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Correlation of Leukocyte Esterase Detection by Reagent Strips and the Presence of Neutrophils*

A Study in BAL Fluid

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Study objective: In the present study, we evaluated the leukocyte esterase (LE) area of a reagent strip designed for urinalysis for the semiquantitative measurement of the percentage of polymorphonuclear neutrophils (PMNs) in BAL fluid.

Design: Prospective. The relative PMN counts (obtained by conventional microscopy and expressed as a percentage of a 500 cell count) of consecutive BAL fluid samples were compared with the corresponding LE categories as read with a urine chemistry reader. LE categories were graded as follows: negative, trace, +, ++, and +++.

Results: A total of 153 BAL fluid samples were included. The mean PMN counts of the negative LE category ($4.1 \pm 4.3\%$; $n = 43$) and the +++ category ($81.8 \pm 16.3\%$; $n = 37$) differed significantly from each other and from the mean PMN counts of the other categories. Within the trace, +, and ++ categories, a considerable overlap of PMN counts was noted. Assignment of a BAL fluid to the negative LE category consistently predicted a PMN count $< 20\%$. At a threshold value of 50% PMNs, the +++ LE category predicted the BAL fluid samples to the correct group (PMNs $> 50\%$ vs $< 50\%$) with a sensitivity of 70.8% and a specificity of 97.1%.

Conclusions: The reagent strips proved to be useful as a rapid test for semiquantitative measurement of the relative PMN counts in BAL fluid. However, the low predictive value for the exclusion of a high PMN count may limit their application. (CHEST 2000; 118:1450-1454)

Key words: BAL; leukocyte esterase; polymorphonuclear neutrophils; reagent strips; ventilator-associated pneumonia

Abbreviations: LE = leukocyte esterase; PMN = polymorphonuclear neutrophil; VAP = ventilator-associated pneumonia

The examination of BAL fluid is routinely used in the diagnosis of ventilator-associated pneumonia (VAP) and in the assessment of interstitial lung diseases. Important diagnostic information on these conditions may be obtained from the BAL fluid differential cell count.¹⁻³ In particular, the number of polymorphonuclear neutrophils (PMNs) is of interest. For example, the BAL fluid PMN number may distinguish between sarcoidosis patients who demonstrate remission and those having a more

severe course of the disease,⁴ and it has been demonstrated that a BAL fluid PMN number of $< 50\%$ has a 100% negative predictive value for histologic pneumonia.⁵ However, the widespread clinical application of BAL fluid cytology is limited by the fact that this procedure is expensive and time-consuming, and relies on specialized technicians.⁶ Furthermore, in most hospitals, facilities for BAL fluid cytology are not available on a 24-h basis. Consequently, we were interested in evaluating a simpler, shorter method of quantification of PMNs, which would be available to most clinical laboratories. Therefore, we evaluated a commercially available reagent strip (Multistix 7; Bayer; Elkhart, IN) for its ability to detect and measure PMNs in BAL fluid samples. The Multistix 7 reagent strip was originally designed for semiquantitation of PMNs in urine by an area for detecting leukocyte esterase (LE) enzyme activity. The aim of this study was to compare the semiquantitative LE categories gener-

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ated by this reagent strip with their corresponding microscopic PMN counts.

MATERIALS AND METHODS

Study Population

During a 16-month period (April 1998 to August 1999), BAL fluid samples obtained from patients in the University Hospital Maastricht were collected. The patients included were suspected of having pneumonia or were suffering from different interstitial lung diseases, including sarcoidosis, extrinsic allergic alveolitis, and idiopathic pulmonary fibrosis.

Sampling Technique

A fiberoptic bronchoscope (Pentax FB-15 h/FB-15X; Pentax Medicals; Tokyo, Japan) was introduced through a special adapter (Swivel Connector; Gibeck Respiration; Upplands Väsby, Sweden) and "wedged" into the affected segmental or subsegmental bronchus. The fluid was instilled into the subsegment through the biopsy channel of the bronchoscope in four 50-mL aliquots of sterile saline solution (0.9% NaCl, room temperature) and immediately aspirated and recovered. The BAL fluid samples were transported to the laboratory within 15 min after collection and analyzed within 1 h on arrival in the laboratory.

