Upsala J Med Sci 99: 187–196, 1994

Use of a Novel Measuring Technique for the Erythrocyte Sedimentation Rate—a Pilot Study in Patients with Neutropenia and Fever

Per Engervall,[1] Anders Kallner[2] and Magnus Björkholm[1]

[1]*Division of Medicine, Section of Hematology and Medical Immunology and* [2]*Department of Clinical Chemistry, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden*

ABSTRACT

In the present study a new technique for measuring the erythrocyte sedimentation rate (ESR) is clinically evaluated. This technique extends the measuring range above that of the traditional ESR by calculating the sedimentation (S) at 60 minutes from data collected between 18 and 24 minutes. Measurement of ESR, S and C-reactive protein (CRP) was performed prospectively three times a week in 25 patients developing 30 fever episodes following chemotherapy for a hematological malignancy.

A good correlation was obtained between S and ESR values up to 90 mm ($r=0.98$; $p<0.0001$). The use of S may allow the clinician to follow an infectious or inflammatory process more accurately than with ESR.

During neutropenia a rise in S preceded fever in all episodes were two samples were obtained before start of fever (n=13). Changes in CRP and S values showed the same pattern in 11 episodes, in 12 CRP preceded S and in 7 episodes there was no correlation between the results. CRP, opposed to S, discriminated between bacteremias and blood culture negative episodes ($p < 0.05$) in samples obtained during the first 72 hours after start of fever. In patients with fever during neutropenia determination of S does not offer any clear advantage to CRP.

INTRODUCTION

The erythrocyte sedimentation may be separated into three distinct phases: aggregation, sedimentation and packing (11). In most cases, the aggregation phase ends within 10 to 15 minutes and the sedimentation phase then continues. In some patients with an increased erythrocyte sedimentation rate (ESR) the sedimentation phase may be short and the packing phase can start well before the standardized reading (60 minutes) takes place. In these cases the reported results do not correctly reflect the degree of activity. In the present report, we evaluate

an approach to include samples with short sedimentation phases into the measuring range by calculating a sedimentation at 60 minutes from the first part of the sedimentation phase (10).

As indicated in a previous paper (11), the classic Westergren ESR (15) is understood as the sedimentation achieved at 60 minutes rather than a sedimentation rate. The kind of quantity measured is thus a length rather than a rate. The quantity used in this study is also a length but it is estimated from the slope and the intercept of the sedimentation versus time. We call the measured quantity Sedimentation (S), and the unit mm (10). Using the suggested calculation procedure, results will be produced which are compatible with results from a Westergren ESR up to 90 mm but can be extended to higher values.

In order to evaluate this method clinically, patients with hematological malignancies and expected very high sedimentations were chosen. These patients experience long periods of neutropenia due to cytoreductive therapy and a majority of the patients will become febrile. Furthermore, 20-40 % of patients with fever and neutropenia have a blood culture proven bacteremia (2).

C-reactive protein (CRP) is commonly measured to monitor the acute phase reaction (9). Several reports have focused on the clinical value of CRP measurements in the surveillance of neutropenic patients with fever (4, 13, 14). Serum CRP concentrations exceeding 100 mg/l have frequently been associated with the presence of infection. There is, however, a wide range of reported values and the ability to discriminate between different etiologic agents (including gram-negative (Gr-) versus gram-positive (Gr+) bacteremias) has been poor (4, 13, 14).

The classic ESR is of limited value in the monitoring of this patient category mainly due to the latency of the sedimentation and lack of ability to correctly measure values exceeding 90 mm.

The primary aim of the present study was to determine whether S is superior to conventional ESR measurements in a clinical setting where ESR values exceeding 90 mm is commonly encountered. A secondary aim was to compare S to CRP determinations in the clinical evaluation of patients with fever during neutropenia.

MATERIAL AND METHODS

Patients

Blood samples were collected from patients with hematological disorders receiving chemotherapy. Patients who developed fever (> 38.0 °C on two occasions with at least a four-hour interval or >38.5 °C on one occasion) during neutropenia (absolute granulocyte count < 0.5 x 10^9/l) were studied. To qualify for inclusion in the present study, one measurement

within one week before start of fever and one within 72 hours after start of fever had to be obtained.

