ORIGINAL ARTICLE

Sedimentation characteristics of leucocytes can predict bacteraemia in critical care patients

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Background: Early detection of blood stream infection can be lifesaving, but the results of blood cultures are not usually available before 24 hours after blood sampling. An earlier indication would lead to the initiation of immediate and adequate antibiotic treatment with obvious advantages for the patient.

Objective: To evaluate the ability of leucocyte count, serum procalcitonin (PCT) concentration, erythrocyte sedimentation rate (ESR), and leucocyte antisedimentation rate (LAR) in predicting the blood culture results in critical care patients.

Methods: 39 consecutive patients with their first febrile episode were investigated prospectively. LAR was determined as the percentage of leucocytes crossing the midline of a blood column upward during one hour of gravity sedimentation. The relevance of the different variables was estimated by likelihood ratio tests and area under receiver operating characteristic curves (AUC).

Results: 23 patients had positive blood culture results and 16 negative. LAR was significantly higher in bacteraemic patients than in non-bacteraemic patients ($p=0.001$), but leucocyte count, ESR and PCT level failed to show significant differences. Leucocyte count, PCT, and ESR yielded low discriminative values with the AUCs of 0.66, 0.64, and 0.52, respectively. LAR provided a likelihood ratio of 3.6 and an AUC of 0.80 (95% confidence interval, 0.64 to 0.95) ($p=0.002$).

Conclusions: The simple LAR test can predict blood culture results and support urgent treatment decisions in critical care patients in their first febrile episode.

Bacteraemia or fungaemia are associated with life threatening conditions that need initiation of immediate and adequate antibiotic treatment. Traditional clinical signs and simple laboratory indices—for example, components of systemic inflammatory response syndrome (core temperature, heart rate, circulating leucocyte count or band forms, and respiratory rate or arterial P_{CO_2})—often lack sensitivity and specificity for diagnosing severe infections.^{1,2} Serum interleukin (IL) 6 and IL8 had a high negative predictive value in discriminating between bacteraemic and non-bacteraemic patients with febrile neutropenia.^{3,4} Similarly, serum procalcitonin (PCT) had a high sensitivity (92–100%) and a lower specificity (34–43%) in the assessment of bacteraemia in community acquired⁵ and mainly hospital acquired² severe infection in adult patients. Neither erythrocyte sedimentation rate (ESR) nor C reactive protein were able to predict bacteraemia positivity or negativity (area under receiver operating curve (AUC) 0.65 and 0.68, respectively) in febrile patients immediately after hospital admission.⁵

We previously reported a simple leucocyte function test measuring the number of upward floating (that is, antisedimenting) leucocytes in a sedimentation tube during one hour of gravity sedimentation.⁶ The leucocyte antisedimentation rate (LAR), as a percentage, indicates the proportion of leucocytes crossing the midline of a blood column in an upward direction during one hour of sedimentation. The upward flotation is driven by the ascending plasma and it is further enhanced by a decrease in the specific gravity of polymorphonuclear leucocytes (PMNs) relative to plasma.⁷ This fall in specific gravity is caused by cell swelling secondary to water uptake occurring during activation of the cells.⁷ It has also been shown that a raised LAR is positively correlated with enhanced cellular adherence,⁸ increased cell volume, and higher vacuole content of PMNs.⁷ LAR can take negative values when the leucocyte

count is smaller in the upper blood column than in the lower one after one hour of sedimentation, indicating increased cellular specific gravity relative to the serum. LAR correlated significantly with serum PCT and C reactive protein concentration in critical care patients with severe sepsis.⁹

No clinical studies have investigated the value of PCT and LAR in predicting bacteraemia in non-neutropenic critically ill adults. Our aim in this study was to assess the ability of PCT and LAR levels to differentiate bacteraemic from non-bacteraemic febrile episodes in critical care patients without previous antibiotic treatment for at least 48 hours.

METHODS

Following regional ethics committee approval and informed consent, 39 consecutive critically ill patients (28 men, 11 women; mean age 56 years, range 37 to 78) entered the study after admission to our eight bed teaching hospital critical care unit (CCU) between January and April 2005. Patients with haematological malignancy or previous antibiotic treatment (except for single shot surgical prophylaxis given half an hour before skin incision) were excluded. Surgical patients were admitted to the CCU immediately after recovery from anaesthesia.

