Clinical Utility of Erythrocyte Sedimentation Rate Test

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ABSTRACT

The Erythrocyte sedimentation test is a common method used worldwide to detect acute inflammation. ICSH-WHO reference and standard methods have been routinely used for many years. However, they require much blood and long testing time over 2 hours, and several simple and rapid methods for what have recently been proposed. This paper describes advantages and disadvantages of these methods, plasma factors affecting ESR and clinical utility of which test.

Key Words ESR; ICSH-WHO Method; Rapid Test; Inflammation Marker

INTRODUCTION

The erythrocyte sedimentation rate test (ESR) is one of the most common and traditional laboratory tests in the world. The ESR reflects both plasma concentration of acute-phase proteins of large molecular size and anemia.¹⁾ The increased mobility of patients and the benefits to laboratories of sharing their experience have led to the need for comparability of measurements between laboratories. Thus, an original ICSH (International Council for Standardization in Hematology) reference method for measuring ESR based on the Fahraeus and Westergren method using diluted blood (4 vol. of blood plus 1 vol. of sodium citrate) in open-ended glass tubing of 300 mm in length, mounted vertically in a rack or stand was developed.²⁾ Modifications, in particular the use of undiluted blood, were recommended as the basis of a new ICSH reference standard. Responding to this recommendation, the ICSH reference method was released in 1988, and is performed with undiluted blood samples with hematocrit of 0.35 or less under standardized conditions in a Westergren open-ended glass pipette that meets ICSH specifications. These undiluted blood samples are anti-coagulated with EDTA (dilution less than 1 %).³⁾ For comparison with the traditional method using diluted blood, a correlation formula can be applied: diluted blood ESR mm = (undiluted blood ESR mm × 0.86) – 12. On the other hand, a new standardized method was proposed by the ICSH, which is designed for use of EDTA-ant coagulated and undiluted blood in pipettes with a 200 mm scale and to avoid spillage of blood or aerosol generation.¹⁾ In 1993, the ICSH recommended specifications for working methods, using diluted or undiluted blood, which may be considered ICSH selected methods for routine use.^{1), 4)} A protocol is outlined for evaluation of such working methods against the ICSH reference method or the new ICSH standardized method.⁵⁾

In contrast to the international movement for ESR testing, Asian countries including Japan have thus far been supporting the use of the traditional Westergren method⁶⁾ for diluted blood as their routine method. The Westergren method is popular in Asian countries because it is simple, reliable, and inexpensive in the routine laboratory. When we searched the literature on ESR in the past 10 years through the PubMed site, over 500 were found to have been released every year. This reflects the fact that the ESR test still has clinical usefulness as a diagnostic tool.

MERITS AND DEMERITS OF CONVENTIONAL WESTERGREN ESR TEST AND RAPID ESR TESTS

In general, the Westergren method is known to detect inflammation markers. It required 2 ml/ diluted sample blood, a standardized Westergren tube, and at least 2-hour testing time. The tube must be stood straight-up and vertically at room temperature. Excessive sample number and time requirement are disadvantages for routine use. Spilling of blood is bio-hazardous for medical technologists. Furthermore, some researchers believe that ESR is less informative and cannot be automated, and that C-reactive protein determination is more useful than ESR tests for detection of acute inflammation,⁷⁾ although ESR testing is legally required for diagnosing and monitoring tuberculosis in Japan.

To compensate for such disadvantages, several types of simplified ESR methods have been proposed (Table 1). The first one developed was the Zeta Sedimentation Ratio (Zeta-fuge), by Dr. Bull.⁸⁾⁻¹⁰⁾ Anti-coagulated blood (0.18 ml) is inserted into an open-ended tiny glass tube (75 mm × 2.3 mm), and the tube is loaded onto a specific centrifuge and centrifuged for 3 min. at room temperature. The results obtained are influenced by temperature, and not by hematocrit, but it is very difficult to determine the sedimentation ratio reading particularly for specimens judged normal by the Westergren method, because such specimens exhibit very small amounts of sedimentation. Furthermore, Zeta-fuge results exhibit a non-linear correlation with Westergren values.¹⁰⁾

The Ves-matic 20/60 (DIESSE Diagnostics Senese; Siena-Italy) is an automatic instrument for the determination of ESR for 20 or 60 samples. One ml/ citrated blood is placed into a specific tube. Its tube loader holds sample tubes at a tilted angle and moves them automatically. The ESR reading is performed automatically, and testing time is less than 30 min. for the Westergren 1-hour value and 40 min. for the Westergren 2-hour value. The results obtained with automated reading can be corrected by test temperature by means of simple keyboard

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Table 1 Inter-methodological comparison of ESR tests.