Cytologic Processing of BAL Fluid Specimens

The volume of the recovered BAL fluid and its macroscopic appearance were recorded. The first fraction, representing the bronchial fraction, was separated for mycobacterial culture and the remaining fractions were pooled in conical 50-mL nonadhesive polypropylene tubes (Product No. 227.261; Greiner; Alphen aan de Rijn, The Netherlands). The total cell count was performed in a Fuchs-Rosenthal hemocytometer chamber. All nucleated cells were counted, and the average value of two successive counts was considered. Cyto centrifugation was done with the Cytospin 3 apparatus (Shandon Scientific; Astmoor, England) as previously described.⁷ The slides were allowed to air dry, stained with the May-Grünwald Giemsa dyes, and subsequently sealed with a cover glass by means of a xylene-free mountant (Histomount; Shandon Scientific). Differential cell counts were made by examining 500 nucleated cells, excluding squamous and bronchial epithelial cells. Cells were counted in a circular pattern around the center of the cyto centrifuge spot,⁸ and the differential cell counts including the PMNs were expressed as a percentage of a 500-cell aliquot.

Rejection Criteria

BAL fluid samples were excluded if the recovery was < 20 mL or if the total cell count was < 60,000 cells/mL, and if the cyto centrifuged preparations showed excessive amounts of RBCs, intercellular debris, or damaged cells precluding adequate recognition of different cell types.^{7,9}

Reagent Strips

Multistix 7 reagent strips were used for semiquantitative assessment of the BAL fluid LE activity. Multistix 7 is a seven-patch test reagent strip that was designed to test urine for glucose, ketones, blood, pH, protein, nitrite, and leukocytes. The reagent strips were read instrumentally, using the Clinitek 50

Urine Chemistry Analyzer (Bayer Corporation). The performance of the reagent strips was checked against the Chek-stix positive and negative controls (Bayer Corporation) at any time a new package was begun. The procedures were performed as described in the package insert and in the Clinitek 50 operating manual.

Reagent strips were removed from the bottle just immediately before they were used for testing. They were dipped directly and briefly into well-mixed pooled BAL fluid specimens. If the BAL fluid specimen depth in the conical tubes was < 5 cm, the specimen was poured into a 10-mL tube. While removing the strips, the edge of the reagent strip was dragged against the rim of the BAL fluid container to remove excess fluid. At that time, the "Start" key of the Clinitek 50 reader was pressed. Within the provided 5-s to 10-s delay, the reagent strip was blotted by gently touching the edge to a paper towel. Subsequently, the reagent strip was placed into the trough of the test strip table of the Clinitek 50 reader and slid along the table until it touched the end of the trough. Readings were performed automatically, and test results were printed by the Clinitek 50 reader. The presence of PMNs was as graded as follows: negative, trace, +, ++, and +++.

To compare the instrumental readings with visual readings, a number of reagent strips were also read against the standards provided on the bottle label, at 2 min after dipping, according to the Multistix 7 package insert. Furthermore, the effect of increasing reaction times for the reagent strips was studied on four BAL fluid specimens that were diluted with 0.9% NaCl until they were read as + by the Clinitek 50 reader. Reagent strips were subsequently entered in the Clinitek 50 reader at increasing delays of 30, 60, and 120 s after dipping.

Statistical Evaluation

For the different LE categories, pair-wise comparisons of the mean PMN counts were assessed for significance by the one-way analysis of variance *post hoc* test for multiple comparisons, with Bonferroni's modification

RESULTS

During the study period, 153 BAL fluid samples obtained in 134 patients were included. Fourteen BAL fluids were excluded on the basis of insufficient recovered volume or a low total cell count, and 22 samples were excluded because of poor microscopic quality. Half of the BAL fluid samples (76 of 153) were obtained from patients in the ICU, and 57 samples (37.2%) were obtained from patients in the Department of Pulmonology. The remaining BAL fluid samples (20 of 153; 13.1%) were recovered from patients in the Departments of Internal Medicine and Surgery. Nearly one third (48 of 153; 31.4%) of the BAL fluid samples displayed PMN counts > 50%.

