Automated Counting of White Blood Cells in Synovial Fluid

Robert de JONGE

Department of Clinical Chemistry, Erasmus MC,University Medical Centre Rotterdam, Rotterdam, The Netherlands.

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INTRODUCTION

This presentation will describe the automated analysis of white blood cells in synovial fluid, research that was initiated by the Clinical Chemistry and Rheumatology Departments in the Erasmus University Medical Centre in Rotterdam, The Netherlands. ***Fig. 1*** shows the routine blood counting section of the laboratory with an analytical line consisting of two SYSMEX XE-2100 fully automated blood cell analysers linked to an SP-100 slide preparation unit.
Synovial fluid analysis forms part of the routine work of the laboratory as an important step in the diagnosis and management of arthritis. The discrimination between non-inflammatory and inflammatory forms of joint swelling is important and many parameters exist, which may differentiate between non-inflammatory and inflammatory joint effusions. Some of these are listed in ***Table 1***.

Fig. 1 *Sysmex HST-302 in the Erasmus MC.*

Table 1 *Features used to distinguish between non-inflammatory and inflammatory joint disease.*

Parameter	Non-inflammatory	Inflammatory
Cytology		
WBC	≤ 2 x 10^9 /L	> 2 x 10^9 /L
PMN	≤ 75 %	> 75 %
Crystals	negative	yes / no
Biochemical		
Glucose	≥ 4.2 mmol /L	< 4.2 mmol /L
Total protein	≤ 30 g /L	> 30 g /L
LDH	≤ 250 U /L	> 250 U /L
RF	negative	yes / no
Associated diseases	Osteoarthritis Traumatic arthritis Overusage Osteochondritis	Infective (septic) arthritis Crystal synovitis (gout) Auto-immune (RA, SLE)

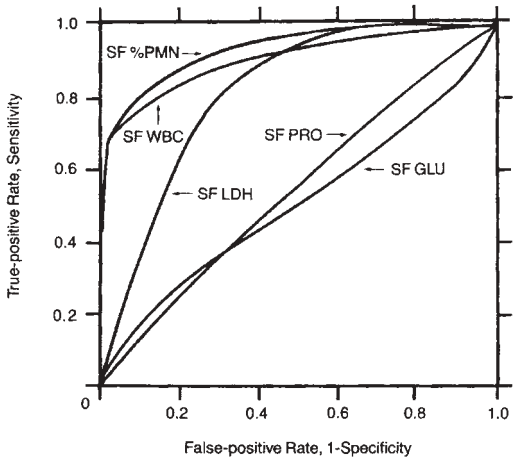


Fig. 2 *ROC analysis of parameters for discriminating inflammatory from non-inflammatory joint disease. (PMN=polymorphonuclear leukocytosis ; WBC=total white cell count ; LDH=lactic dehydrogenase ; PRO=total protein ; GLU=glucose.)*

Table 2 *Schmerling et al., JAMA 1990.*

SF test	Sensitivity	Specificity	ROC area
Total WBC count	0.84	0.84	0.91
Neutrophils %	0.75	0.92	0.94
Glucose	0.20	0.84	0.51
Protein	0.52	0.56	0.54
LDH	0.83	0.71	0.81

Schmerling and colleagues [1] studied these parameters by means of Receiver Operator Characteristic (ROC) analysis to determine which tests were most useful (***Fig. 2***, ***Table 2***).

ROC analysis characteristics show that the total leukocyte count and the percentage neutrophil count were the best discriminators between inflammatory and non-inflammatory effusions. Chemistry analyses proved to be quite poor discriminators with the exception of lactic dehydrogenase which lay somewhere in the middle. Thus it is clearly demonstrated that synovial fluid cytology forms a vital step in the evaluation of a painful and swollen joint.

THE CURRENT STATUS OF SYNOVIAL FLUID CELL COUNTING

Currently in most clinical laboratories the standard method for synovial fluid cell counting remains the haemocytometer chamber and the microscope. This method, however, has many problems, not least of which are the large intra- and interlaboratory variations in result [3,4] leading to erroneous classification of fluids as either inflammatory or non-inflammatory. Amer et al [5] assessed the utilization of synovial fluid analysis by means of a postal survey among rheumatologists and orthopaedic surgeons in the UK on synovial fluid analysis in general. Only microbiological tests and polarized light microscopy (PLM) were used regularly. Respondents were confident in microbiological assays but not in cell counts nor PLM unless they were undertaking these procedures themselves. This was the reason the rheumatologists visited our laboratory in Rotterdam; they were not satisfied with the results from the laboratory. The laboratory was keen to undertake automated counting because this practice potentially could improve performance. However, the technique is considered inaccurate because of a number of adverse reports in the literature. Particulate matter, especially fat globules, can lead to false elevation of leukocyte counts with aperture impedance counters [6,7]. There are, however, reports indicating a good correlation between manual and automated counts using an impedance counter [8,9,10]. There do not appear to be any reports on the use of flow cytometry based cell counters for this purpose.

