

Erythrocyte Sedimentation Rate Measurements by TEST 1 Better Reflect Inflammation Than Do Those by the Westergren Method in Patients With Malignancy, Autoimmune Disease, or Infection

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Abstract

We compared the TEST 1 (Alifax, Padova, Italy) and Westergren methods of measuring the erythrocyte sedimentation rate (ESR) to assess inflammation. The ESR was measured by both methods in 154 blood samples from patients with malignancy ($n = 69$), autoimmune disease ($n = 44$), or infection ($n = 41$). Total protein, albumin, and C-reactive protein (CRP) levels were measured in each plasma sample, and albumin and α_1 -, α_2 -, β_1 -, β_2 -, and γ -globulin fractions were measured by capillary electrophoresis. TEST 1 ESR values were significantly lower than the Westergren values, by 10.9 mm/h. We found that the correlations of TEST 1 ESR values with inflammatory protein levels (total protein, globulin, CRP, and α_1 -, α_2 -, β_2 -, and γ -globulin) were better than those obtained using the Westergren method. These findings indicate that ESR measurements by TEST 1 reflect inflammation better than do those by the Westergren method in patients with malignancy, autoimmune disease, or infection.

The erythrocyte sedimentation rate (ESR) is widely used as a screening or monitoring test for patients with acute or chronic inflammatory diseases.¹ The International Council for Standardization in Haematology (ICSH) selected the Westergren method as the reference technique for measuring ESR.¹ This method, however, takes 60 minutes and results in difficulty in quality control.^{2,3} There are many variables in the Westergren method: specimen collection, time and temperature of specimen storage, sedimentation equipment, and methodological variables.^{1,4} The ICSH and Clinical and Laboratory Standards Institute describe procedures for preventing errors in the ICSH reference method.^{1,4}

Recently, an automated ESR measurement instrument, TEST 1 (Alifax, Padova, Italy) has been marketed. This system measures the ESR in small volumes (150 μ L) of blood using a microagglutination method that assesses the interaction of RBCs with inflammatory plasma proteins and requires only 20 seconds.^{5,6} To confirm system accuracy, carefully performed comparisons of this technique against the reference procedure are required.^{1,4} Tests should be performed in parallel on at least 100 samples from patients with a wide variety of diseases and with ESR results distributed evenly in a wide range.

In response to stressful or inflammatory states that occur during infection, injury, surgery, trauma, and other causes of tissue necrosis, the levels of certain proteins, called acute phase reactant proteins, are elevated. These include α_1 -antitrypsin, α_1 -acid glycoprotein, haptoglobin, ceruloplasmin, complement proteins, fibrinogen, C-reactive protein (CRP), and immunoglobulins.⁷ The important process of erythrocyte rouleaux formation is dependent on the concentration of acute phase proteins.⁴ We evaluated the capability of

TEST 1 to reflect inflammation by determining correlations between plasma proteins that increase during inflammatory conditions and TEST 1 ESR values.

Materials and Methods

Blood samples anticoagulated with K₃EDTA (Becton Dickinson, Franklin Lakes, NJ) were routinely obtained from hospitalized and ambulatory patients at the Asan Medical Center, Seoul, Korea, and processed for analysis. The concentration of K₃EDTA was 1.8 mg/mL, and we used 3-mL EDTA tubes. All samples were obtained under standardized conditions (in the morning after a night of fasting) and tested within 4 hours of venipuncture, according to ICSH recommendations. Between October 2007 and November 2007, we selected 154 blood samples from patients with malignancy (n = 69), autoimmune disease (n = 44), or infection (n = 41); all patients had TEST 1 ESR values of 20 mm/h or more and hematocrit values between 33% and 35% (0.33-0.35) ■ **Table 1**.

Westergren Method

The Westergren method was performed according to ICSH specifications on undiluted blood samples anticoagulated with K₃EDTA using glass pipettes (Greiner Bio-One, Kremsmuenster, Austria). During sedimentation, the pipettes were mounted vertically on appropriate supporting racks and kept at room temperature, which never exceeded 25°C.

