Automated counting of white blood cells in synovial fluid

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Objectives. To evaluate the performance of automated leucocyte (white blood cell; WBC) counting by comparison with manual counting.

Methods. The number of WBC was determined in heparinized synovial fluid samples by the use of (i) a standard urine cytometer (Kova) and a microscope (reference method) and (ii) a haematology analyser (Sysmex XE-2100; WBC/BASO and DIFF channels). Imprecision within and between days was determined by replicate analysis of a low (level A; WBC \( \sim 0.560 \times 10^9/l \)) and a high (level B; WBC \( \sim 1.081 \times 10^9/l \)) dedicated synovial fluid control (Quantimetrix).

Results. The WBC count of the DIFF channel was highly correlated with the WBC count of the microscopic reference method \( (r = 0.99; \text{WBC analyser} = 0.870 \times \text{WBC reference method} + 0.413). \) In contrast, no agreement existed between WBC counts generated by the WBC/BASO channel of the analyser and the reference method \( (r = 0.52; \text{WBC analyser} = 0.008 \times \text{WBC reference method} + 0.079). \) Within-day imprecision (4–7%) and between-day imprecision (10%) of the haematology analyser were smaller than the within-day imprecision (12%) and the between-day imprecision (20–22%) of the manual reference method. For manual counting, inter-observer coefficients of variation were 35.9% (control level A) and 21.0% (control level B).

Conclusions. The WBC count in synovial fluid can be reliably determined using the DIFF channel of the Sysmex XE-2100. Automated counting of WBC in synovial fluid offers more precise and faster results than manual counting.

Key words: Synovial fluid, Sysmex XE-2100, Automated, Leucocytes.

Synovial fluid analysis forms a vital step in the diagnosis [1, 2] and management [3] of arthritis. The determination of the number of leucocytes (white blood cells; WBC) in synovial fluid is the most important tool (together with the percentage of polymorphonuclear cells) to discriminate between inflammatory and non-inflammatory forms of joint swelling [1, 2, 4–6]. A total WBC count \( > 2 \times 10^9/l \) is indicative of inflammatory joint diseases, such as infectious (septic) arthritis, crystal synovitis (gout, pseudogout) and autoimmune arthritis (e.g. rheumatoid arthritis, psoriatic arthritis). A WBC count \( < 2 \times 10^9/l \) points to a non-inflammatory origin (e.g. osteoarthritis, post-traumatic) of the joint swelling.

In the clinical laboratory, the reference method for counting the number of WBC in synovial fluid is by the use of a cytometer and a microscope [1]. Surprisingly, and in contrast to its central place in the diagnosis of arthritis, the reliability of this test has been little studied. The available quality control studies indicate that there is great variation among and within laboratories in manual counting of WBC in synovial fluid [7, 8], sometimes leading to erroneous classification of fluids as either inflammatory or non-inflammatory. Automated WBC counting of synovial fluid potentially could improve performance. However, automated cell counting of synovial fluid is considered inaccurate [8]. Fat globules, crystals, synovial cells, chondrocytes and fragments of cartilage in synovial fluid could result in artificially elevated WBC counts using the Coulter counter (impedance method) [9, 10]. In contrast, other reports observed good correlations between automated (Coulter) counting and manual counting [11–13]. To our knowledge, no study has evaluated the performance of the synovial fluid WBC count of the newest generation of flow cytometry-based cell counters. Therefore, we tested whether the WBC count in synovial fluid can be determined reliably using a routine haematology analyser (Sysmex XE-2100).

Materials and methods

Preparation of synovial fluid samples

Synovial fluid samples were obtained from the affected knees of patients with a swollen knee joint. Residual material was used for this study after informed consent. Synovial fluid was collected in heparin tubes. Upon arrival in the laboratory, the tubes containing synovial fluid were mixed, diluted 10 times in phosphate-buffered saline (pH 7.4) containing 0.01 mol/l phosphate, and mixed again. Diluted synovial fluid was analysed directly (within 1 h) using a cytometer/microscope as well as the haematology analyser; undiluted synovial fluid was analysed only on the Sysmex XE-2100 haematology analyser (Goffin Meyris, Etten-Leur, The Netherlands). Microscopic examination of diluted synovial fluid was performed at a magnification of 400×.