Blood samples (15 ml) for aerobic and anaerobic blood cultures (BacT/ALERT, Biomerieux, Durham, North Carolina, USA) were taken under aseptic conditions from the femoral vein when axillary temperature exceeded 38.4°C for the first time after admission to the CCU. Two sets of culture bottles were incubated for a maximum of seven days in an automatic analyser. In case of growth, Gram stains were prepared and

Abbreviations: AUC, area under receiver operating characteristic curve; CCU, critical care unit; ESR, erythrocyte sedimentation rate; IL, interleukin; LAR, leucocyte antisedimentation rate; PCT, procalcitonin; PMN, polymorphonuclear leucocyte; ROC, receiver operating characteristic

cultures were processed for identification of the organisms and susceptibility testing. Immediately after blood culture samples were taken, arterial blood was also drawn into EDTA and Na citrate anticoagulated tubes (Vacutainer, Becton Dickinson, Meylan, France) to measure leucocyte count, PCT, ESR, and LAR. Thereafter, indwelling intravascular catheters with an insertion time of more than five days were removed and replaced with new ones. After blood culture tests had been taken, empirical antibiotic treatment was initiated if objective signs of chest, urinary tract, or wound infection were present. If fever returned, blood cultures were repeated but the results were disregarded for the purposes of the current study when antibiotic treatment had already been initiated. Bacteraemia was diagnosed if microbiological growth was detected within four days in at least one blood culture bottle taken from the first culture sample without antibiotic treatment.

Serum PCT was measured by immunoluminometric assay (LUMitest, Brahms Diagnostika, Berlin, Germany). LAR was determined by measuring leucocyte count in the upper (U) and lower (L) half of the sedimentation tube after one hour of gravity sedimentation.⁶ The formula $LAR = 100 \times (U - L) / (U + L)$ was used for calculating the percentage of leucocytes crossing the midline of the sedimentation blood column upward during one hour of sedimentation. The inter-assay coefficient of variation for both PCT and LAR measurements was less than 5%.

The data were analysed using Statistical Package for Social Sciences (SPSS version 10.0) software for Windows. The Mann-Whitney U test was used for investigating significance levels between bacteraemic and non-bacteraemic subgroups. Statistical significance was assumed at $p < 0.05$. The number of patients required was calculated by power analysis according to PCT results from our previous pilot studies on a similar population. Therefore, for type I $\alpha = 5\%$ and type II (power) of 90% we needed 38 patients.

RESULTS

In all, 45 febrile episodes occurring in 39 patients were evaluated (28 men; median age 56 years, range 37 to 78). Thirty two patients were admitted to the CCU after major operations (oesophageal tumour resection in 11, total gastrectomy in six, hemicolectomy in six, Whipple's operation in five, and lung lobe resection in four), and seven were non-surgical patients. Twenty three patients were categorised as bacteraemia positive and 16 as bacteraemia negative. The

non-surgical patients were evenly distributed between the bacteraemia positive and negative groups (four and three patients, respectively). Cultures with only one bacterial species were found in 19 cases and with two species in three cases; polymicrobial infection was detected in one patient. Gram negative organisms were isolated in 16 cases (*Pseudomonas aeruginosa* in eight, *Escherichia coli* in five, *Acinetobacter* species in four, *Enterobacter* species in three, and *Haemophilus influenzae* in one). Gram positive microorganisms were isolated in eight cases (*Staphylococcus aureus* in three, coagulase negative staphylococci in two, *Staphylococcus epidermidis* in two, and *Enterococcus* species in one). *Candida* was cultured in one case. The laboratory variables (leucocyte count, PCT, ESR, and LAR) in patients with more than one bacterial isolate ($n = 4$) were not significantly different from the values in remaining bacteraemic patients ($n = 19$).

Leucocyte count, PCT, and ESR were raised both in the bacteraemic and non-bacteraemic patient subgroups with no significant differences between them (median values: $16.6 \times 10^3/\mu\text{L}$, $11.2 \times 10^3/\mu\text{L}$; 110 mm/h , 100 mm/h ; and $3.8 \mu\text{g/L}$, $2.0 \mu\text{g/L}$; respectively) but LAR was higher in bacteraemic than in non-bacteraemic patients, at 27.5% v 7.5% , respectively ($p = 0.002$). In the 23 blood culture positive patients, 15 had high PCT level ($\geq 2.5 \mu\text{g/L}$) and 21 had a raised LAR ($\geq 15\%$). Of 16 non-bacteraemic patients, seven had non-pathological PCT results (sensitivity 65%, specificity 44%, likelihood ratio 1.2) and 12 had a low LAR (sensitivity 91%, specificity 75%, likelihood ratio 3.6). Figure 1 presents the receiver operating characteristics (ROC) curves for PCT and LAR values. The area under ROC curves for PCT was 0.64, (95% confidence interval, 46 to 0.82) (NS), and for LAR, 0.80 (0.64 to 0.95) ($p = 0.002$).