■ **Table 1**
Distribution of Diagnoses in 154 Patients

Diagnosis	No. of Cases
Malignancy (n = 69)	
Colorectal cancer	15
Breast cancer	10
Stomach cancer	12
Lung cancer	7
Leukemia	6
Lymphoma	5
Pancreatic cancer	4
Other	10
Autoimmune disease (n = 44)	
Rheumatoid arthritis	22
Systemic lupus erythematosus	6
Crohn disease	5
Ulcerative colitis	3
Behçet disease	3
Other	5
Infections (n = 41)	
Viral hepatitis	10
Tuberculosis	8
Pneumonia	6
Pancreatitis	3
Cholangitis	3
Other	11

TEST 1 Method

The TEST 1 analyzer, a closed automated analyzer, determines the length of sedimentation reaction in blood in a standard-size primary tube with a perforating stopper. The principle of measurement is the study of the aggregation capacity of RBCs by telemetry. The tubes are placed in appropriate racks, and their contents are rotated slowly for about 2 minutes. The sample loader simultaneously accepts 4 racks containing 15 tubes each. By using a closed aspiration needle, the blood is directly drawn from the collection tube, distributed in a capillary, and centrifuged at about 20g. The sensing area temperature is maintained at 37°C. The system uses an infrared ray microphotometer with a light wavelength of 950 nm and performs 1,000 readings during 20 seconds. The electrical impulses, collected using a photodiode detector, are directly correlated to the aggregation of RBCs present at each capillary level. For each sample, an aggregation and sedimentation curve is obtained. A mathematical algorithm converts the raw data obtained from evaluation of optical density signals into ESR results, which are transformed to comparable Westergren values. The system operates at a rate of 180 specimens per hour in continuous loading, providing a result every 20 seconds, and requires 150 µL of blood for each sample.^{3,5,6,8}

Measurement of Inflammatory Plasma Proteins

Total protein, albumin, and CRP concentrations in each plasma sample were measured at Seoul National University Hospital, Seoul, Korea. The globulin concentration was calculated by subtracting the albumin from the total protein concentration. The fractions of albumin and α_1 -, α_2 -, β_1 -, β_2 -, and γ -globulin were measured by capillary electrophoresis.

Statistical Analysis

We compared the correlations of Westergren and TEST 1 ESR values with the concentration of each inflammatory plasma protein. All statistical analyses were performed using SPSS 13.0 software (SPSS, Chicago, IL) and MedCalc, version 9.5.2.0 (Mariakerke, Belgium). The correlations of TEST 1 data and those of the Westergren method were evaluated by linear regression and paired *t* tests. Data were also compared by using Bland-Altman analysis.⁹ Accuracy was assessed by determining 95% confidence intervals for the mean differences between methods. Linear regression was used to estimate correlations between ESR measurements and the concentration of each plasma protein. *P* values less than .05 were considered statistically significant.

Results

Comparison Between TEST 1 and Westergren Methods

ESR was measured in 154 blood samples by the TEST 1 and Westergren methods. The mean \pm SD ESR was 46.1 \pm

22.3 mm/h (range, 20-108 mm/h) for TEST 1 and was 57.1 ± 25.3 mm/h (range, 10-118 mm/h) for the Westergren ($P < .0005$). There was a significant correlation between TEST 1 and Westergren measurements ($r^2 = 0.386$; $P < .0005$). Bland-Altman analysis showed a negative mean bias, equal to -10.95 mm/h (limits of agreement, -29.9 to 51.8), indicating that the TEST 1 values were lower than were the Westergren values (95% confidence interval, -14.27 to -7.63) **Figure 1**.