STUDY AIMS

The study aims were really threefold; (1) to evaluate the performance of the XE-2100 for synovial fluid counting by comparison with the routine Kova urine cytometer / microscopy method as reference; (2) to evaluate the precision of both methods; and (3) to evaluate the stability of leukocytes in synovial fluid.

STUDY METHODS

1. Comparison : The design of the study was quite simple. Synovial fluid from the knee was aspirated and anticoagulated with heparin. This was mixed and diluted x10 in phosphate buffered saline (PBS) and the leukocytes counted by the reference chamber method and by the XE-2100. The WBC/BASO channel of the XE-2100 is designed to give the total leukocyte count for EDTA anticoagulated human whole blood. A different reagent system is employed in the DIFF channel, which with human whole blood provides a 4-part differential leukocyte count. The total leukocyte count in the DIFF channel as well as the ratio between the leukocyte counts in the DIFF and WBC/BASO channels can be read from the service screen (***Fig. 3***). Of course the sum of the leukocyte components in the DIFF channel should equal the total leukocyte count obtained from the WBC/BASO channel (ratio = 1). However when counting in synovial fluid this does not occur, there being a very substantial difference between the two counts, the WBC/BASO count being very much lower (see example in ***Fig. 3***: ratio = 150).

Which instrument count is correct? The comparison of the WBC/BASO count with the chamber count is shown in ***Fig. 4a*** indicating very poor agreement between the two methods with the instrument giving very low and often zero counts (r = 0.65; slope = 0.007). The picture is totally different, however, when comparing the DIFF channel count with the chamber count (***Fig. 4b***). Here there is good agreement (r = 0.99; slope = 0.87). The instrument gives a slightly lower count than the chamber method. The DIFF channel counts for diluted and undiluted synovial fluid show excellent agreement (***Fig. 4c***).

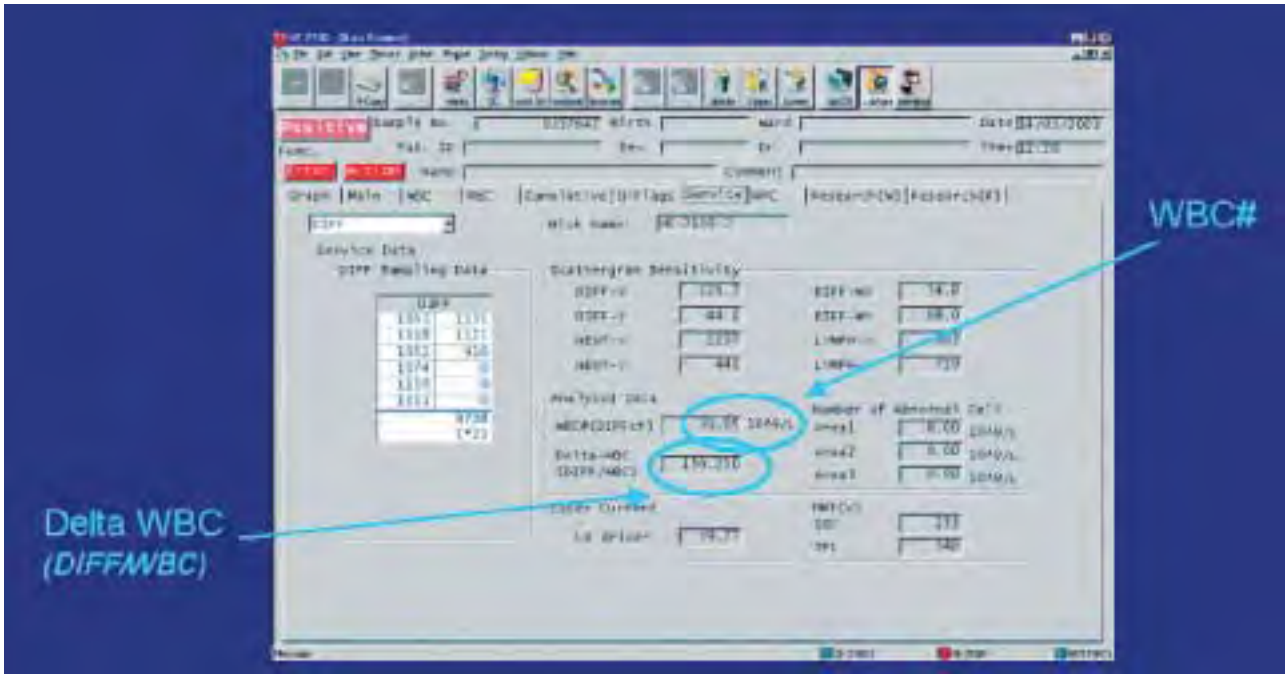


Fig. 3 *Service screen from the XE-2100 showing the count difference between the DIFF WBC and the WBC count from the WBC/BASO channel.*

