

# Evaluation of buffy-coat microscopy for the early diagnosis of bacteraemia

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**SUMMARY** Three hundred and sixty samples of blood from 230 hospital patients were examined and compared with the results of simultaneous blood culture to determine the value of buffy-coat microscopy in detecting bacteraemia.

One observer found 86 positive smears, 12 of which were from patients with positive blood cultures and 74 from patients with negative blood cultures. The buffy-coat smear was negative in 274 specimens, 8 of which yielded positive blood cultures. A second observer considered that only 34 of the buffy coats were positive. Only six of these were associated with positive blood cultures. Although there was a statistically significant association between positive smears and positive blood cultures, the procedure has little practical value because of the high incidence of false positives and negatives.

Microscopy of stained leucocytes for bacteria in buffy-coat smears has been one of a number of techniques recommended for the rapid diagnosis of bacteraemia.<sup>1-6</sup> It has been said to detect 35% of bacteraemic adults and 70% of bacteraemic neonates.<sup>5-7</sup>

Using the methylene blue staining technique of Faden,<sup>6</sup> we examined a large series of buffy-coats from unselected blood samples taken at the same time as blood for culture to determine whether any association exists between positive smears and bacteraemia, and to assess the value of this procedure in clinical practice.

## Material and methods

### SAMPLES

Three hundred and sixty samples of blood from 230 hospital patients were examined. Few specimens were received from children and none from neonates, because of the difficulty in obtaining a large enough blood sample. A record was made of the clinical history, therapy and progress of each patient. Four ml of blood was placed in a sequestrene container, at the same time as 7.5 ml of blood was inoculated for culture.

### EXAMINATION OF BUFFY-COAT

The method used was essentially that described by

Brooks.<sup>5</sup> A 1 ml aliquot of blood was centrifuged for 10 min at 2500 rpm in a thoroughly clean Wintrobe tube. The bulk of the plasma was removed with a Pasteur pipette and the buffy-coat layer was aspirated along with the remaining plasma on to a clean slide. The buffy-coat smears were allowed to dry in air, heat fixed, and stained with methylene blue for 2 min. Care was taken to use fresh stain to reduce the likelihood of artefacts. All smears were examined for at least 5 min by observer I (MJC) under an oil-immersion objective (magnification  $\times 1000$ ). The presence of intracellular inclusions, or any extracellular stained particles that appeared to be bacteria, was recorded as a positive finding. Smears which were designated positive by observer I were examined several days later by the second observer (CJN) who had access to the blood culture results. A diagnosis of bacteraemia was made on the combined clinical and blood culture findings. A preliminary examination showed that washings from the sequestrene bottles which were employed were sterile and free of stainable bacteria.

### BLOOD CULTURE

Blood culture sets comprised two bottles, one of Castenada's Medium (Southern Group Laboratories), and the other Thiol Broth (Difco). Subcultures from both of the bottles were made routinely at 24 h and at five days, and also more frequently if turbidity or other macroscopic signs of bacterial growth were observed. In cases of suspected

endocarditis, cultures were incubated for up to three weeks.

## Results

The observers disagreed over the classification of smears. Observer I examined 360 specimens, 86 gave positive results on buffy-coat examination and 274 negative results (Table 1). Observer II considered that only 34 of the 86 smears designated as positive by observer I contained microorganisms. Eight of these 34 smears had positive blood cultures, the remainder were negative. As observer II did not examine all of the buffy-coats, the following further analysis of all results is based on the findings of observer I.

Table 1 Overall results of buffy-coat microscopy and blood culture

| Blood culture result | Buffy-coat microscopy |          |       |
|----------------------|-----------------------|----------|-------|
|                      | Positive              | Negative | Total |
| Positive             | 14                    | 26       | 40    |
| Negative             | 72                    | 248      | 320   |
| Total                | 86                    | 274      |       |

Only 14 of 86 positive buffy-coats came from patients with positive blood cultures; the remaining 72 were negative. Twenty-six of the 274 specimens that gave negative results on buffy-coat examination yielded positive blood cultures; the remaining 248 were negative.

The bacteria from the 40 positive blood cultures obtained from our series have been classified in Table 2. Eighteen cultures grew *Staphylococcus epidermidis* and two grew *Bacillus* spp. We examined the clinical details of cultures positive for these organisms and found that they did not support a diagnosis of bacteraemia, because only one of the two blood culture bottles yielded organisms in each case, and because the patients appeared either clinically not to have sepsis or to have obvious sepsis attributable to other organisms. These criteria are similar to those of Faden.<sup>6</sup> None of these patients had renal transplants or prosthetic heart valves. Excluding

the 20 cultures positive for *Staphylococcus albus* and *Bacillus* spp, there were 20 remaining positive blood cultures that supported a diagnosis of bacteraemia. The following analysis of the results is based on the exclusion of *Staphylococcus albus* and *Bacillus* spp from the positive cultures (revised results in Table 3).