Correlations Between Inflammatory Plasma Protein Levels and ESR Values

Overall, in all 154 patient samples, there were significant correlations between total protein, globulin, CRP, α_1 -, α_2 -, and β_2 -globulin levels and Westergren method data and between total protein, globulin, CRP, α_1 -, α_2 -, β_2 -, and γ -globulin and TEST 1 results **Table 2**. TEST 1 values showed a significant correlation with γ -globulin concentrations,

whereas the Westergren data did not. In addition, the correlation coefficients were higher for TEST 1 data than for Westergren results. This finding was also observed when we compared correlations according to patient diagnoses **Table 3**, **Table 4**, **Table 5**, and **Figure 2**. Only the TEST 1 method showed significant correlations with total protein, globulin, α_2 -, and β_1 -globulin concentrations in samples from patients with autoimmune diseases and with α_1 -globulin in patients with infections.

Discussion

The ESR does not measure an analyte but rather a physical phenomenon that depends on a large number of variables. In this regard, the Westergren method may be affected by sources of variability, such as dilution of blood

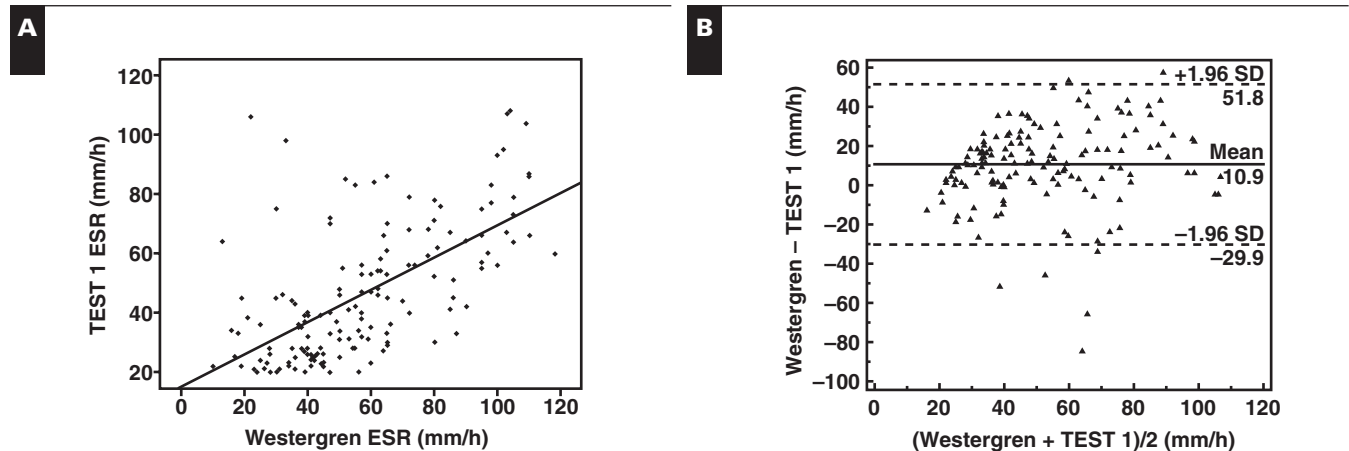


Figure 1 Comparison of TEST 1 and Westergren methods for erythrocyte sedimentation rate (ESR) measurements. **A**, Scattergram of correlations between the methods ($r^2 = 0.386$; $P < .0005$; $y = 0.547x + 14.881$). **B**, Plot of the differences between ESR measurements (y-axis) vs average ESR values (x-axis) ($n = 154$). Dotted lines denote the limits of agreement.

Table 2 Correlations Between TEST 1 and Westergren ESR Values and Concentrations of Inflammatory Plasma Proteins in Samples for 154 Patients*