Thirty-five fever episodes in 25 patients (13 women and 12 men) with a median age of 56 years (range 26-77 years) were identified. They were all receiving multiagent chemotherapy for acute myelocytic leukemia (n=15), acute lymphoblastic leukemia (n=4), chronic myelocytic leukemia (n=2) or ablative chemotherapy/total body irradiation followed by autologous bone marrow support (n=4).

One fever episode from each etiologic category (i.e. outcome of blood cultures, see below) per patient was selected for analysis. In patients with more than one episode within the same category (n=5), the episode with the shortest time to the first sample obtained after start of fever was selected. Thus, a total of 30 fever episodes were included in the final analysis

Samples

Samples for analysis were obtained when blood was drawn for routine studies, i.e., three times a week in the majority of patients. All ESR/S samples were collected using the Seditainer tube (120 mm long vacuum tube, Becton-Dickinson) containing 1.3 ml trisodium citrate. This device aspirates 5.2 ml of blood.

Sample selection

Samples obtained within one week before and within two weeks after start of fever were included in the study. A median of 7 samples were obtained for each fever episode (range 3-9). Samples were grouped in 3–day (72 hours) intervals in relation to the start of fever, i.e., 1-3 and 4-6 days before, and 0-2, 3-5, 6-8, 9-11 and 12-14 days after start of fever. In episodes with more than one sample obtained within the same time-interval the highest value was selected for analysis.

Characteristics of fever episodes

Fever episodes were divided into three categories depending on the outcome of blood cultures obtained at start of fever: Gr-, Gr+ and blood–culture–negative (BCN) episodes. To minimize the risk of false positive bacteremias, two out of three cultures with coagulase-negative staphylococci had to be positive (4). Seven Gr-, 8 Gr+ bacteremias and 15 BCN episodes were included. No episode with a mixed bacteremia was documented.

Measurement procedures

The sedimentation was measured in a Sedimatic 100 automatic reader (Analysinstrument, Stockholm, Sweden). This instrument was described in a previous report (11). In the present study the instrument was modified to calculate a S from values measured between 18 and 24 minutes in addition to transforming the reading at 60 minutes to the corresponding Westergren value.

CRP was measured in serum using a laser nephelometer and reagents from Behringwerke-Hoechst (Germany) (7).

Calculations and statistics

The sedimentation rate for each sample was calculated from readings taken between the 18th and 24th minute using the method of least squares. Using the obtained regression line, the S in arbitrary units at 60 minutes was calculated. By this method, sedimentations far above what can theoretically be measured can be obtained (10).

Due to the non-parametric distribution of CRP, S and ESR, median values and ranges are given, and, for comparison of median values, the Mann-Whitney's U non-parametric test was used. For correlation between different variables Spearman Rank correlation coefficient was utilized. A p value < 0.05 was considered significant.

RESULTS

Sedimentation and ESR

In samples with ESR ≤ 90 mm (n=159) there was a strong positive correlation with S values ($r=0.98$; $p<0.0001$). ESR can not reliably be measured above a value of 90 mm due to the length of the tube. Therefore, the comparison with S cannot be extended further. In 14 episodes, one or more S values were above 90 mm, and in 12 of these episodes two or more consecutive S values > 90 mm were registered, thus making it possible to monitor the sedimentation above the normal measuring range for the ESR (fig. 1).
In all but one episode, the S and ESR rose after start of fever.

Sedimentation and CRP

In 11 of the 30 episodes, S and CRP showed the same pattern of variation, in 12 episodes the change (increase or decrease) in CRP preceded that of S with one sample (i.e. 24-72 hours), and in the remaining 7 no strict correlation between the two variables was established.