	Westergren	Zeta-fuge	Ves-matic	Test-1	Quick-Eye	HOK405
Sample	citrated/ EDTA	EDTA	citrated	EDTA	citrated/ EDTA	citrated
Blood volume	2 ml	0.18 ml	1 ml	0.15 ml	1.4 ml	2 ml
Test time	60, 120 min.	3 min.	20, 40 min.	32 sec.	20, 40 min.	6,9 min.
Automatic processing	no	no	yes	yes	yes	no
Technical simplicity	relatively simple	relatively simple	simple	simple	simple	relatively simple
Correlation with Westergren method	1.0000	0.9487	0.9680*	0.9538	0.9828	0.9112
Laboratory safety	no	no	yes	yes	yes	no
Graphic analysis	no	no	no	no	yes	no
Temperature control	needed	needed	not needed	not needed	not needed	needed
Others	low reliability and incorrect reading procedures	difficulty in meniscus reading	throughput 30/h	throughput 110/h	throughput 30/h	difficulty in meniscus reading

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operation.¹¹⁾

The Test 1 automated system was developed by Plebani's group (SIRE Analytical System, Uden-Italy).¹²⁾ EDTA blood (150 μ l) is first rotated 2 minutes, aspirated through an automated piecing system, distributed in a capillary and then centrifuged at about 20 g. The system uses an infrared ray micrometer with a light wavelength of 950 nm. Its sensing area temperature is maintained at 37°C. Electrical impulses collected using a photodiode detector are directly correlated to the concentrations of RBCs present at capillary level. Its analytic throughput is a rate of 110 specimens per hour in continuous loading, providing a finding every 32 seconds. In our evaluation, the values of TEST 1 were lower than those obtained by the Westergren method.

The Quick-Eye was developed by Nagase Co. (Tokyo-Japan).^{13), 14)} EDTA or citrated blood is placed into a specific short glass tube, and tubes are loaded on a tilted holder. The holder is moved automatically, and red cell sedimentation is monitored every 40 sec. using a photodiode light detector. The Westergren 1-hour value is measured at 20 min. and the Westergren 2-hour time is measured at 40 min. after sample loading. The values correlated well with Westergren values. The meniscus between RBCs and plasma is separated clearly enough that its reading is very reliable. One characteristic of the Quick-Eye is that it is able to draw a graphic curve of the blood sedimentation process (Fig. 1). The graph is informative for observers and researchers in determining the sample specificity of sedimentation.

The HOK405 (Drager & Heerhorst GmbH & Co. KG) uses the same principle as the Westergren method. A Westergren glass tube filled with citrated blood is set on a tube holder, and

Fig. 1 Sedimentation curve obtained with Quick-Eye.

the holder is set to tilt manually at 60 degrees from the vertical line. The sedimentation distance is read at the meniscus position of the glass tube at 6 min. for the Westergren 1-hour value and at 9 min. for the Westergren 2-hour value after tilting of the tubes. However, reading of the meniscus is very difficult, and the values obtained are somewhat lower than Westergren values.¹⁵⁾

Details on the functions of these methods are listed in Table 1.

FACTORS INVOLVING ACCELERATION OF BLOOD SEDIMENTATION RATE

Although ESR accelerating factors are already well known

Table 2 Correlation between ESR and blood components.

Accelerator	Latent time	Sedimentation rate
Hemoglobin	<i>0.70</i>	<i>-0.57</i>
Albumin	0.31	-0.47
alpha-1 globulin	-0.49	<i>0.68</i>
alpha-2 globulin	-0.48	<i>0.70</i>
beta globulin	-0.33	0.46
gamma globulin	-0.49	0.54
Total protein	-0.21	n.s.
White cell count	n.s.	0.31
Rheumatoid factor	-0.23	0.44
CRP	-0.41	<i>0.74</i>

Latent time and Sedimentation rate were automatically calculated by a build-in computer in Quick-eye ($N=111$)
The number in each box represents correlation coefficient ($=r$). n.s.; not significant.
Italics show high r -value.

from clinical and rheological considerations, all such findings were obtained by obsolete laboratory testing methods. Recent technology enables more accurate and precise test results. However, there is no study on which factors using recent kinds of reliable laboratory data. Thus, we studied which factors using the Quick-Eye sedimentation curves and sedimentation rate values (Table 2). Complete blood count and blood clinical chemistry data were studied for comparison.

