Referenties

1. NHG-standaard *Diep Veneuze Trombose*. 2008, M86.
2. ten Cate-Hoek AJ, Toll DB, Büller HR, Hoes AW, Moons KG, Oudega R, Stoffers HE, van der Velde EF, van Weert HC, Prins MH, Joore MA. Cost-effectiveness of ruling out deep venous thrombosis in primary care versus care as usual. J Thromb Haemost. 2009; 12: 2042-9.
3. Büller HR, ten Cate-Hoek AJ, Hoes AW, Joore MA, Moons KG, Oudega R, Prins MH, Stoffers HE, Toll DB, van der Velde EF, van Weert HC. Safely ruling out deep venous thrombosis in primary care. Ann Intern Med. 2009; 150: 229-35.
4. Wells PS, Anderson DR, Rodger M, Stiell I, Dreyer JF, Barnes D, et al. Excluding pulmonary embolism at the bedside without diagnostic imaging: management of patients with suspected pulmonary embolism presenting to the emergency department by using a simple clinical model and d-dimer. Ann Intern Med. 2001; 135: 98-107.
5. Oudega R, Hoes AW, Moons KG. The Wells rule does not adequately rule out deep venous thrombosis in primary care patients. Ann Intern Med. 2005; 143: 100-7.
6. Toll DB, Oudega R, Bulten RJ, Hoes AW, Moons KG. Excluding deep vein thrombosis safely in primary care. J Fam Pract. 2006; 55: 613-8.
7. CBO-richtlijn Diagnostiek, behandeling en zorg voor patiënten met een beroerte. 2008.
8. Janssens PMW, Schipper MH. Is het een goede keus om met 'point of care testing' plus transport van monsters het laboratorium van een klein algemeen ziekenhuis te vervangen? Ned Tijdschr Klin Chem Labgeneesk. 2010; 35: 54-9.
9. Moonen AF, Thomassen BJ, Knoors NT, van Os JJ, Verburg AD, Pilot P. Pre-operative injections of epoetin-alpha versus post-operative retransfusion of autologous shed blood in total hip and knee replacement: a prospective randomised clinical trial. J Bone Joint Surg Br. 2008; 90: 1079-83.
10. Chew DP, Bhatt DL, Lincoff AM, et al. Defining the optimal activated clotting time during percutaneous coronary intervention: aggregate results from 6 randomized, controlled trials. Circulation. 2001; 103: 961-6.

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Evaluation of the HemoCue WBC analyzer to count leucocytes in body fluids

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Hemocytometric analysis of body fluids aids in the management and diagnosis of several diseases. Counting of white blood cells (WBC) in synovial fluid discriminates between inflammatory and non-inflammatory forms of joint swelling (1). Differential counting of WBC and erythrocytes (RBC) in cerebrospinal fluid (CSF) forms important and rapid available information in the diagnosis of meningitis, encephalitis and neuroinflammatory diseases like multiple sclerosis. Bacterial peritonitis is suspected when a large number of polymorph nucleated cells (PMN >250 x 106/L) (2) or WBC (WBC >100 x 106/L with ≥50% PMN) (3) are present in ascites and in continuous ambulatory peritoneal dialysis (CAPD) fluid, respectively. Microscopic analysis has been the gold standard for determination of the (differential) WBC and RBC counts in fluids but suffers from high imprecision (4), long turnaround times, and requirement of skilled personnel and mostly is not available 24 hours a day. Automated hemocytometric analysis may be the answer to these problems. Dedicated body-fluid modules have been developed by some manufacturers and are available on two commercial hemocytometers (5, 6). However, these machines are relatively costly and are mostly only available in central laboratory facilities. Moreover, the aspirated volume is relatively large (ca. 130 µL) and the matrix of some fluids such as drain fluids, synovial fluids, and broncheo alveolar lavage (BAL) fluids mostly is complex and not always suitable for automated hemocytometric analysis. Furthermore, no POC-instrument is on the market today to count WBC in body fluids. HemoCue recently launched a very small POC-instrument to count WBC in blood. We investigated whether this POC-analyzer also can be used to count WBC in body fluids.