DISCUSSION

This study suggests that microbial invasion of blood stream may affect the sedimentation properties of leucocytes in a heterogeneous population of febrile critical care patients. This association was not confounded by other variables such as PCT level, leucocyte count, or ESR. The enhanced leucocyte antisedimentation phenomenon may be explained by previous observations made on the systemic host response to bacteraemia.^{2-5 10 11} The presence of invading microorganisms in the blood stream is associated with a significant increase in the plasma level of various circulating soluble inflammatory mediators such as lipopolysaccharide,¹² C reactive protein,^{2 3 5 13 14} tumour necrosis factor α , IL6, IL8, activated complement fragments,^{3 4 10 14} and PCT.^{2 3 5} These molecules are multifunctional cytokines and most of them are produced through activation of monocytes and macrophages, which play a central role in both specific and non-specific immunity against bacterial, viral, and fungal infections. Other leucocyte subtypes (T and B lymphocytes, PMNs) are also involved in the inflammatory process, themselves being sources and targets of the cytokines.¹⁵ In bacteraemia, while the plasma levels of cytokines are significantly raised,^{2-5 10 11} it is very likely that most of the leucocytes become activated.^{15 16} In a previous experiment we proved that PMN activation was positively correlated with increased cellular volume,⁷ which decreases the specific gravity of PMNs and results in enhanced ascension during gravity sedimentation. The leucocyte antisedimentation test reflects the activation of circulating monocytes and granulocytes challenged by systemic antigens. LAR result can aid decision on the start of antibiotic treatment in critical care patients at their first febrile episode.

We attempted to reduce the likelihood of false negative blood culture results by disregarding those samples that were taken after the initiation of antibiotic treatment. The

Figure 1 Receiver operating characteristics curves of serum procalcitonin (PCT) and leucocyte antisedimentation rate (LAR) for prediction of bacteraemia results. Area under the curve for PCT is 0.64 (NS) and for LAR, 0.80 ($p = 0.002$).

Take home messages

- The leucocyte antisedimentation rate (LAR) reflects a decrease in the specific gravity of polymorphonuclear leucocytes (PMNs) secondary to water uptake occurring during activation of the cells, and is positively correlated with enhanced cellular adherence.
- An increased LAR is correlated with positive blood culture results.

probability of systematic pre-test errors which can cause false positive results (for example, by contamination of the culture material) seemed low because of the small proportion of coagulase-negative staphylococci among the Gram-positive bacteria. This might have been a beneficial effect of the meticulously sterile blood sampling technique that was applied during femoral vein puncture and blood sample transport to the culture bottle. The ratio of Gram negative to Gram positive isolates seems high compared with previous reports.^{13 17 18} This might reflect the difference in patient populations: all our patients had been admitted to hospital for more than 48 hours before the first blood sample was taken, and in this way all infections were nosocomial, while the other reports were made mainly on community acquired infections.^{3 17 18}

Other prediction rules for bacteraemia have also been developed.^{17–19} The prognostic models used simple variables obtained from the patients' clinical history. The models either predicted mainly post-bloodstream infection mortality rather than bacteraemia itself,¹⁸ or performed poorly in the case of Gram negative bacteraemia.¹⁷

This study has potential limitations. First, the intravenous volume intake of our patients might have influenced the specific gravity of patients' plasma. The higher the fluid intake before blood sampling, the lower is the plasma specific gravity. However, we can assume that the patients with true bacteraemia must have needed a higher intravenous fluid intake than non-bacteraemic cases, and the more diluted plasma would tend to decrease the upward flotation of leucocytes and so make the result even more significant. Second, the study did not attempt to examine the role of leucocyte subpopulations in LAR, nor did it pay attention to the ratio of immature granulocytes. Ansari-Lari and co-workers have proved that percentage of immature granulocytes is a very specific predictor of both infection and positive blood culture results.¹¹

In conclusion, our study suggests that the simple leucocyte antisedimentation test may be a good positive predictor of bacteraemia in critically ill patients presenting their first febrile episode without preceding antibiotic treatment. A

larger scale study is needed to evaluate more fully the usefulness of LAR in the detection of community acquired and hospital acquired septicaemias in non-CCU patient cohorts.