The latent time was strongly influenced by hemoglobin content in red cells ($r = 0.70$), followed by alpha 1 fraction ($r = -0.49$; its largest component is antitrypsin, at 300 mg/dl), alpha 2 fraction ($r = -0.48$; its largest component is haptoglobin, at 540 mg/dl), gamma globulin ($r = -0.49$; its concentration is 800–1,800 mg/dl), and CRP (a component of the gamma globulin fraction, the concentration of which is less than 1 mg/dl in normal condition and increases 10 to 1,000 times in cases of acute inflammation). Although hemoglobin had less effect on the sedimentation rate, sedimentation rate was influenced by CRP, antitrypsin, and haptoglobin. CRP had the strongest effect on acceleration of ESR, while gamma globulin had a less strong effect. Fibrinogen distributing between beta and gamma fractions has been considered a strong accelerator of ESR. In our study, fibrinogen concentration was correlated with 1-hour ESR value with an r -value of only 0.47. The Latent time is affected by hemoglobin content. In other words, hemoglobin content affects rouleaux formation strongly, while the sedimentation rate itself will be affected by combined actions of major acute-phase reactants consisting of alpha 1 fraction, alpha 2 fraction and CRP.

DIFFERENCES BETWEEN ESR AND CRP IN CLINICAL SIGNIFICANCE

ESR has been believed to reflect acute-phase response. However, it has been used for diagnosing and monitoring tuberculosis, a chronic form of inflammation, for many years in Japan.¹⁵⁾ Hypergammaglobulinemia, such as *that in* liver cirrhosis and rheumatoid arthritis^{16), 17)} demonstrates acceleration

Fig. 2 Mechanism of ESR acceleration.

of ESR, although gamma globulin is not an acute-phase reactant. CRP and fibrinogen increase in plasma when acute inflammation occurs, and can accelerate ESR. Red cell count and hematocrit are accelerators, although not acute phase reactants.¹⁸⁾ Thus, ESR accelerators can be divided into 3 groups: those with high content of acute-phase proteins, hyperglobulinemia and anemia.

In our Quick-Eye curve analysis, initial latent time of ESR correlated strongly with hemoglobin, while sedimentation rate correlated strongly with CRP and alpha-1 and alpha-2 globulin fractions (Table 2). Between Westergren 1-hour value and fibrinogen concentration, an r of 0.5517 ($n = 45$) was demonstrated in our experiments, which was not high. Thus, the initial latent time of the curve reflects rouleaux formation rate and zeta potential of red cells, and the sedimentation rate reflects interaction between red cell shape and plasma components.^{19)–21)}

ESR is surely a non-specific and non-diagnostic test, but is very useful in detecting disease conditions and performance status in chronic inflammation.^{22)–26)} Compared with ESR, both CRP and white cell count are the best markers for acute inflammations. Mobilization of white cells and release of CRP is due to acute inflammatory response, while acceleration of ESR is an overall outcome of acute and chronic inflammatory changes and anemia (Fig. 2). The correlation between CRP and white cell count was 0.367 ($n = 248$), but the correlation between CRP and neutrophil count was 0.392 ($n = 248$).²⁷⁾ White cell count determination cannot reflect both CRP and ESR. The half-life of CRP is short, but those of gamma globulin and red cells are long. Acceleration of ESR is often observed in non-inflammatory conditions. Therefore, we should not compare ESR and CRP in the same table.^{22)–29)}

FUTURE OF THE ESR TEST

ESR is a simple, inexpensive and reliable laboratory test that clinician have used for decision-making for over 70 years.^{30)–32)} Despite this fact, many have criticized the test because of its lack of specificity and inadequacy for laboratory automation. CRP is often used as a substitute for ESR. However, the mech-

anism of acceleration of ESR is different from that of elevation of CRP. Since ESR is a comprehensive sign of inflammation and anemia, it should be used correctly and appropriately. Currently, various types of rapid ESR test systems are marketed. After confirming the precision and accuracy of the system you choose, such a conventional test system should be used for health check-up and promotion, referring to the ICSH reference method.

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