

THE ESR TEST: AN OLD TEST WITH NEW CONTENTS

ESR TEST: STARI TEST SA NOVOM NAMENOM

Elisa Piva, Mario Plebani

Department of Laboratory Medicine, Padua University School of Medicine, Padua, Italy

Summary: The erythrocyte sedimentation rate (ESR) remains one of the most widely used laboratory tests. Its clinical usefulness and interpretation are in the monitoring of inflammatory diseases, in particular rheumatoid arthritis, temporal arteritis and polymyalgia rheumatica. At present, the reference method for measuring the ESR proposed by the International Committee for Standardization in Haematology (ICSH) utilizes EDTA-anticoagulated-undiluted blood to perform the test using the method described by Westergren in 1921. Current interest in the methodology focuses on the development of an automated closed system that allows the determination of the sedimentation rate with selected working methods, using a single sample for more than one haematological test, improving the bio-hazardous aspects of the testing procedures. As a consequence, standardization becomes necessary. ESR results should be reliable, despite the increased number of different methods and testing variables. Control materials and External Quality Assurance Schemes are now available, and should be used. In conclusion, innovative techniques may improve the appropriateness and usefulness of ESR in clinical practice, but in addition, they need to guarantee the traceability of results in comparison to the reference method in order to ensure comparability of results among different clinical laboratories.

Keywords: erythrocyte sedimentation rate, reference and selected procedures, quality control, external quality assurance schemes

The »old« ESR test

In 1897, the Polish pathologist Biernacki first announced that »the rate at which red corpuscles

Kratak sadržaj: Brzina sedimentacije eritrocita (ESR) ostaje jedan od najčešće korišćenih laboratorijskih testova. Kliničku primenu i korisnost ovaj test ima u praćenju inflamatornih bolesti, naročito reumatoidnog artritisa, temporalnog arteritisa i reumatske polimijalgije. Referentni metod za merenje ESR predložen od strane Međunarodnog komiteta za standardizaciju u hematologiju koristi punu krv sa antikoagulansom EDTA za izvođenje testa pomoću metode koju je 1921. opisao Westergren. Trenutno zanimanje za metodologiju fokusirano je na razvoj automatizovanih zatvorenih sistema koji omogućavaju određivanje brzine sedimentacije uz odabrane radne metode, koje koriste jedan uzorak za više hematoloških testova i unapređuju bio-hazardne aspekte postupka testiranja. Usled toga, standardizacija postaje neophodna. Rezultati ESR moraju biti pouzdani uprkos povećanom broju različitih metoda i varijabli za testiranje. Danas su dostupni kontrolni materijali i šeme za osiguranje spoljašnjeg kvaliteta i treba ih koristiti. Dakle, inovativne tehnike mogu dalje opravdati korisnost ESR u kliničkoj praksi, ali pored toga moraju garantovati sledivost rezultata u poređenju sa referentnom metodom kako bi se obezbedila uporedivost rezultata između više kliničkih laboratorija.

Ključne reči: brzina sedimentacije eritrocita, referentne i odabrane procedure, kontrola kvaliteta, šeme za osiguranje spoljašnjeg kvaliteta

separated could help identify a patient's disease and that the increased sedimentation rate of blood from ill individuals was due to the presence of fibrinogen« (1). Later, in 1918 the Swedish pathologist and haematologist Robert (Robin) Sanno Fåhræus furthered Biernacki's work, initially using the erythrocyte sedimentation rate (ESR) as a pregnancy test. Along with the first descriptions by Fåhræus, Alf Vilhelm Albertson Westergren, a Swedish internist, developed the test method that is named after him (2–3).

Since the 19th century, the erythrocyte sedimentation rate has become one of the most common

Address for correspondence:

Prof Mario Plebani
Dipartimento Medicina di Laboratorio
Azienda Ospedaliera-Università di Padova
Via Nicolò Giustiniani 2
35128 Padova, Italy
Fax 39-049-663240
e-mail: mario.plebani@unipd.it

laboratory tests. The erythrocyte sedimentation rate (ESR) is the laboratory test that assesses the acute phase response to inflammation and provides clinicians with a useful »sickness index« (4). Over the years, only a few modifications have been made to the original description. For example, the use of sodium citrate-anticoagulated specimens has not been modified. Introduced by Westergren, the manual technique was recommended as the method of reference by the International Council for Standardization in Haematology (ICSH) and the Clinical and Laboratory Standard Institute (CLSI) (5–6). It remains the benchmark against which all new instruments and methodologies should be tested for validation.