Twelve of the 86 positive buffy coats came from positive blood cultures, the remaining 74 were negative. Eight of the 274 specimens that gave negative results on buffy coat examination yielded positive blood cultures, the other 266 were negative. The association between positive buffy-coat microscopy and bacteraemia was highly significant (Table 3;  $\chi^2 = 15.2$ ;  $p < 0.0001$ ).

Table 3 Revised results of buffy-coat microscopy and blood culture excluding *Staphylococcus epidermidis* and *Bacillus* spp

| Blood culture result | Buffy-coat microscopy |          |       |
|----------------------|-----------------------|----------|-------|
|                      | Positive              | Negative | Total |
| Positive             | 12                    | 8        | 20    |
| Negative             | 74                    | 266      | 340   |
| Total                | 86                    | 274      |       |

$$\chi^2 = 15.2, p < 0.0001.$$

Sensitivity, specificity, and predictive values were determined as described by Galen and Gambino.<sup>10</sup> Sensitivity was defined as the incidence of true positive results observed in patients shown to have bacteraemia by blood culture; specificity as the incidence of true negative results in patients with negative blood cultures. The predictive value of a positive test was defined as the percentage of positive results that were true positives when a test was applied to all patients in the series. In our series, the sensitivity of the technique was 60%, the specificity 78%, and the predictive value 14%.

There was no significant difference between the detection values of cocci and bacilli (Table 2). The number of bacteria-like particles seen in positive smears of bacteraemic patients ranged from 1 to 10 particles for each observer. The largest numbers

Table 2 Bacteria isolated from blood cultures

| Organism   | No of positive cultures | No of positive buffy-coats |             |
|--|-------------------------|----------------------------|-------------|
|  |                         | Observer I                 | Observer II |
| Gram-positive cocci (excluding <i>Staphylococcus epidermidis</i> ) | 17                      | 10                         | 5           |
| Gram negative bacilli (excluding <i>Bacillus</i> spp)              | 4                       | 3                          | 2           |
| <i>Staphylococcus epidermidis</i> and <i>Bacillus</i> spp          | 20                      | 2                          | 0           |
| Total  | 40*                     | 14*                        | 6*          |

\*Totals are reduced by 1 because one culture grew both Gram-positive cocci and Gram-negative bacilli.

were seen in patients with *Staphylococcus aureus* septicaemia.

## Discussion

Buffy-coat microscopy has been used successfully in detecting bacteraemias of the order of  $3 \times 10^5$  organisms/ml,<sup>4</sup> although positive smears may be associated with as few as 50 staphylococcal colony forming units/ml.<sup>5</sup> In our hands 60% of bacteraemia were detected by the test, a rate similar to that of Faden.<sup>6</sup> Our findings of 85% false positives is higher than most other published results, although Studer<sup>9</sup> had similar results to ourselves. We found that the sensitivity of the smear was considerably improved when cultures containing probable contaminants were excluded.

We used methylene blue stain as results have not proved superior using both Giemsa's and Gram's stain.<sup>10</sup> Staining with acridine orange may have produced a better result since this technique has a high sensitivity and slides can be overstained with Gram's stain.<sup>11</sup>

Carlson and Anderson<sup>8</sup> showed that interpretation varied considerably according to whether the observer had access to clinical information. They found that when screening was performed blindly, there was no association between positive smears and bacteraemia. Our experience of buffy-coat examination has shown that a high percentage of false positive results occur and that there is considerable variability.

Carlson and Anderson<sup>8</sup> could not show any relation between numbers of inclusions and quantitative blood culture. They concluded that these particles were most likely to be artefacts of the staining process; but Struder<sup>9</sup> considered that repeated findings of intragranulocytic organisms in the buffy-coats of patients with fever and sterile blood cultures might indicate that they had persistent bacteraemia. We believe that artefacts account for

some but not all the findings, as there is a highly significant association between positive microscopy and bacteraemia.

In conclusion, we have found that buffy-coat microscopy produced statistically significant results in the detection of bacteraemia in a large series. However, this method is of little practical use and may be misleading because of the low predictive value of a positive result.

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