Protein	Mean, g/dL (g/L)	Westergren ESR			TEST 1 ESR		
		Regression Equation	r^2	P	Regression Equation	r^2	P
Total protein	7.3 (73)	$y = 7.850x - 0.147$	0.031	.029	$y = 11.385x - 36.858$	0.084	2.65×10^{-4}
Albumin	4.5 (45)	$y = 3.126x + 43.124$	0.003	.476	$y = 0.423x + 44.238$	0.000	.913
Globulin	2.8 (28)	$y = 8.775x + 32.256$	0.025	.049	$y = 17.025x - 2.022$	0.123	8.58×10^{-6}
CRP	18 (171.4) [†]	$y = 2.731x + 52.161$	0.114	1.84×10^{-5}	$y = 2.534x + 41.567$	0.127	5.97×10^{-6}
α_1 -Globulin	0.3 (3)	$y = 89.646x + 26.347$	0.100	6.60×10^{-5}	$y = 117.672x + 5.794$	0.222	7.25×10^{-10}
α_2 -Globulin	0.8 (8)	$y = 51.558x + 17.335$	0.130	4.40×10^{-6}	$y = 69.554x - 7.483$	0.305	1.16×10^{-13}
β_1 -Globulin	0.4 (4)	$y = -11.447x + 62.045$	0.001	.689	$y = 10.795x + 41.433$	0.001	.668
β_2 -Globulin	0.8 (8)	$y = 57.998x + 8.187$	0.185	2.53×10^{-8}	$y = 81.324x - 22.421$	0.469	1.12×10^{-22}
γ -Globulin	1.2 (12)	$y = 5.532x + 50.199$	0.007	.307	$y = 12.819x + 30.197$	0.047	.007

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
 * Bold type indicates significantly different correlation findings.
[†] Given as mg/L (nmol/L).

Table 3
Correlations Between TEST 1 and Westergren ESR Values and Concentrations of Inflammatory Plasma Proteins in 69 Patients With Malignancy*

Protein	Mean, g/dL (g/L)	Westergren ESR			TEST 1 ESR		
		Regression Equation	r ²	P	Regression Equation	r ²	P
Total protein	7.2 (72)	y = 13.151x - 37.983	0.076	.022	y = 10.125x - 28.662	0.059	.044
Albumin	4.6 (46)	y = 3.739x + 40.011	0.003	.637	y = -2.999x + 58.414	0.003	.664
Globulin	2.6 (26)	y = 18.851x + 7.452	0.092	.011	y = 19.949x - 8.024	0.136	.002
CRP	16 (152.4) [†]	y = 3.400x + 51.642	0.166	.001	y = 3.411x + 39.044	0.221	4.51 × 10⁻⁵
α ₁ -Globulin	0.3 (3)	y = 115.974x + 18.427	0.143	.001	y = 117.125x + 5.463	0.192	1.64 × 10⁻⁴
α ₂ -Globulin	0.8 (8)	y = 61.467x + 9.526	0.211	7.26 × 10⁻⁵	y = 58.539x - 0.783	0.253	1.08 × 10⁻⁵
β ₁ -Globulin	0.4 (4)	y = -89.612x + 96.905	0.061	.040	y = -52.565x + 67.912	0.028	.171
β ₂ -Globulin	0.8 (8)	y = 59.401x + 7.642	0.198	1.26 × 10⁻⁴	y = 76.831x - 19.481	0.438	5.92 × 10⁻¹⁰
γ-Globulin	1.1 (11)	y = 20.408x + 35.070	0.076	.021	y = 18.469x + 24.594	0.083	.017

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

* Bold type indicates significantly different correlation findings.

[†] Given as mg/L (nmol/L).

Table 4
Correlations Between TEST 1 and Westergren ESR Values and Concentrations of Inflammatory Plasma Proteins in 44 Patients With Autoimmune Disease*