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REFERENCES

- 1 American College of Chest Physicians. Society of Critical Care Medicine: consensus conference definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992;**20**:864–75.
- 2 Liadot S, Dayer E, Praz G, et al. Usefulness of procalcitonin serum level for the diagnosis of bacteremia. *Eur J Clin Microbiol Infect Dis* 2001;**20**:524–7.
- 3 von Lilienfeld-Toal M, Dietrich MP, Glasmacher A, et al. Markers of bacteremia in febrile neutropenic patients with hematological malignancies: procalcitonin and IL-6 are more reliable than C-reactive protein. *Eur J Clin Microbiol Infect Dis* 2004;**23**:539–44.
- 4 Kern WV, Heiss M, Steinbach G. Prediction of gram-negative bacteremia in patients with cancer and febrile neutropenia by means of interleukin-8 levels in serum: targeting empirical monotherapy versus combination therapy. *Clin Infect Dis* 2001;**32**:832–5.
- 5 Chirouze C, Schuhmacher H, Rabaud C, et al. Low serum procalcitonin level accurately predicts the absence of bacteremia in adult patients with acute fever. *Clin Infect Dis* 2002;**35**:156–61.
- 6 Bogar L, Horvath JA, Tekeres M. Leucocyte motion during gravity sedimentation of whole blood. In: List WF, Müller MM, McQueen MJ, editors. *Advances in critical care testing*. The 1996 IFCC-AVL Award. Berlin: Springer, 1997:13–19.
- 7 Bogar L, Tarsoly P, Jakso P. Characteristics of light and heavy polymorphonuclear leukocytes. *Clin Hemorheol Microcirc* 2002;**27**:149–53.
- 8 Bogar L, Tekeres M. Leucocyte flotation during gravity sedimentation of the whole blood. *Clin Hemorheol Microcirc* 2000;**22**:29–33.
- 9 Bogar L, Tarsoly P. Leucocyte sedimentation and inflammatory complications after thoraco-abdominal operations [abstract]. *Biorheol* 1999;**36**:91–2.
- 10 Groeneveld ABJ, Bossink AWJ, van Mierlo GJ, et al. Circulating inflammatory mediators in patients with fever: predicting bloodstream infection. *Clin Diagn Lab Immunol* 2001;**8**:1189–95.
- 11 Ansari-Lari MA, Kickler MA, Borowitz MJ. Immature granulocyte measurement using Sysmex XE-2100. Relationship to infection and sepsis. *Am J Clin Pathol* 2003;**120**:795–9.
- 12 Hurley JC. Reappraisal with meta-analysis of bacteremia, endotoxemia, and mortality in Gram-negative sepsis. *J Clin Microbiol* 1995;**33**:1278–82.
- 13 Wyllie DH, Bowler IC, Peto TE. Bacteremia prediction in emergency medical admissions: role of C reactive protein. *J Clin Pathol* 2005;**58**:352–6.
- 14 Byl B, Deviare J, Saint-Hubert F, et al. Evaluation of tumor necrosis factor- α , interleukin-6 and C-reactive protein plasma levels as predictors of bacteremia in patients presenting signs of sepsis without shock. *Clin Microbiol Infect* 1997;**3**:306–16.
- 15 Spittler A, Razenberg M, Kupper H, et al. Relationship between interleukin-6 plasma concentration in patients with sepsis, monocyte phenotype, monocyte phagocytic properties, and cytokine production. *Clin Infect Dis* 2000;**31**:1338–42.
- 16 Akira S, Hirano T, Taga T, et al. Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF). *FASEB J* 1990;**4**:2860–7.
- 17 Bates DW, Sands K, Miller E, et al. Predicting bacteremia in patients with sepsis syndrome. *J Infect Dis* 1997;**176**:1538–51.
- 18 Vales EC, Abreira V, Sanchez JCC, et al. A prediction model for mortality of bloodstream infections: Bedside analysis with the Weibull function. *J Clin Epidemiol* 2002;**55**:563–72.
- 19 Jaimes F, Arango C, Ruiz G, et al. Predicting bacteremia at the bedside. *Clin Infect Dis* 2004;**38**:357–362.