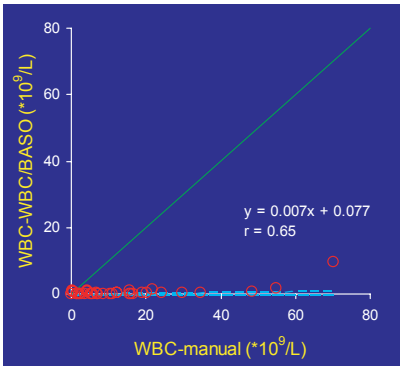


Fig. 4a *WBC/BASO count vs manual count.*

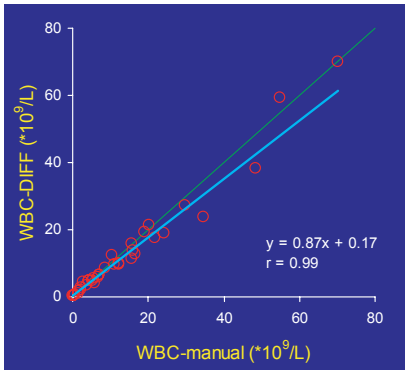


Fig. 4b *WBC-DIFF count vs manual count.*

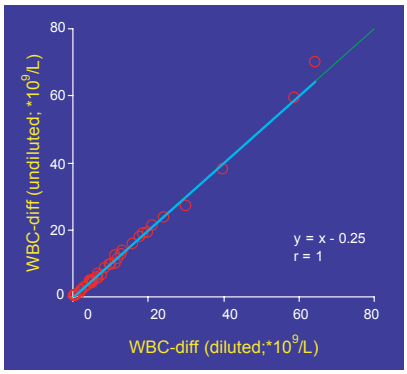
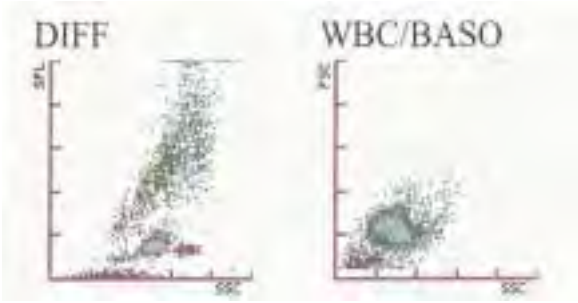
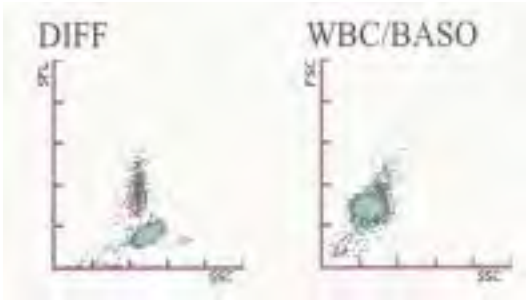


Fig. 4c *DIFF count undiluted vs DIFF count diluted.*



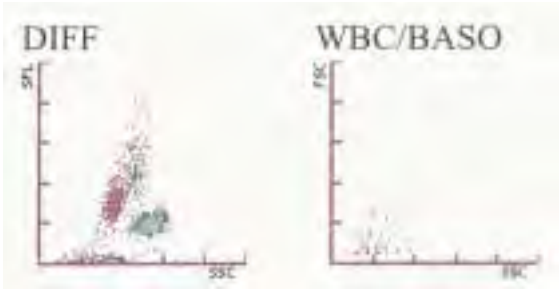
WBC = 5.50*10⁹/L WBC = 5.79*10⁹/L

Fig. 5a *WBC counts in pleural effusion. In the DIFF channel the count is 5.5×10⁹ /L and in the WBC/BASO channel it is 5.79×10⁹ /L, reasonably good agreement.*



WBC = 5.50*10⁹/L WBC = 5.59*10⁹/L

Fig. 5b *WBC counts in peritoneal effusion. In the DIFF channel the count is 5.5×10⁹ /L while in the WBC/BASO channel it is 5.59×10⁹ /L, very good agreement.*



