the Department of Infectious Diseases, Sahlgrenska University Hospital, during the period January 1, 2010 to December 31 2012. Data on CSF cell count and final diagnosis was obtained. The study was approved by the regional ethical review board at the University of Gothenburg.

Analyses

Manual CSF cell counting was performed on a Fuchs–Rosenthal hemocytometer after 1:2 dilution with methylene blue. Cells were counted in 32 1 mm^2 areas. All counts were done in duplicate by two experienced laboratory technicians. The average value of the results of the two manual examinations was used for comparisons between the methods. Automated CSF cell counting was performed on a Siemens ADVIA 2120i instrument within 1 h of sampling using the ADVIA 2120i CSF Assay according to the manufacturer's instructions (Siemens AG, Erlangen, Germany). The method requires 300 μL of CSF and reports counts for erythrocytes, lymphocytes, monocytes and granulocytes, which are differentiated on the basis of size, absorbance and light scattering characteristics. The instrument is in routine use at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, is regularly assessed by internal and external quality control programs and has an inter-assay coefficient of variation of 10–12%. All analyses were done by experienced and certified laboratory technicians.

Statistics

Reference ranges were calculated according to guidelines by the International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI), CSLI C28-A3. Reference ranges were calculated using non-parametric methods. Where possible, ranges were also calculated with the robust method according to CSLI C28-A3 [9]. For the reference range section, data are presented as the mean (standard deviation, SD). Reference range calculations were performed using MedCalc 12.4 (MedCalc Software, Ostend, Belgium). For the differential diagnosis section, data are presented as the median (range). Analyses were made using non-parametric methods. Differences between groups were analyzed with the Mann–Whitney *U* test. Correlations were analyzed using the Spearman rank order correlation. P values of <0.05 were considered significant. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, USA).

Results

Initial evaluation

121 consecutive CSF samples were used for the evaluation. Correlation between manual and automated cell counts was excellent for erythrocytes, Spearman $r = 0.98$ ($P < 0.0001$) (Fig. 1A). The correlation was lower for leukocytes (Spearman $r = 0.82$, $P < 0.0001$). This was caused by erythrocytes in blood stained samples being misclassified as leukocytes. The limit for this artifact was found to be an erythrocyte count of 250 cells/μL. The correlation analysis for leukocyte counts was redone on CSF samples with an erythrocyte count <250 cells/μL ($n = 98$). On this material the correlation was good (Spearman $r = 0.87$, $P < 0.0001$), also for samples with a low leukocyte count (Fig. 1B,C). In the very low cell range (cell counts <3 cells/μL) the correlation was weaker.

Reference range section

CSF was sampled from 94 neurologically healthy volunteers. Fourteen individuals were excluded from further analysis: two because the CSF did not reach the laboratory within 1 h, and 12 because they had erythrocyte counts >250 cells/μL. Among the 80 included individuals, the mean age was 67 years (SD 15.3), mean lymphocyte count was 0.93 cells/μL (SD 0.94), mean monocyte count was 0.32 cells/μL (SD 0.52), and mean granulocyte count was 0.05 cells/μL (SD 0.22). The 95th percentile was 3.0 cells/μL for lymphocytes, 1.0 cell/μL for monocytes and 1.0 cell/μL for granulocytes (Fig. 2). Using the robust method, the 95% right-sided reference interval was 2.4 cells/μL for lymphocytes. It was not possible to use the robust method for reference range calculations for monocytes or granulocytes due to the large number of samples with the same value. There was no significant correlation between age and cell count for any of the cell types (data not shown).

Differential diagnosis section

During the period reviewed, 878 CSF samplings were performed on 771 patients. In 234 of the samples lymphocytes and/or monocytes were elevated (>4 cells/μL). These 234 samples came from 175 patients. In cases where one patient had several CSF samples with elevated lymphocytes and/or monocytes, the first one was chosen for the study. Thus, 175 CSF cell counts from 175 patients were further analyzed. Patients were grouped according to diagnosis in the following groups (number of patients): bacterial meningitis (20), HIV infection (33), Lyme neuroborreliosis (39), viral CNS infection excluding HIV (60), other infectious disease (9) and non-infectious disease (14). Diagnoses were determined according to standard clinical criteria at the Department of Infectious Diseases, Sahlgrenska University Hospital and coded according to ICD-10 [13]. Background data and cell counts for each of the groups are shown in Table 1. Included diagnoses for the groups of bacterial meningitis, viral CNS infection excluding HIV, other infectious disease and non-infectious disease are shown in Table 2. Scatter plots of the cell counts for the various diagnostic groups are shown in Fig. 3. Comparisons were done between the groups Lyme neuroborreliosis and viral CNS infection excluding HIV. There were no significant differences between these two groups regarding cell counts;

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