There was a positive correlation between S and CRP in samples obtained 0-2 days after start of fever ($r=0.4$, $p<0.01$). This correlation was strong for Gr- ($r=0.6$; $p<0.05$) and Gr+ ($r=0.6$; $p<0.05$) bacteremias, while no correlation in the BCN group was found ($r=0.1$; $p=0.7$).
The increase in S, following start of fever, as response to bacteremias was delayed compared to the rise in CRP (Tables 1 and 2). In samples obtained during the first 72 hours after start of fever CRP discriminated between blood culture negative (BCN, median 73 mg/, range 12-187 mg/l) and blood culture positive (BCP) episodes (Gr+ and Gr- combined, median 125 mg/l, range 62-205 mg/l; $p < 0.05$, Table 1). There was, however, no difference in median CRP values between Gr- and Gr+ infections (Table 1), nor did the proportion of high CRP (> 100 mg/l) values differ between the two groups (5/7 Gr- versus 5/8 Gr+). There was no statistically

significant difference in S values between BCP and BCN episodes in the first time interval after start of fever (Table 1). A tendency to higher S values (> 100 mm) in the Gr- group was found (3/7 Gr- versus 1/8 Gr+). The peak S value occured in samples obtained during 3-5 days after start of fever and in BCP episodes the median S value (95 mm, range 31-112 mm) was numerically higher than that recorded in BCN episodes (68.5 mm, range 10-130 mm; p=0.055, Table 1).

In 13 episodes, samples were obtained in both the interval 36 hours preceding start of fever (minus 36 hours to 0) and in the 72 hours preceding this interval, i.e. 108 to 37 hours before start of fever. In all episodes the S values rose (Wilcoxon signed-rank test $p < 0.01$) and median S values increased from 22 mm (range 1-38 mm) in the latter interval to 43 mm (range 2-86 mm; $p<0.05$) in the former time interval.
The increase in CRP was less pronounced (median 19.5 mg/l, range 5-71 mg/l to 30 mg/l, range 5-95 mg/l, the two time intervals respectively; p=0.4). In four episodes, the CRP values were unchanged or decreased between the two time intervals (Wilcoxon signed-rank test p=0.1).

Table 1
C-reactive protein (mg/l) concentrations and *sedimentation (mm, italic)* in relation to etiology of fever and time-interval.

Time-interval (days before and after start of fever)	Gram-negative (n=7) median (range)	Gram-positive (n=8) median (range)	BCN[1] (n=15) median (range)
3-1	32 (5-93) *43 (2-70)*	5 (3-42) *22 (8-66)*	10 (5-111) *30 (12-66)*
0-2	125 (62-205) *86 (30-143)*	123 (65-197) *65 (17-106)*	73* (12-187) *57 (34-119)*
3-5	72 (51-135) *99 (53-112)*	78 (38-241) *90 (31-104)*	74 (5-351) *69 (10-130)*
6-8	49 (29-159) *87 (30-115)*	67 (17-151) *71 (25-111)*	45 (6-348) *75 (34-134)*

* $p < 0.05$ in comparison to gram-positive episodes
[1]Blood culture negative

Case report

The relationship between cytostatic treatment, fever, ESR, S, and CRP values in on typical patient with repeated S values above 90 mm are shown in Fig.1.
A 54-year-old man received his third induction course of multi-drug chemotherapy for an acute myelocytic leukemia. He was doing well for 8 days after chemotherapy when chills and fever started (day 0, Fig. 1). Antibiotic therapy was initiated with amikacin and trimethoprim-sulfamethoxazole. Blood cultures showed growth of gram-negative bacteria (*Proviencia* sp.) sensitive to the empirical therapy. His temperature gradually normalized until day 5 but fever

returned the next day (day 6). The patient was otherwise in a good clinical condition. After 8 days, therapy was changed to imipenem/cilastatin but fever persisted until the granulocyte count rose after another 5 to 6 days (days 13 and 14).

The ESR increased to 90 mm the day fever started and then remained at this maximum level throughout the whole study period. The variation in S mirrored the clinical course although CRP reacted faster with a decline after changing to imipenem/cilastatin therapy.

