A rapid method to estimate Westergren sedimentation rates

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The erythrocyte sedimentation rate (ESR) is a nonspecific but simple and inexpensive test that was introduced into medical practice in 1897. Although it is commonly utilized in the diagnosis and follow-up of various clinical conditions, ESR has several limitations including the required 60 min settling time for the test. Herein we introduce a novel use for a commercially available computerized tube viscometer that allows the accurate prediction of human Westergren ESR rates in as little as 4 min. Owing to an initial pressure gradient, blood moves between two vertical tubes through a horizontal small-bore tube and the top of the red blood cell (RBC) column in each vertical tube is monitored continuously with an accuracy of 0.083 mm. Using data from the final minute of a blood viscosity measurement, a sedimentation index (SI) was calculated and correlated with results from the conventional Westergren ESR test. To date, samples from 119 human subjects have been studied and our results indicate a strong correlation between SI and ESR values ($R^2=0.92$). In addition, we found a close association between SI and RBC aggregation indices as determined by an automated RBC aggregometer ($R^2=0.71$). Determining SI on human blood is rapid, requires no special training and has minimal biohazard risk, thus allowing physicians to rapidly screen for individuals with elevated ESR and to monitor therapeutic responses. © 2009 American Institute of Physics.

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I. INTRODUCTION

The erythrocyte sedimentation rate (ESR) was introduced into medical practice in 1897 and is often referred to as the Biernacki or Fahraeus–Westergren test. It describes the distance in millimeters that red blood cells (RBC) settle in a vertical tube during a specified period of time, usually 1 h. The Westergren manual method is considered as the reference methodology.¹

The ESR measurement is simple, inexpensive and is thus a frequently utilized test for patients with acute or chronic inflammatory processes.¹ Elevated ESR is also common in infections, can serve as a useful aid in the diagnosis of various clinical conditions, and has been shown to correlate with an unfavorable prognosis in neoplastic diseases and coronary artery disease.^{2–5} In addition, serial ESR measurements allow monitoring the efficacy of therapeutic interventions (e.g., in rheumatoid arthritis). Although ESR is a commonly ordered laboratory test, it has several limitations: (1) slow turnaround time since the measurement alone takes 60 min to perform; (2) it is an open tube system and thus has biohazard risks; and (3) inaccurate results are possible due to nonvertical tube positioning or inaccurate/delayed reading. Several automated systems have been developed that analyze multiple samples in parallel; they tend to improve accuracy but are expensive, mostly available in large medical centers and *still* require 60 min to obtain an ESR result.

An innovative blood viscometer has recently been developed (Rheolog™, Rheologics, Inc., Exton, PA) that utilizes a closed, U-shaped disposable test kit.^{6–8} This device is able to accurately monitor the position of RBC columns in two vertical tubes as a function of time, and thus we have developed a novel method to predict one hour ESR values using data

collected during the third, final minute of viscosity testing. This new derived parameter is termed a sedimentation index (SI). Because ESR is governed primarily by RBC aggregate formation, we have also compared RBC aggregation parameters with the SI.

II. MATERIALS AND METHODS

A. Blood samples

Blood samples were collected by sterile antecubital venipuncture into ethylenediaminetetraacetic acid (EDTA; 1.5 mg/ml) from 72 apparently healthy adult volunteers, 33 patients with stable angina (SA) or acute coronary syndromes (ACS), and 14 subjects with severe sepsis who were admitted to the USC-LAC Medical Center (119 subjects: 28 males and 91 females; average age 64 ± 10 yr). No specific inclusion or exclusion criteria were employed for the present study. All tests were completed within 4 h and the study was approved by the Institutional Review Board of the University of Southern California.

B. ESR measurement

ESR was measured using 200 mm long disposable glass Westergren tubes (Curtin Matheson Scientific, Houston, TX) at room temperature (22 ± 1 °C) and with blood at native hematocrit (Hct). RBC sedimentation in the vertical tubes was recorded at 60 min and results expressed as mm/hour. All blood samples were mixed thoroughly immediately prior to testing.

C. SI

A fully automated tube viscometer (Rheolog™) was utilized to determine the SI, with all SI measurements made at

FIG. 1. (Color online) Association between calculated SIs and Westergren ESR ($R^2=0.9219$). The dashed line indicates that an upper limit of SI=8 might be utilized as a single cutoff point to identify subjects with pathologically elevated ESR.

native Hct. In this device, blood is introduced into a disposable assembly consisting of a horizontal small-bore tube with a vertical “riser” tube at each end. Blood flow through the tube is driven by the pressure gradient created by initially unequal heights of blood in the riser tubes. Two pairs of light-emitting diode (LED) arrays and charge-coupled devices (CCDs) are positioned parallel with the vertical tubes. In the presence of RBC, LED light is blocked from reaching the detectors and thus the number of CCD sensors not receiving light is directly proportional to the height of a RBC column. Data from the optical detectors are collected every 20 ms by a microcomputer at 12 pixels/mm; thus allowing the top of the RBC column in the vertical tubes to be determined with a resolution of 0.083 mm. RBC column height information is collected for 3 min and utilized to calculate blood viscosity versus shear rate.^{9,10} Based on our experience, the hydrostatic pressure in the vertical tubes generally equalizes within the first 120–130 s of the viscosity test and thus blood flow through the small-bore tube ceases. Since stasis or very low shear conditions promote RBC aggregation, sedimentation occurs primarily during the final 60 s of the measurement. The process is simple to monitor because RBC-free or RBC-poor plasma allow LED light to reach the CCD sensors. The difference between the *total* number of inactive pixels (i.e., the sum of inactive pixels in the two riser tubes), as measured at 120 and 180 s, is determined and the magnitude of this difference termed the SI.