WBC = 4.35*10⁹/l WBC = 0.06*10⁹/l

Fig. 5c *Synovial fluid. DIFF count = 4.35×10⁹ /L and WBC/BASO = 0.06×10⁹ /L.*

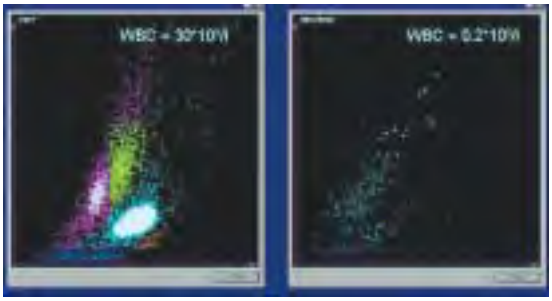


Fig. 5d *Synovial fluid. DIFF scattergram of the left (WBC count=30×10⁹/L). WBC/BASO scattergram on the right (WBC=0.2×10⁹/L).*

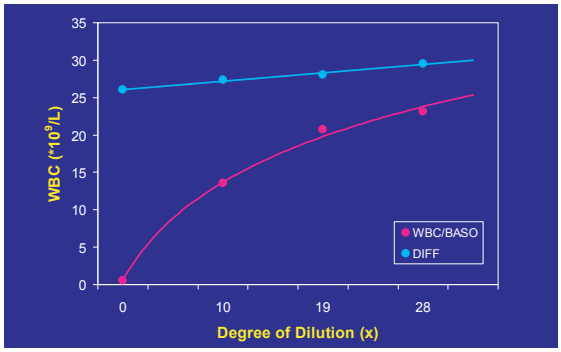


Fig. 6 *Effect of dilution on automated WBC counts.*

Why should such discrepancy occur between the DIFF and the WBC/BASO channels? Is this a phenomenon specific for synovial fluid? The behaviour of pleural fluid (***Fig. 5a***) where there is good agreement between the two counts, and peritoneal effusion (***Fig. 5b***) where there is also good agreement between the two counts, is compared with two examples of synovial fluid (***Fig. 5c*** and ***5d***). The conclusion must be that the phenomenon is related to the matrix of the synovial fluid.

In an attempt to explain this anomaly, a specimen of synovial fluid was serially diluted 30 times and WBC counts performed in both instrument channels at each dilution. The effect of this is shown in ***Fig. 6***. As the dilution increases, the effect on the WBC/BASO becomes less and the counts virtually equate at the highest dilution. This confirms the hypothesis that the difference is related to the composition of the synovial fluid matrix. As the matrix is replaced the difference disappears. Synovial fluid is an ultrafiltrate of plasma and its high viscosity is caused by hyaluronate. When synovial fluid is treated with hyaluronidase at 37℃ for one hour and then measured in the WBC/BASO channel there is an increased count whereas the DIFF channel count remains unchanged. This is illustrated in ***Fig. 7***. The conclusion is that polymerization of hyaluronate (mucin clot) occurs in the acidic environment of the WBC/BASO channel (pH ~ 3.4) whereas the DIFF channel has a pH of 7.4.

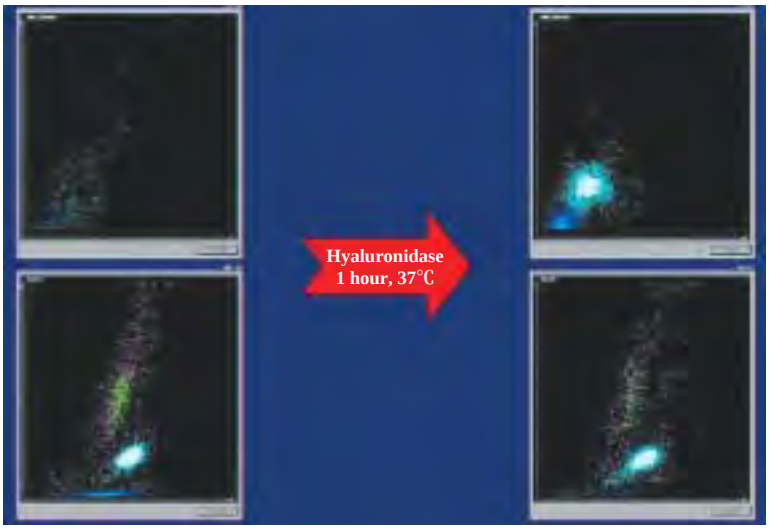


Fig. 7 *Effect of hyaluronidase treatment. The upper panels are the WBC/BASO scattergrams and the lower panels the DIFF scattergrams. Note the increase in counts in the WBC/BASO channel after treatment with hyaluronidase.*