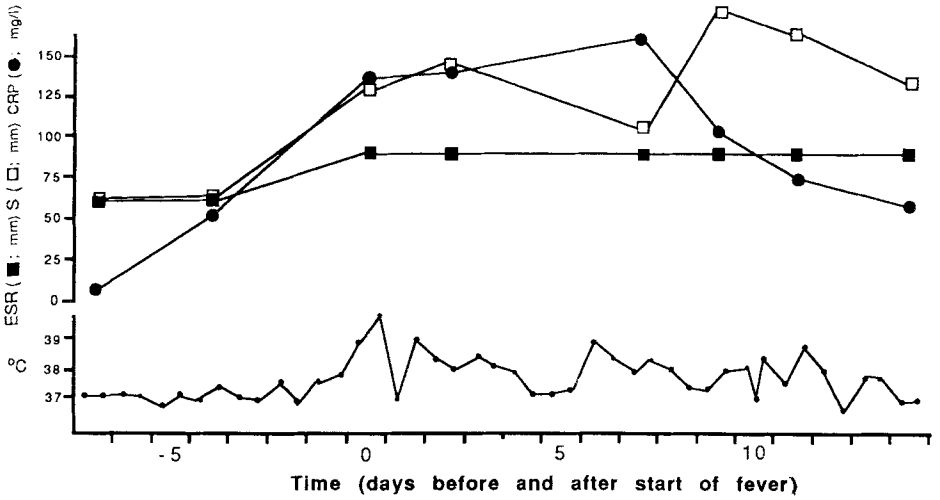

Fig. 1 CRP, S, and ESR levels in a 54-year-old male with acute myelocytic leukemia. Start of fever at day 0. Blood cultures showed growth of gram-negative bacteria (*Proviencia* sp.).

DISCUSSION

Several approaches can be attempted to circumvent the physical limitations of the ESR. The development of cheap and easily programmed electronic devices makes it possible to perform calculations on measured values which were not previously possible. In the present paper, we use a scheme in which the sedimentation rate at 18 to 24 minutes forms the basis for such calculations (10). By adjusting an algorithm to fit the linear phase of the sedimentation, values equivalent with the conventional ESR, up to 90 mm, were obtained (10). Higher sedimentations were calculated on a continuous scale.

The patients in this series were all erythrocyte transfusion dependent and hemoglobin concentrations varied during the clinical course. This variation may of course have affected the

S in some patients. However, there was no difference in hemoglobin concentrations in the different time intervals between the three groups (Gr-, Gr+ bacteremias and BCN episodes (data not shown).

In the ESR range of 0-90 mm, S correlated well with ESR, thus allowing the clinician to evaluate the S value in the same way as the classic ESR. In 14 of the 30 episodes with neutropenic fever, the S rose above the measuring range for the ESR. Thus, S is a more appropriate way of measuring the sedimentation in these patients. As also demonstrated by the case reports, there may be useful additional information in the variation of the S in the range above 90 mm.

The rapid rise of CRP, within six to ten hours after tissue damage, and the short half life (4-6 hours) of CRP makes it more suitable for monitoring fast changes in inflammatory/infectious activity (13). This was also demonstrated by the episodes in this study where a change in CRP preceded a change in S and ESR and also by the delayed peak value for S in response to bacteremia. Using 72–hour intervals S in most episodes was found to reflect changes in the patients' clinical status. However, the oscillations, especially falling values, were not as prominent as for CRP.

The observation that S values increased before start of fever is interesting. CRP values obtained during the same intervals did not show such a consistent pattern. Thus, in this regard there may be an advantage for the sedimentation compared to CRP.

The median S value in the Gr- group was numerically higher than in the Gr+ group in the first 72 hours after start of fever (Table 1). There was also a tendency to a higher proportion of S values > 100 mm in the Gr- group. In contrast, CRP values were almost identical for Gr- and Gr+ bacteremias, respectively, during the same time-interval.

The main cytokine responsible for release of CRP is interleukin-6 (IL-6), followed by tumor necrosis factor (TNF) and interleukin-1 (IL-1; 3,12) . In a study of children with hematological malignancies and neutropenic fever, higher IL-6 levels were seen in patients with Gr- bacteremia (8). Similar findings are reported in patients with hematological disorders and neutropenic fever where TNF values were higher in Gr- than in Gr+ bacteremias (5). However, in this study, and other, no difference in CRP values between Gr- and Gr+ bacteremia was seen.

Hypothetically, the S reaction, which is the final response to of many inflammatory reactions, may be more sensitive to these cytokine responses than CRP and thus explaining the tendency to higher S values in Gr- bacteremias. Extended studies will elucidate if there is a difference in the S response between Gr- and Gr+ bacteremias.