Methods

The following body fluids were prospectively studied: CSF, pleural fluid, ascites, CAPD fluid, and synovial fluid. CSF was delivered in plain tubes, synovial fluid in heparin-anticoagulated tubes and the other fluids in EDTA-anticoagulated tubes. Because only material was used that was leftover from routine analysis, informed patient consent was not required. Routine body fluids were mixed and first counted using the Sysmex XE-5000 Body-Fluid (BF) Module (Sysmex, Etten-Leur, The Netherlands) in the open-manual mode. Directly after, fluids were measured on the HemoCue WBC analyzer (HemoCue Diagnostics B.V., Waalre, The Netherlands). After mixing, fluid was pipetted on parafilm and ca. 10 µL of fluid was drawn into a single-use microcuvette by capillary action. In the microcuvette, red blood cells were lysed (saponin) and WBC stained (methylene blue). An image of the stained WBC was

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taken automatically after introduction of the microcuvette in the analyzer and WBC were counted within 3 minutes by the software image analysis. Agreement between methods was analyzed by creating Bland & Altman difference plots and Passing & Bablok regression analyses in Analyse-it for Excel.

Results

Combining all fluids, good agreement was observed between both analyzers: HemoCue = 0.93 × XE-5000 BF-mode + 86 (n=56; WBC in 10^6/L; figure 1). The HemoCue WBC analyzer counted significantly less WBC in the higher ranges and more WBC in the lower ranges because the 95% confidence interval (95% CI) of the slope (0.90 - 0.99) did not include 1 and the 95% CI of the intercept (43 - 137) did not include 0. The mean bias was - 297 × 10^6/L and non-significant (95% CI: - 716 - 122 × 10^6/L; figure 2). The slightly negative bias was mostly caused by some extreme outliers in the higher WBC counts, because in the lower cell counts the HemoCue in general counted more cells than the XE-5000. Exclusion of 5 extreme high WBC counts (>10000 × 10^6/L) resulted in a non-significant positive bias of 47 × 10^6/L (95% CI: - 52 - 146 × 10^6/L); similarly, exclusion of 5 outliers did not dramatically change the regression results (HemoCue = 0.95 × XE-5000 BF-mode + 82; n=51; 95% CI slope: 0.90 - 1.04 × 10^6/L, 95% CI intercept: 18 - 134 × 10^6/L). Good agreement was also observed between both analyzers for the individual fluids ascites (HemoCue = 0.99 × XE-5000 BF-mode + 145; n=24; WBC in 10^6/L) and pleural fluid (HemoCue = 0.89 × XE-5000 BF-mode + 102; n=15; WBC in 10^6/L). For the other individual fluids, too few samples were measured to perform Passing-Bablok regression analyses.

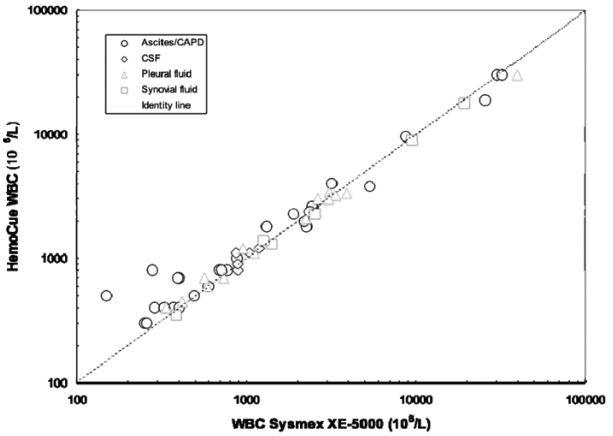

Figure 1. HemoCue vs. XE-5000 BF-mode. ○ Ascites/CAPD; ◇ CSF; △ Pleural fluid; □ Synovial fluid; ····· identity line.