The ESR test: the phenomenon

Erythrocyte sedimentation remains a partially understood phenomenon. It is transient, confined to fresh blood. ESR is the measurement of the suspension stability of red blood cells in plasma under specified test conditions. If the descent of the plasma-red cell interface is plotted against time, it forms a typical sigmoid curve with three distinct phases. The initial portion of the curve, the lag phase, reflects red cells rouleau formation. In this phase, there is little sedimentation and the size of the aggregates is dependent on plasma (extrinsic) factors, mainly acute phase proteins and cellular (intrinsic) factors.

Erythrocyte aggregation is critical for the outcome of sedimentation. During the second, decantation phase, the plasma-red cell interface falls more rapidly. During the final phase, the cell aggregates pile up at the bottom of the tube. The two main determinants of ESR are the degree of red cell aggregation and the haematocrit (packed cell volume). The Westergren method evaluates the rate at which erythrocytes settle as measured by the distance that red blood cells fall in a tube within a specific amount of time. The sedimentation time for each phase may vary from patient to patient so the ESR test result will depend on varying contributions from the three phases. This method measures the final contributions from the three phases, i.e. the fall of erythrocytes at 60 minutes (7).

The »new« ESR test

Given that this method estimates the amount of sedimentation at a fixed time-interval, IFCC-IUPAC has deemed the term »sedimentation rate« to be improper and has decided to re-define ESR as »length of sedimentation reaction in blood« (LSRB) (6). This definition recognizes that the Westergren method and its modified versions measure neither the kinetics nor the rate of erythrocyte sedimentation, but only the final phenomenon described by Fåhræus.

The Westergren method is not a »practical« routinary technique; it remains the reference against which all currently marketed systems should be compared. The new millennium has consolidated ESR automation, with an improvement of working analytical techniques, laboratory workflow, biohazard and standardization. The new marketed automated instruments should show good correlation to Westergren reference method as well as advantages like safety for operators, reduced turn-around time and reduced analytic imprecision. However, the variety of methods have increased variation in the reported results making it more difficult to compare results from different clinical laboratories. Thus, there is an obvious need to evaluate all the proposed working methods against the reference in order to document their reliability, accuracy and robustness.

Some automated techniques address other important issues; in particular, they attempt to measure the rate of red cell sedimentation by selecting appropriate time intervals, or by employing multiple readings in a selected phase of the reaction (7–10). Some instruments require anticoagulated blood with sodium citrate, whereas the most recent ones measure ESR from EDTA undiluted samples (reference specimen) (11). The EDTA-anticoagulated sample has several advantages: (i) it preserves the red blood cell morphology, (ii) it does not interfere with mechanisms that lead to erythrocyte sedimentation, (iii) it increases specimen stability, and (iv) it does not incur problems related to sample dilution with sodium citrate, in view of the fact that the ratio between blood and anticoagulant is of great importance and failure to respect the standard may explain specimen rejection in routine practice. The reference range must be estimated, as it is a pre-requisite for reducing the number of false positive results. Reference ranges should be established for sex and for span of ages that are significantly different, as this will allow the appropriate use of the ESR test (12).

Since the ESR was developed using manual and semi-automated methods, the procedure has been judged so simple that it could be performed without quality control (13). According to this view, the ESR was considered a semi-quantitative test instead of a haematological analysis with great clinical importance in different diseases. Accreditation and regulatory requirements have prompted clinical laboratories to adopt internal and external quality control procedures that cannot be ignored. The poor suitability of stabilized materials for ESR measurement is well recognized (14). In 1993 the official documents of ICSH and NCCLS described how patient specimens can be used for quality control; the specimen should be collected in EDTA, have a packed cell volume of 0.35 or less, and an elevated ESR in the range of 15 to 105 mm/h. Before testing, these must be inverted, at least, 16 times (5–6). The primary objective of quality control in the clinical laboratory is to ensure

that the analytical values are sufficiently reliable to be used in the care of patients. In 2002 we described a procedure based on the use of fresh human whole blood for the daily quality control of ESR and we demonstrated that this procedure is reliable and inexpensive. Therefore, we concluded that fresh samples do not represent a useful warning for the drift control or Levey-Jennings plot and »efforts should now be made to assure that adequate control materials and procedures are provided« (14). A number of commercially available quality control materials now exist, but usually these ESR-checks are stabilized whole blood specimens, not suitable for use in all analyzers and should not be considered »commutable« for simulating human whole blood. Getting this data, for TEST1, which assesses the sedimentation rate rather than the final fall of erythrocytes after a fixed time means that available quality control material looks »commutable« for simulating the human whole blood (15). With the development of stable control materials, a new scenario for inter-laboratory proficiency testing and External Quality Assurance programmes becomes feasible, ensuring compliance with accreditation requirements.