In conclusion, S determinations will allow the clinician to follow an infectious or inflammatory process with more accuracy than with the use of the ESR. This could have both diagnostic and therapeutic implications in patients with high ESR where the ESR are commonly used in monitoring disease activity (e.g. auto-immune and other inflammatory disorders). For patients with fever during neutropenia, determination of S provides more information than ESR but offers no clear advantage to CRP in the clinical evaluation of the patient. Future studies will evaluate the possible benefit of S in other patient categories with very high ESR.

REFERENCES

1 Dofferhoff, A.S.M., Bom,V.J.J., de Vries-Hospers, H.G., van Ingen, J., vd Meer J., Hazenberg, B.P.C., Mulder, P.O.M. & Weits, J. : Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute-phase proteins during the treatment of severe sepsis in humans. Crit Care Med **20**: 185-193, 1992.

2 Engervall, P., Stiernstedt, G., Günther, G. & Björkholm, M.: Trimethoprim-sulfamethoxazole plus amikacin as first-line therapy and imipenem/cilastatin as second empirical therapy in febrile neutropenic patients with hematological disorders. J Chemotherapy **4**: 99-106, 1992.

3 Geiger, T., Andus, T., Klapproth, J. Hirano, T., Kishimoto, T. & Heinrich, P.C.: Induction of rat acute-phase proteins by interleukin-6 in vivo. Eur J Immunol **18**: 717-721, 1988.

4 Gozzard, D.I., French, E.A., Blecher, T.E. & Powell, R.J.: C-reactive protein levels in neutropenic patients with pyrexia. Clin Lab Haemat **7**: 307-315, 1985.

5 Günther, G., Gårdlund, B., Lindberg, M. Hast, R. & Wretlind, B.: Endotoxin and tumor necrosis factor in patients with hematological malignancies and septicemia. Proceedings, The annual meeting of the Swedish medical association, Stockholm 182, 1992.

6 Hack, C.E., Groot de, E.R., Felt-Boersma, R.J.F., Nuijens, J.H., van Schinjndel, S., Eerenberg-Belmer, A.J.M., Thijs, L.G. & Aarden, L.A.: Increased plasma levels of interleukin-6 in sepsis. Blood **74**: 1704-1710, 1989.

7 Harmoinen, A, Hällström, O. & Grönroos, P: Rapid quantitative determination of C-reactive protein using laser-nephelometer. Scand J Clin Lab Invest **40**: 293-295, 1980.

8 Heney, D., Lewis, I.J., Evans, S.W., Banks, R., Bailey, C.C. & Whicher, J.T.: Interleukin-6 and its relationship to C-reactive protein and fever in children with febrile neutropenia. J Infec Diseases **165**: 886-890, 1992.

9 ICSH. Guidelines on selection of laboratory tests for monitoring the acute phase response. J Clin Pathol **41**: 1203-1212, 1988.

10 Kallner A., Engervall P. & Björkholm, M.: Kinetic measurement of the erythrocyte sedimentation rate. Upsala J Med Sci. In print this issue.

11 Kallner, A.: On the temporal development of erythrocyte sedimentation rate using sealed vacuum tubes. Am J Hematol **37**: 186-189, 1991.

12 Le, J. & Vilcek, J. Biology of disease: Interleukin-6: A multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab Invest **61**: 588-602, 1989.

13 Rose, P.E., Johnson, S.A., Meakin, M., Mackie, P.H. & Stuart, J.: Serial study of C-reactive protein during infection in leukemia. J Clin Pathol **34**: 263-6, 1981.

14 Starke, I.D., De Beer, F.C., Donnelly, P.J., Catovsky, D., Goldman, J.M., Galton, D.A.G. & Pepys, M.B.: Serum C-reactive protein levels in the management of infection in acute leukemia. Eur J Cancer Clin Oncol **20**: 319-325, 1984.

15 Westergren, A.: The technique of the red cell sedimentation reaction. Am Rev Tuberc **14**: 94-100, 1926.

Correspondence: Per Engervall, MD, Division of Medicine, Section of Hematology and Medical Immunology, Karolinska Hospital, S-171 76 Stockholm, Sweden.

Telephone No: +46-8-729 38 72 Fax No: +46-8-31 73 03