Rapid diagnosis of inflammatory synovial fluid with reagent strips

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Abstract

Objective. To determine the usefulness of reagent test strips for screening inflammatory synovial fluid (SF).

Methods. Consecutive patients undergoing diagnostic arthrocentesis, attending the Department of Rheumatology of a large tertiary care hospital were evaluated. All SF specimens obtained were tested using two techniques: (i) white blood cell (WBC) count with the differential according to standard practice (which is considered the gold standard) (an inflammatory SF was defined as a WBC count > 2000 cells/mm³); and (ii) reagent strips used to test urine (Multistix 8 SG, Bayer Diagnostics) for the presence of leucocytes (a positive test was defined as a strip showing more than a trace for leucocytes). Sensitivity, specificity, predictive values and likelihood ratio (LR) of the reagent strip in diagnosing inflammatory SF were determined.

Results. Two hundred and eight samples of SF were tested. The results of using the reagent strip were: sensitivity 76.9% (95% CI, 66.0–85.7%), specificity 86.9% (95% CI, 79.9–92.2%); positive LR, 5.88 (95% CI, 3.71–9.31) and negative LR, 0.27 (95% CI, 0.18–0.40). In 13 of the 19 false negative results, the differential cell count showed a predominance (>50%) of lymphocytes.

Conclusion. This study suggests that, in daily practice, the evaluation of SF by reagent strips could be of use to discriminate between inflammatory and non-inflammatory SF.

KEY WORDS: Synovial fluid, Reagent test strip, Diagnosis, Inflammation.

Analysis of synovial fluid (SF) is widely recognized as an important part of the diagnostic evaluation of patients with arthritis and joint effusion [1–4]. In particular, white blood cell (WBC) count in SF allows classification of SF as non-inflammatory or inflammatory [2, 3, 5, 6]. Prompt analysis is necessary because delay may lead to false negative results [7, 8]. When SF is removed from the joint, its WBC count decreases with time, and mildly inflammatory fluids with WBC counts up to 6000 cells/mm³ can decrease to a non-inflammatory range of <2000 cells/mm³ after only 6 h [8]. From a practical point of view, analysis within a short time can be difficult to obtain, and frequently SF analysis is not performed in primary care due to organizational problems. Consequently, we were interested in evaluating a simple method of quantification of WBC which would be available immediately at the point of care. Reagent strips originally designed for semi-quantitation of leucocytes in urine have been used to measure leucocytes in various body fluids such as bronchoalveolar fluid and cerebrospinal fluid [9, 10].

Our aim was to determine the performance of a commercially available reagent test strip in discriminating between inflammatory and non-inflammatory SF.

Patients and methods

SF samples obtained from consecutive patients undergoing arthrocentesis at the Department of Rheumatology of Cochin Hospital and sent to the hospital laboratories during a 6-month period were analysed. The specimens had been collected in tubes containing EDTA. All samples were divided into two equal portions and examinations were made within 2 h of receipt of the SF sample. Using one portion of each specimen, the WBC count was measured by manual leucocyte counting using 0.3% saline as diluent. The
other portion of each specimen was tested for the presence of leucocytes with commercial reagent strips (Multistix 8 SG, Bayer Diagnostics) that were originally designed to test urine for blood, pH, protein, nitrite and leucocytes. The reagent strip was dipped directly into the tube. The leucocyte pad changes colour according to the concentration of leucocytes. Two minutes after dipping, the sample is graded as negative, trace positive, + positive, ++ positive or +++ positive by comparison with a standard colour chart found on the container’s label. The Multistix was considered positive if the leucocyte esterase pad was more than trace positive. This analysis was performed by an investigator unaware of the WBC count and of the diagnosis.

The sensitivity, specificity, positive predictive and negative predictive value and positive and negative likelihood ratio (LR+ and LR–) were estimated using the microscopic WBC count as the gold standard, and a graphic representation was presented as proposed by Brenner [11]. The cut-off for the WBC count (>2000 cells/mm³) was used to classify patients as having inflammatory or non-inflammatory SF.

To assess the reproducibility of dipstick results, 50 consecutive SF samples were independently assessed by three readers and kappa coefficients were calculated.

Results

There was excellent agreement among the three readers, with a kappa coefficient of 0.97 (95% CI, 0.90–1.00; reader 1 vs reader 2), 0.96 (95% CI, 0.88–1.00; reader 2 vs reader 3) and 0.92 (95% CI, 0.81–1.00; reader 1 vs reader 3).

Two hundred and eight SF samples were obtained. According to the laboratory results, 78 of the 208 (37.5%) SF samples were inflammatory using a 2000 cells/mm³ cut-off value. Diagnoses of the patients included, based on standard clinical, radiological and serological criteria, are presented in Table 1. The results obtained with the reagent strips are shown in Fig. 1.