Protein	Mean, g/dL (g/L)	Westergren ESR			TEST 1 ESR		
		Regression Equation	r ²	P	Regression Equation	r ²	P
Total protein	7.3 (73)	y = 7.241x + 2.331	0.032	.243	y = 13.036x - 51.802	0.110	.028
Albumin	4.4 (44)	y = 8.657x + 16.923	0.026	.295	y = -0.700x + 46.269	0.000	.931
Globulin	2.9 (29)	y = 4.254x + 42.850	0.006	.613	y = 24.326x - 26.810	0.212	.002
CRP	14 (133.3) [†]	y = 3.940x + 49.634	0.129	.017	y = 2.708x + 39.431	0.064	.098
α ₁ -Globulin	0.3 (3)	y = 99.229x + 21.827	0.143	.011	y = 111.007x + 5.969	0.188	.003
α ₂ -Globulin	0.8 (8)	y = 28.298x + 33.739	0.036	.216	y = 96.854x - 29.899	0.446	7.31 × 10⁻⁷
β ₁ -Globulin	0.4 (4)	y = 91.683x + 14.834	0.047	.160	y = 156.223x - 25.414	0.142	.012
β ₂ -Globulin	0.8 (8)	y = 79.926x - 9.377	0.260	4.02 × 10⁻⁴	y = 96.575x - 34.715	0.399	4.22 × 10⁻⁶
γ-Globulin	1.3 (13)	y = -2.144x + 57.898	0.001	.842	y = 19.166x + 18.083	0.080	.063

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

* Bold type indicates significantly different correlation findings.

[†] Given as mg/L (nmol/L).

Table 5
Correlations Between TEST 1 and Westergren ESR Values and Concentrations of Inflammatory Plasma Proteins in 41 Patients With Infection*

Protein	Mean, g/dL (g/L)	Westergren ESR			TEST 1 ESR		
		Regression Equation	r ²	P	Regression Equation	r ²	P
Total protein	7.4 (74)	y = 1.965x + 44.479	0.002	.785	y = 10.145x - 23.048	0.082	.069
Albumin	4.3 (43)	y = 0.518x + 56.755	0.000	.949	y = 7.771x + 18.502	0.038	.220
Globulin	3.1 (31)	y = 3.159x + 49.205	0.002	.759	y = 8.058x + 26.883	0.025	.319
CRP	25 (163.1) [†]	y = 1.751x + 54.573	0.063	.113	y = 1.418x + 48.238	0.066	.105
α ₁ -Globulin	0.4 (4)	y = 52.657x + 39.762	0.035	.241	y = 114.322x + 10.091	0.263	.001
α ₂ -Globulin	0.8 (8)	y = 50.531x + 19.574	0.107	.036	y = 66.641x - 0.159	0.298	2.22 × 10⁻⁴
β ₁ -Globulin	0.4 (4)	y = 33.779x + 44.953	0.011	.508	y = 38.787x + 35.703	0.024	.336
β ₂ -Globulin	0.9 (9)	y = 47.169x + 16.708	0.130	.020	y = 77.833x - 17.941	0.566	1.41 × 10⁻⁸
γ-Globulin	1.4 (14)	y = -6.409x + 68.179	0.008	.589	y = -1.205x + 53.535	0.000	.898

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

* Bold type indicates significantly different correlation findings.

[†] Given as mg/L (nmol/L).