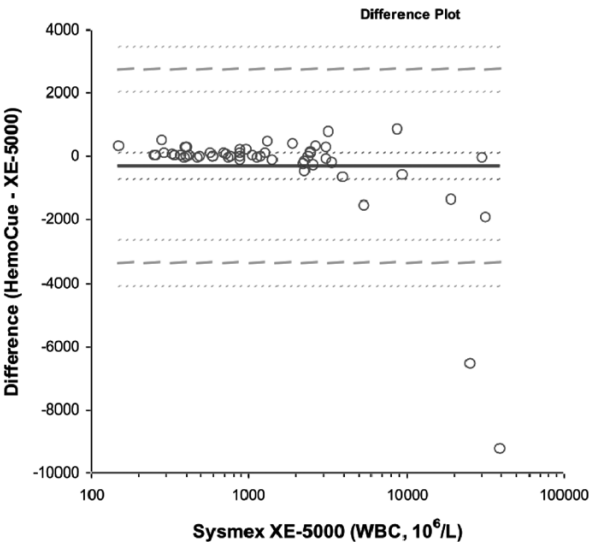

Figure 2. Bland & Altman bias plot for the HemoCue WBC analyzer versus Sysmex XE-5000 BF-mode. —— Bias (-297); ····· 95% CI; - - - - 95% Limits of agreement (-3362.1 to 2768.9).

Conclusion

In this study, we show that the HemoCue WBC analyzer agrees well with the Sysmex XE-5000 BF-mode and allows to counting WBC in body fluids. The HemoCue WBC analyzer could be of use in POC settings such as at the emergency department, in situations where skilled personnel or financial resources are scarce (e.g., peripheral locations, underdeveloped countries), or when the sample matrix or volume does not permit automated counting in the clinical chemistry laboratory. The use of the HemoCue WBC analyzer is limited because of several reasons. First, like all other blood modes on automated cell counters, false-positive counts are generated when large cells (macrophages, mesothelial cells, tumor cells) are present. Second, the measuring range (300 - 30000 × 10^6/L) does not allow cell counting in clinically relevant ranges in CSF (most counts will be below 300 ×10^6/L) and CAPD fluid (decision cut-off at WBC >100 ×10^6/L). Third, no differential is performed, which is important information in the diagnosis of bacterial peritonitis in ascites (PMN >250 x 10^6/L) or CAPD fluid (WBC>100x10^6/L with ≥50% PMN) and to distinguish bacterial meningitis from other causes of meningitis in CSF fluid.

References

1. Shmerling RH, Delbanco TL, Tosteson AN, Trentham DE. Synovial fluid tests. What should be ordered? JAMA. 1990; 264 (8): 1009-14.
2. Runyon BA. Management of adult patients with ascites due to cirrhosis: an update. Hepatology. 2009; 49 (6): 2087-107.
3. Piraino B, Bailie GR, Bernardini J, Boeschoten E, Gupta A, Holmes C, et al. Peritoneal dialysis-related infections recommendations: 2005 update. Perit Dial Int. 2005; 25 (2): 107-31.
4. De Jonge R, Brouwer R, Smit M, de Frankrijker-Merkestijn M, Dolhain RJ, Hazes JM, et al. Automated counting of white blood cells in synovial fluid. Rheumatology (Oxford). 2004; 43 (2): 170-3.
5. Aune MW, Becker JL, Brugnara C, Canfield W, Dorfman DM, Fiehn W, et al. Automated flow cytometric analysis of blood cells in cerebrospinal fluid: analytic performance. Am J Clin Pathol. 2004; 121 (5): 690-700.
6. De Jonge R, Brouwer R, de Graaf MT, Luitwieler RL, Fleming C, de Frankrijker-Merkestijn M, et al. Evaluation of the 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 (5): 665-75.