Methods

Diluted synovial fluid was analysed using a standard urine cytometer (Kova Glasitic 10, Hycor Biomedical, Penicuik, UK). The total number of WBC in 10 small grids was counted and the
results converted to WBC $\times 10^9$/l. This microscopic evaluation was considered the reference method. Synovial fluid was also analysed in the open-manual mode with the XE-2100 haematology analyser. WBC counts ($10^9$/l) were read from both the WBC/BASO (basophil) channel and the DIFF (differential) channel (service screen). To determine agreement between methods, paired results of 38 patients were obtained.

Imprecision studies

Precision studies were performed using a low (control level A; WBC $\sim 0.560 \times 10^9$/l) and a high (control level B; WBC $\sim 1.081 \times 10^9$/l) dedicated synovial fluid control (Synovialscopics synovial fluid control; Quantimetrix, Redondo Beach, CA). This control material also contained red blood cells, calcium pyrophosphate dehydrate (control A) and monosodium urate (control B) crystals. WBC were predominantly lymphocytes in control A and predominantly neutrophils in control B. For between-day imprecision (inter-assay imprecision), total WBC counts for both control levels were determined using the haematology analyser (DIFF channel only) and the haemocytometer/microscope for 20 non-consecutive days. For within-day imprecision (intra-assay imprecision), 10 replicates of both control levels were assayed on the same day using both methods. All studies were performed by the same medical technician (MS) and synovial control fluid was not diluted before analysis. On the same day, 10 different medical technicians determined the WBC count of both control levels using the reference method for determination of the inter-individual variation. The coefficient of variation (CV) for each parameter was calculated.

Statistics

To validate the synovial fluid WBC count on the Sysmex XE-2100 haematology analyser, Pearson’s correlation coefficients were calculated between the WBC count generated by the haematology analyser and the WBC count determined by cytometry/microscopy (reference method). Agreement between methods was determined using Passing–Bablok regression analysis.

Results

Method comparisons

Regression analysis between different methods is shown in Fig. 1. There was a poor correlation between the microscopic WBC count and the WBC count of the WBC/BASO channel of the haematology analyser ($r = 0.52$, $P = 0.001$; linear regression line equation, $y = 0.008x + 0.079$) (Fig. 1A). In contrast, the WBC count of the DIFF channel was highly correlated with the WBC count of the microscopic reference method ($r = 0.99$, $P < 0.0001$; linear regression line equation, $y = 0.870x + 0.413$) (Fig. 1B).

Discussion

Method comparison

The Sysmex XE-2100 uses two different WBC-counting channels: the so-called WBC/BASO channel and the DIFF channel. The former is generally applied for total WBC counting and selective basophil (BASO) counting, whereas the DIFF channel is the source of information for counting neutrophils, lymphocytes, monocytes and eosinophils. Also, in the service screen of the DIFF channel, a different total WBC count (sum of the five-part DIFF) is presented together with the ratio of both WBC counts. When this ratio between the two WBC counts is not close to 1, the result of the differential WBC analysis is not displayed (error messages: ‘DIFF Channel Error’ and ‘RBC Lyse Resistance’). The WBC/BASO channel of the Sysmex XE-2100 is the standard channel for reporting total WBC counts. In this study, no
correlation was present between the WBC count generated by the WBC/BASO channel and the reference method, and falsely low results were reported by the analyser (Fig. 1A). Often, the error message ‘WBC Abnormal Scattergram’ was generated. To our surprise, good agreement was observed between the WBC count generated by the DIFF channel and the microscopic reference method (Fig. 1B). Exclusion of the high outlier visible in Fig. 1 did not result in significant changes in the observed regressions or correlations. Although good agreement was observed, automated WBC counts (DIFF channel) were lower than manual WBC counts (Fig. 1B). This is in contrast to studies using the Coulter counter (impedance method), in which automated counts were always higher than manual counts [11–13]. The positive bias observed with impedance-based WBC counting can possibly be accounted for by particulate matter (e.g. fat globules) that are registered as WBC [10].