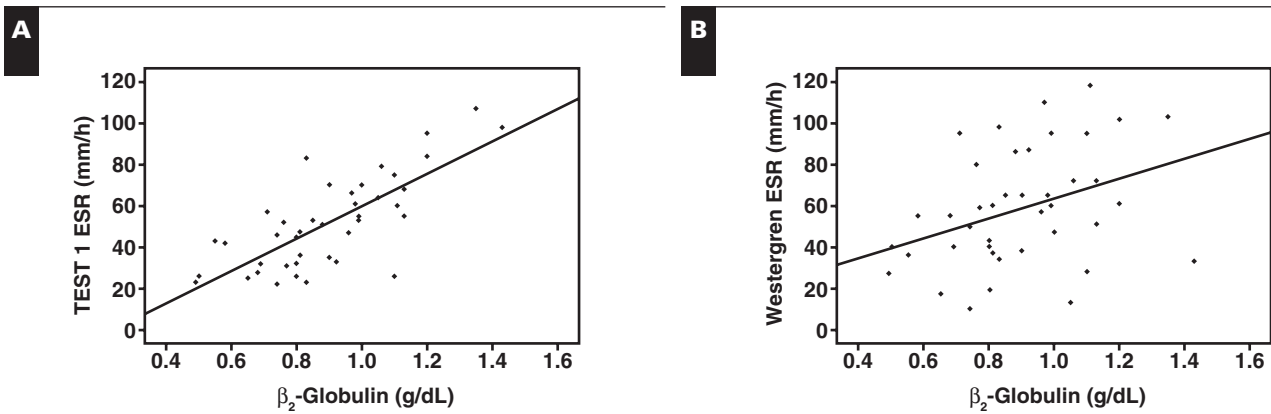


Figure 2 Correlations of TEST 1 erythrocyte sedimentation rate (ESR) values (**A**) and Westergren ESR values (**B**) with β_2 -globulin concentrations in samples of patients with infection ($n = 41$) (**A**, $r^2 = 0.566$; $P = 1.41 \times 10^{-8}$; **B**, $r^2 = 0.130$; $P = .020$). Values for β_2 -globulin are given in conventional units; to convert to Système International units (g/L), multiply by 10.0.

with anticoagulants (eg, citrate), hematocrit values, the internal diameter and length of the column, column material, temperature, and the time from venipuncture.⁶ Several new methods of measuring ESR have improved the technical, as well as the biohazardous, aspects of the testing procedure. These include automated and semiautomated instruments, which shorten testing time. Careful comparison of each new technique with the reference procedure is required to confirm the accuracy of any new method.

The TEST 1 has been extensively evaluated and has shown good correlation with ICSH-recommended methods.^{5,6,8,10} For comparison, we selected blood samples with hematocrit values between 33% and 35% (0.33-0.35). We found that TEST 1 measurements of the ESR were significantly lower than those of the Westergren method. An earlier study, which reported a significant difference between these methods, found no significant bias in groups with clinically relevant ESR values.⁸ Romero et al⁶ reported that, in samples with an ESR of more than 60 mm/h, TEST 1 values were significantly lower than those obtained with the Westergren method and suggested that the Westergren method might overestimate the ESR in samples with low hematocrit values. In addition, the Fabry formula failed to correct the differences when applied to the Westergren ESR values obtained in the comparison study, indicating that, in addition to hematocrit values, other variables may be involved.⁶

Erythrocyte sedimentation remains an only partly understood phenomenon. Three phases—the lag or aggregation phase, decantation or precipitation phase, and packing phase—can be distinguished in the sedimentation process. The all-important process of erythrocyte rouleaux formation is dependent on the concentrations of acute phase proteins and, to a lesser degree, the globulins.⁴ Fibrinogen is the most

abundant acute phase protein with greatest impact on ESR,^{10,11} appearing in the β_2 region during capillary electrophoresis of plasma proteins.¹¹ We found that the β_2 -globulin fraction was increased in all samples and that this fraction of β_2 -globulin showed the highest correlation coefficient (r^2) with the ESR data from both methods.

We found that the TEST 1 ESR values correlated better with inflammatory plasma protein concentrations than did the Westergren ESR data. In all fractions showing a significant correlation with both methods, the TEST 1 method showed higher correlation coefficients (r^2) and lower P values than did the Westergren method. When we analyzed samples subdivided according to patient diagnosis, we also found that the TEST 1 method showed higher correlation coefficients (r^2) than did the Westergren method. Although only Westergren data were significantly correlated with β_1 -globulin levels in samples from patients with malignancy and with CRP levels in patients with autoimmune diseases, the correlation coefficients (r^2) were not high (0.061 and 0.129, respectively). Only the TEST 1 method was significantly correlated with globulin and α_2 - and β_1 -globulin concentrations in samples from patients with autoimmune diseases and with α_1 -globulin levels in samples from patients with infection, and these correlation coefficients (r^2) were high (0.212, 0.446, 0.142, and 0.263, respectively).

We have shown that the correlations between TEST 1 ESR values and the level of each plasma protein that increases under inflammatory conditions were better than those for Westergren ESR data. These findings indicate that TEST 1 ESR measurements better reflect the presence of inflammation than do Westergren data in patients with malignancy, autoimmune disease, or infection.

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