Using the 2000 cells/mm³ cut-off, inflammatory SF was correctly identified with the strip method in 60 of 78 cases. This yields a sensitivity of 76.9% (95% CI, 66.0–85.7%), a specificity of 86.9% (95% CI, 79.9–92.2%), a LR+ of 5.88 (95% CI, 3.71–9.31) and a LR– of 0.27 (95% CI, 0.18–0.40). Nineteen false negatives were observed with the 2000 cells/mm³ cut-off. The median WBC count for these patients was 3000 (range 2000–25 000 cells/mm³) and only four of them had >6000 cells/mm³ (6300, 15 000, 22 000 and 25 000 cells/mm³). Among these false negatives, nine out of 18 had <3000 leucocytes/mm³, and five of the nine remaining patients had >50% of lymphocytes (Table 2).

Discussion

The data presented suggest that the reagent strip is a potentially useful test to discriminate inflammatory from non-inflammatory SF if a laboratory test is not available.

The false negative results observed in this study were predominantly in SF samples that had a high percentage of lymphocytes. Eleven of these patients with 18 false negative results had <2000 neutrophils/mm³ and 13 out of 19 had <3000 neutrophils/mm³. These results are not surprising: the Multistix 8 reagent strip is designed for semi-quantitation of polymorphonuclear neutrophils (rather than leucocytes) by detecting leucocyte esterase enzyme activity. Leucocyte esterase enzyme activity is detectable in polymorphonuclear neutrophils and monocytes but not in lymphocytes [12].

Table 1. Diagnosis of the 208 consecutive patients undergoing SF analysis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients (%)</th>
<th>Number of patients with laboratory WBC ≥ 2000 cells/mm³</th>
<th>Number of patients with reagent strip WBC ≥ 2000 cells/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoarthritis</td>
<td>113 (54.3)</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Gout or calcium pyrophosphate deposition disease</td>
<td>6 (2.9)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>30 (14.4)</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>Spondylarthropathy</td>
<td>23 (11.0)</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>6 (2.9)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Miscellaneous diagnoses*</td>
<td>12 (5.8)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No diagnosisb</td>
<td>18 (8.7)</td>
<td>17</td>
<td>9</td>
</tr>
</tbody>
</table>

*aIncludes osteonecrosis, osteochondromatosis, pigmented villonodular synovitis, meniscal tears and post-traumatic effusions.

*bIncludes monoarthritis of unknown significance.
SF testing at the site of primary care using reagent strips has the potential benefits of not requiring a laboratory and providing immediate results. These benefits may be offset by reduced accuracy compared with a laboratory result obtained in optimal conditions (i.e. short interval between joint aspiration and analysis, trained personnel, etc.). Furthermore, the results obtained are limited to the WBC count, which is only one element of a complete SF analysis that includes microbiological culture and crystal analysis. However, in daily practice, the conditions in which WBC counts are made are probably not optimal. A survey of 42 hospitals revealed that in many laboratories the frequency of SF analysis is low, with a median of 1.5 per month and with numerous hospital laboratories performing one or less per month [13]. Consequently, the ability of some laboratories to perform routine SF analysis is doubtful. As an example in this study, the WBC count reported for a single fluid studied in 26 laboratories ranged from 2467 to 12 000 cells/mm$^3$.

Secondly, in daily practice the time elapsed between joint aspiration and WBC count is frequently >3 h and can reach 12 h or more, particularly if the sample arrives at the laboratory during a weekend or off-hours, or if SF is shipped by the laboratory to a central facility. Consequently, WBC count tests performed in routine practice are probably not fully accurate.

Although SF analysis is relatively cheap, effective, simple and reliable, SF analysis is one of the least used tests in rheumatology [14]. According to some authors, the reason is that too few laboratories offer the investigation and too few clinicians request it [14]. It has also been proposed to abandon routine SF analysis [15], on the basis that SF analysis for cells and/or crystals does not aid diagnosis or management in patients with already established rheumatological diagnoses, while constituting an additional cost. Thus, the use of reagent strips could be considered an attractive alternative.

Our study has several limitations. First, our patients represent the recruitment of a tertiary care hospital and we cannot exclude that the results could be different in another setting with a different prevalence of inflammatory SF. Secondly, we did not assess the visual appearance of the SF. Visual appearance could be used alone or in combination with dipsticks as a screening procedure to distinguish inflammatory and non-inflammatory SF.

The best way to distinguish inflammatory and non-inflammatory SF remains the laboratory WBC count. However, when a laboratory is not available, particularly in patients with previously established diagnoses, the use of reagent strips could be considered a useful alternative that can be carried out quickly enough to guide initial management. Further studies are needed to assess the performance of such strips in other settings, particularly primary cases, taking into account both the differences in recruited patients and the different laboratories involved in daily practice.

### References

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