In Figure 1, the semiquantitative LE categories are plotted against the corresponding numbers of BAL fluid PMNs. As can be seen in Figure 1, a considerable overlap of individual PMN counts was noted among the LE categories of trace, +, and ++. The respective mean PMN counts of these categories did not differ significantly (mean \pm SD

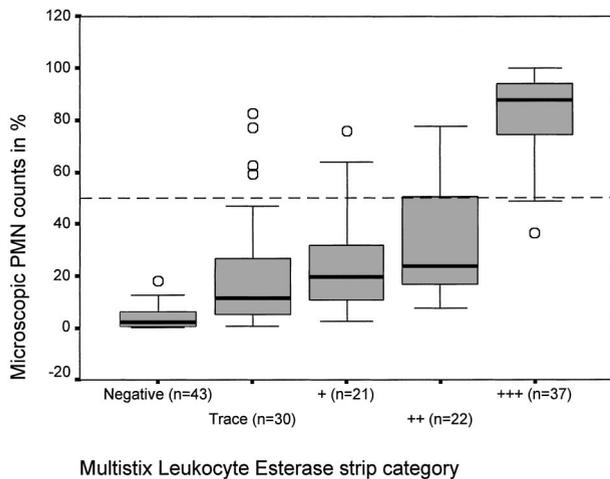


FIGURE 1. Box-plot graph representing the semiquantitative LE categories generated by the reagent strips for the 153 BAL fluid samples, plotted against the percentages of PMNs counted microscopically. The dotted line represents the 50% PMN count.

PMN counts, $20.2 \pm 22.8\%$, $27.1 \pm 21.5\%$, and $32.8 \pm 21.4\%$ respectively). However, the mean PMN count of the negative LE category ($4.1 \pm 4.3\%$; $n = 43$) differed significantly from the mean PMN counts of the trace category ($p = 0.003$) and the ++ and +++ categories ($p < 0.001$). Assignment of a BAL fluid to the negative LE category consistently predicted a PMN count of $< 20\%$. Conversely, the mean PMN count of the +++ LE category ($81.8 \pm 16.3\%$; $n = 37$) largely exceeded the mean PMN counts of any other category ($p < 0.001$). At a threshold of 50% PMNs, the +++ LE category predicted the BAL fluid samples to the correct group (PMNs $> 50\%$ vs $< 50\%$) with a sensitivity of 70.8% and a specificity of 97.1%. Given the prevalence of 31.4% for a PMN count $> 50\%$ in the present study, the +++ LE category had a positive predictive value of 91.9% and a negative predictive value of 87.9%.

With respect to the 50% PMN count, 17 of 153 LE categories were inconsistent with the microscopic findings. Three BAL fluid samples were assigned to the +++ LE category but showed PMN counts $< 50\%$, resulting in a 2.9% false-positive ratio. In two of these BAL fluids, borderline PMN counts were noted (48.0% and 49.2%, respectively); for the remaining BAL fluid (with a PMN count of 36.2%), no explanation for this false-positive event was found. Another 14 BAL fluid specimens showed PMN counts $> 50\%$ but were assigned to LE categories ++ ($n = 6$), + ($n = 4$), and trace ($n = 4$). Apart from three BAL fluid samples (in the ++ category) with a borderline PMN count, four BAL fluid samples with an elevated protein level (> 300

mg/L) were noted. For the remaining seven BAL fluid samples, no cause for the false-negative result was found.

The LE categories that were visually read on 40 BAL fluid samples corresponded well with those generated by the Clinitek 50 reader, except for an occasional discrepancy in the trace or + categories. By contrast, accurate timing proved to be critical for obtaining reliable results, as the readings of the BAL fluid samples shifted from the LE + category to the +++ category when the delay of entering the reagent strip into the Clinitek 50 reader reached 120 s. Likewise, visual readings at < 2 min or > 2 min after dipping caused false results into a too-low or a too-high LE category, respectively.

DISCUSSION

Urine reagent strips have been used for semiquantitation of PMNs in other body fluids with varying results.¹⁰⁻¹³ To our knowledge, they have not been evaluated in BAL fluid samples before. The present prospective study demonstrated that the Multistix 7 reagent strip provided a rapid method for the prediction of low ($< 20\%$) and high ($> 50\%$) PMN counts in BAL fluid samples, by the negative and the +++ LE categories, respectively.