It is important to emphasize that the calculation of SI is based upon determining the position of the RBC-plasma interface in *both* vertical tubes, and thus zero flow through the small-bore tube is not absolutely essential. Rather, any upward movement of the RBC interface in the downstream vertical tube due to residual flow over the 60 s period is detected and used to correct the downward movement in the upstream vertical tube. Thus slow or “creeping” flow through the tube while SI is determined does *not* vitiate the results.

D. RBC aggregation

RBC aggregation was evaluated for 50 subjects with a native Hct of $40\% \pm 1\%$ using a Myrenne MA-2 Aggregometer (Myrenne GmbH, Roetgen, Germany) operating at room temperature. This device provides two dimensionless aggregation indices: (1) “M” reflects RBC aggregation at stasis and (2) “M1” indicating RBC aggregation at very low shear (3 s^{-1}). Both indices increase with enhanced RBC aggregation.^{11,12}

III. RESULTS AND DISCUSSION

A. ESR and SI

In the present study, 1 hr Westergren rates (ESR) were measured and SIs were calculated for 119 randomly selected human subjects in order to validate a novel screening method developed to rapidly predict one hour sedimentation rates. Blood samples were tested at native Hct (average: $38.7\% \pm 4.4\%$) for ESR and to calculate SI; a subgroup with a native Hct of 40% was used to test for RBC aggregation. Based on the age range and gender distribution of our study population, an ESR value of 30 mm/hr was chosen as the upper limit of the normal range.¹³

One hour sedimentation rates ranged between 1 and 91 mm/hr with an average value of 27 mm/hr. Of the 72 apparently healthy individuals, 68 had normal ESR values. As expected, sedimentation for septic patients was the highest and subjects with SA or ACS had intermediate results. Calculated SI data ranged between 1 and 68 with an average pixel number of 12.5. As shown in Fig. 1, SI values increased with increasing ESR, with the data well-fitted by a polynomial curve

$$\text{ESR} = -0.016(\text{SI})^2 + 2.095\text{SI} + 8.8792, \quad R^2 = 0.9219. \quad (1)$$

Considering the nonlinear relationship between the two variables, the sensitivity of SI was relatively low for samples with normal ESR and was found to range between 1 and 8 pixels. Importantly, *all* subjects with normal sedimentation

FIG. 2. (Color online) Association between calculated SIs and Myrenne M1 aggregation indices ($R^2=0.71$). $N=50$; Hct= $40\% \pm 1\%$.

rates (i.e., <30 mm/hr) had SI indices of 8 or less. On the contrary, SI exceeded 8 pixels for *all* but three subjects with an elevated 1 hr ESR. For these three individuals, we documented a borderline SI of 8 and only slightly elevated sedimentation rates (i.e., 31, 33, and 35 mm/hr). Based on these findings, our data suggest that an SI of 8 may be used as a single cutoff point to identify individuals with ESR values exceeding the upper limit of the normal range.

SI increased sharply for Westergren rates beyond 30 mm/hr (Fig. 1). As a consequence, minor changes in ESR above the normal range were accompanied by significant changes in SI. The high sensitivity of our new method in the pathologically elevated ESR range suggests that it might also be utilized as a rapid test to follow the efficacy of therapeutic interventions when combined with clinical evaluation. For example, the activity of various autoimmune diseases (e.g., temporal arteritis and polymyalgia rheumatica) and their response to therapy might be followed conveniently in the outpatient setting with the screening result available within 5 min.

B. Association between RBC aggregation and SI

Next we examined the association between the Myrenne M and M1 indices and the SI value for a subset of patients with a native Hct of 40%. As shown in Fig. 2, we found a close association between SI and M1 indices with the data well-fitted with a polynomial curve ($R^2=0.71$). A similar, although weaker correlation was documented between SI and the M values ($R^2=0.45$; data not shown). The higher correlation for M1 than for M is likely explained by the different measurement techniques: M is a measure of RBC aggregation at stasis, while M1 reflects aggregate formation in a dynamic environment, similar to that in the Westergren tube and also in the tube viscometer.

C. Summary

In summary, SI of a human blood sample can be determined quickly, reliably, and easily using data collected during the final minute of a blood viscosity measurement. Performing the test is relatively inexpensive, requires no special training and has minimal biohazard risk. This newly developed method would allow physician to rapidly screen for individuals with elevated sedimentation rates and, as with the conventional Westergren ESR, it should also be useful to monitor disease activity for patients with chronic medical conditions. Studies involving patients with a wide variety of medical conditions will be necessary in order to further validate our novel methodology.

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