Diluted and undiluted WBC counts on the haematology analyser (DIFF channel) were also highly correlated ($r = 1.00$, $P < 0.0001$; linear regression line equation, $y = x - 0.3$, results not shown) and hence prediluting synovial fluid samples is not strictly necessary. Storage of synovial fluid for 24 h at 4°C resulted in a non-significant decrease of 10% in the automated (DIFF channel) WBC count (results not shown).

Falsely low WBC counts in the WBC/BASO channel were produced by the synovial fluid matrix. The low pH of the inorganic surfactant used in the WBC/BASO channel (pH 3.4) resulted in mucin clotting (polymerization of hyaluronate [14]) and falsely low WBC counts because, after hyaluronidase treatment, the WBC count in the WBC/BASO channel dramatically increased and equalled the WBC count of the DIFF channel (results not shown). Because the surfactant used in the DIFF channel is not acidic (pH 7.3), mucin clotting does not occur and therefore this channel correctly counts the number of WBC in synovium. Similar results were obtained using the Advia 120 cell counter (Bayer, M¨yrdrecht, The Netherlands) (BASO vs PEROX channel, results not shown).

**Imprecision studies**

At both control levels, the within-day and between-day imprecision of the automated WBC count (DIFF channel) was good (Table 1). Imprecision of the manual count was poor and about twice as large as the automated analyses (Table 1). These results are not surprising because about seven times more cells are counted by the haematology analyser compared with manual counting. In the WBC/BASO and DIFF channels of the XE-2100, blood or fluids are diluted 1:50 and 1:51 respectively, with surfactant and staining solutions, after which the number of cells in 40 µl is counted by flow cytometry. For example, ~700 cells are counted by the Sysmex XE-2100 compared with 100 cells with our manual counting procedure in a synovial fluid sample containing $1 \times 10^9$ WBC/l (synovial fluid control B). For within-run imprecision, CVs for manual synovial fluid WBC counts of 1–18% (WBC > $1.5 \times 10^9$/l) and 20–62% (WBC < $0.3 \times 10^9$/l) have been reported before [7, 13]. Precision studies of the manual WBC count were performed by one trained medical technician in this study. It should be remembered that, in practice, the microscopic evaluation of synovial fluid is performed by a team of trained medical technicians. When 10 trained medical technicians manually analysed the same synovial fluid control samples A and B in the present study, CVs were fairly large (＞20%) and comparable to the variation between two technicians (21%) reported earlier [13]. Similarly, a maximal difference of 25% in the mean manual WBC counts between two technicians was observed in the study of Schumacher et al. [7] and a large inter-laboratory CV of 30% was observed by Hasselbacher [8]. Thus, the better analytical precision of automated synovial fluid WBC counts may improve the low confidence that clinicians have in these results at present [15]. The cell counter offers the advantage that its performance is monitored regularly by internal and external quality control programs. Furthermore, the accuracy of automated (and manual) synovial fluid WBC counts can now be followed using the dedicated synovial fluid controls that have become available recently.

In conclusion, determination of the WBC count in synovial fluid using the DIFF channel of the Sysmex XE-2100 haematology analyser is highly correlated with microscopic evaluation. Automated WBC counting is faster and analytically more precise than manual counting. Moreover, the dedicated synovial fluid controls now make quality control of automated analysis of synovial fluid WBC counting possible.

The authors have declared no conflicts of interest.