One might argue that the present study population was heterogeneous, comprising BAL fluid samples obtained from patients receiving ventilation, as well as from patients who were immunosuppressed or suffering from interstitial lung diseases. However, the aim of this study was the validation of the Multistix 7 reagent strip as a prediction for the BAL fluid PMN count, irrespective of the conditions affecting these counts. Elevations of the BAL fluid PMN count may occur in several clinical conditions, and the relative numbers of PMNs are associated with disease severity in disorders such as *Pneumocystis carinii* pneumonia,¹⁴ sarcoidosis,⁴ ARDS,¹⁵ and VAP.⁶ In diagnostic practice, rapid estimation of the BAL fluid PMN count will be of most diagnostic gain in the intensive care setting, *ie*, when VAP and ARDS are suspected. BAL fluid specimens obtained in this setting are generally processed in the microbiological laboratory, where cytologic expertise is not always available. Furthermore, in cases of VAP and ARDS, high BAL fluid PMN counts are to be expected,^{15,16} and the need for prompt administration of antibiotics in case of VAP calls for a rapid test.¹⁷ In addition, in our experience, up to 40% of BAL fluid specimens sampled in the ICU arrive at the laboratory during weekends or off-hours. For these reasons, a rapid and reliable screening test for the prediction or the exclusion of high BAL fluid PMN counts is mandatory.

For a screening test to be adopted in the diagnostic laboratory, both a high sensitivity and a high specificity are required.¹⁸ Considering the LE + + + category in its ability to predict a > 50% PMN count, it is clear that the Multistix 7 reagent strip fulfills only the specificity criterion. This will be of particular importance when the reagent strip will be used in a setting with a high prevalence of elevated BAL fluid PMNs, such as in the ICU. In that case, the positive predictive value, or the chance that a BAL fluid assigned to the + + + LE category actually will display a > 50% PMN count, will increase. However, the negative predictive value, or the chance that a BAL fluid assigned to one of the other categories (except the negative category) actually has PMN counts < 50%, will decrease. By consequence, the low predictive value in the exclusion of high PMN counts will be the limiting factor of the reagent strips in this circumstance.

Given the concern on the moderate sensitivity, we looked for explanations for the false-negative events with respect to the 50% PMN threshold. For urinary samples, factors that may contribute to false-negative results include high protein levels,¹⁰ and, according to the Multistix 7 package insert, elevated glucose concentrations, the presence of cephalosporins or tetracyclines in high concentrations, and the presence of colored substances such as nitrofurantoin. In the present study, elevated protein levels were found in three of four false-negative specimens of the trace category, but they were equally observed among the correctly predicted samples. Although we presently did not score the use of antibiotics prior to bronchoscopy, we do not expect these agents to be responsible for false-negative readings, as BAL fluid generally represents a 1:10 to 1:100 dilution of the alveolar epithelial lining fluid.¹⁹ Furthermore, no readings that were obscured by heavily bloodstained specimens were presently found, and we repeatedly observed that high eosinophil counts (> 40%) did not interfere with the reagent strip readings. For increase of the sensitivity of the Multistix 7 reagent strip in the prediction of high PMN counts, further investigation of the possible causes of false-negative readings is required. In addition, we suggest further technical developments of the LE reagent strip area to include an extension of the reading scale, in an attempt to differentiate and grade the PMN counts of the trace, +, + + categories.

In the present study, we did not intend to evaluate the other reagent areas of the Multistix 7 reagent strip. In retrospect, most of these tests proved not to be reliable or clinically useful, except for the pH and the protein tests. The nitrite test, which points to the presence of Gram-negative bacteria, may not be used in this setting because dietary nitrate is not

present in BAL fluid specimens. For purposes of the present study, the reagent strip could be simplified to the area of interest. One might equally consider the addition of areas for estimation of the other BAL fluid cell populations, *eg*, an area detecting nonspecific esterase enzyme activity, which in BAL fluid is confined to the alveolar macrophage population.²⁰

We further found the Multistix 7 reagent strips convenient to use. The readings were easy to perform, although accurate timing proved to be essential for obtaining reliable results. Visual readings that were consistent with the instrumental readings and a long package shelf life made the occasional use of the strips easier. The Clinitek 50 reader was a reliable apparatus that required little maintenance and was easy to calibrate.

In conclusion, the Multistix 7 reagent strip designed for urinalysis may be used as a rapid test for semiquantitative estimation of the relative PMN count in BAL fluid samples, in those conditions in which facilities for cytologic examination are not available. The LE categories generated by the reagent strip may distinguish BAL fluid samples displaying elevated (> 50%) PMN count with a high specificity but at the cost of a low sensitivity. Further study on the false-negative events and refinements of the reading scale are desired for optimal use of this reagent strip in the present setting.

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