Table 3 *Imprecision studies for both manual and instrument methods (numbers in cells/μL).*

Method	Control	Within-day imprecision		Between-day imprecision	
		Mean ± SD	CV(%)	Mean ± SD	CV(%)
Cytometer/microscope	Level A	577 ± 67	11.7	675 ± 151	22.4
	Level B	1020 ± 128	12.25	1300 ± 263	20.3
Hematology analyzer	Level A	672 ± 4.6	6.6	692 ± 69	10.0
(DIFF channel)	Level B	1176 ± 43	3.7	1172 ± 117	10.0

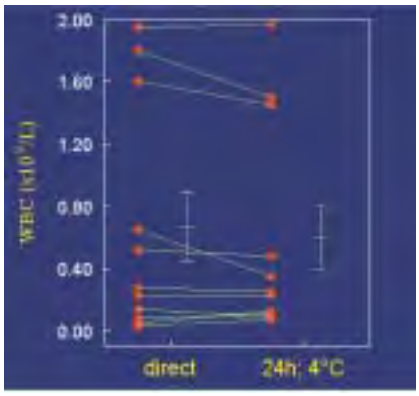


Fig. 8 *Stability of diluted synovial fluid.*

2. Imprecision : For imprecision studies a dedicated commercial synovial fluid control material was used (available from Quantimetrics). This is a two level control: level A has ~ 500 cells per microlitre predominantly lymphocytes plus some calcium pyrophosphate crystals; level B contains ~ 1000 cells per microlitre predominantly neutrophils plus some sodium urate crystals. These materials were used to determine within-day imprecision and between-day imprecision (***Table 3***).

As expected the coefficients of variation for the instrument are very much less than for manual methods which is not surprising since the instrument counts many more cells. In addition, the inter-individual variation was assessed by allowing 10 technologists to manually count the same samples. For the level A control material the CV was 36 % and for the level B control material the CV was 21 %.

3. Synovial fluid stability: Finally the stability of diluted synovial was assessed by measuring diluted specimens directly and then after 24 hours at 4℃. As shown in ***Fig. 8*** there is a small statistically insignificant decrease in the total count.

CONCLUSIONS

This evaluation has permitted the following conclusions:

1. The leukocyte count in synovial fluid can be reliably determined using the DIFF channel of the SYSMEX XE-2100
2. The WBC/BASO channel of the SYSMEX XE-2100 produces falsely low synovial fluid leukocyte counts
3. Automated counting is more precise and faster than manual counting
4. Diluting synovial fluid before automated analysis is not strictly necessary
5. Dedicated synovial fluid control materials are available
6. The leukocyte count is stable in diluted heparinized synovial fluid stored at 24 ℃ for 24 hours.

References

1. *Schmerling R.H., Delbanco T.L., Tosteson A.N. et al (1990) Synovial fluid tests. What should be ordered? JAMA 264, 1009-1014*
2. *Terčič D., Božič B. (2001) The basis of synovial fluid analysis. Clin Chem Lab Med 39, 1221-1226*
3. *Schumacher H.R., Sieck M.S., Rothfuss S. et al (1986) Reproducibility of synovial fluid analyses. A study among four laboratories. Arthritis Rheum 29, 770-774*
4. *Hasselbacher P. (1987) Variation in synovial fluid analysis by hospital laboratories. Arthritis Rheum 30, 637-642*
5. *Amer H., Swan A., Dieppe P. (2001) The utilization of synovial fluid analysis in the UK. Rheumatology 40, 1060-1063*
6. *Dieppe P.A., Crocker P.R., Corke C.F. et al (1979) Synovial fluid crystals. QJ Med 48, 533-553*
7. *Vincent J., Korn J.H., Podewell C. et al (1980) Synovial fluid pseudoleukocytosis. Arthritis Rheum 23, 1399-1400*
8. *Haskard D.O., Revell P.A. (1984) Methods of assessing the synovial fluid cell count. Clin Rheumatol 3, 319-322*
9. *Atilola M.A., Lumsden J.H., Rooke F. (1986) A comparison of manual and electronic counting for total nucleated cell counts on synovial fluid from canine stifle joints. Can J Vet Res 50, 282-284*
10. *Salinas M., Rosas J., Iborra J. et al (1997) Comparison of manual and automated counts in EDTA preserved synovial fluids. Storage has little influence on results. Ann Rheum Dis 56, 622-626*