Conclusion

While the increasing number of automated techniques have improved analytical precision and accuracy, the open question is whether these technologies have been developed merely to maintain and promote the use of an obsolete and useless test.

ESR has little value as a screening test for hidden diseases. The routine use of ESR without non-evidence-based medical procedure was considered an indicator of the presence of unconscious defensive medicine among hospital internists (16). Indiscriminate requests for ESR in healthy subjects and in

other clinical situations are inappropriate. Other laboratory tests show greater clinical efficiency (e.g., C-reactive protein in the first 24 hours after tissue damage), and the phenomenon of erythrocyte sedimentation is known to be affected by physiological and pathological factors including anaemia or abnormally shaped red cells, independent of acute phase reactants. While some laboratory scientists have described ESR as an »obsolete test«, much evidence has been collected to demonstrate its usefulness in clinical practice (17). Since 1990 ESR is indicated in the diagnosis and therapeutic monitoring of temporal arteritis and polymyalgia rheumatica (18–20). It may be helpful in resolving conflicting clinical evidence in patients with rheumatoid arthritis and in the evaluation and management of patients with specific autoimmune, inflammatory or infectious disorders (e.g. pelvic inflammatory disease, bacterial endocarditis, septic arthritis and osteomyelitis) (21–22). Recently, the relation between erythrocyte sedimentation rate (ESR) and the risk of developing coronary heart disease or fatal cerebrovascular accident was assessed in the Reykjavik Study, while another study has evaluated ESR as a screening tool for the presence of low-grade inflammation in people with atherothrombotic risk factors (23–24).

Continuing development in technologies allows the in-depth investigation of the kinetics of red cell aggregation and biological variables affecting the erythrocyte sedimentation. By improving the clinical appropriateness, ESR will not become an obsolete test for the next future.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Biernacki EF. Samoistna sedymentacja krwi jako naukowa, praktyczno-kliniczna metoda badania. *Gazeta Lekarska*, 1897; 17: 962–96.
2. Fåhræus R. The suspension-stability of the blood. *Acta Med Scand* 1921; 55: 1–228.
3. Westergren A. Studies of the suspension stability of the blood in pulmonary tuberculosis. *Acta Med Scand* 1921; 54: 247–82.
4. Koepke JA. Welcome Innovation in Erythrocyte Sedimentation Testing. *Am J Clin Pathol* 2002; 118: 11–2.
5. International Committee for Standards in Haematology. Recommendations for measurement of erythrocyte sedimentation rate. *J Clin Pathol* 1993; 46: 198–203.
6. NCCLS. Reference and selected procedures for the erythrocyte sedimentation rate (ESR) test; approved standard, 4th ed. H2-A4. Wayne, PA: NCCLS, 2000.
7. Pure and Applied Chemistry; Properties and units in general chemistry. IUPAC, 2000; 72: 945.
8. Plebani M, De Toni S, Sanzari MC, Bernardi D, Stokreiter E. The TEST 1 automated system: a new method for measuring the erythrocyte sedimentation rate. *Am J Clin Pathol* 1998; 110: 334–40.
9. Happe MR, Battafarano DF, Dooley DP, Rennie TA, Murphy FT, Casey TJ, et al. Validation of the Diesse Mini-Ves erythrocyte sedimentation rate (ESR) analyzer using the Westergren ESR method in patients with systemic inflammatory conditions. *Am J Clin Pathol* 2002; 118: 14–7.

10. Romero A, Munoz M, Ramirez G. Length of sedimentation reaction in blood: a comparison of the test1 ESR system with the ICSH reference method and the sedisystem 15. *Clin Chem Lab Med* 2003; 41: 232–7.
11. Ajubi NE, Bakker AJ, Van den Berg GA. Determination of the length of sedimentation reaction in blood using the TEST 1 system: comparison with the Sedimatic 100 method, turbidimetric fibrinogen levels, and the influence of M-proteins. *Clin Chem Lab Med* 2006; 44: 904–6.
12. Piva E, Sanzari MC, Servidio G, Plebani M. Length of sedimentation reaction in undiluted blood (erythrocyte sedimentation rate): variation with sex and age and reference limits. *Clin Chem Lab Med* 2001; 39: 451–4.
13. Piva E, Fassina P, Plebani M. Determination of the length of sedimentation reaction (erythrocyte sedimentation rate) in non-anticoagulated blood with the Microtest 1. *Clin Chem Lab Med* 2002; 40: 713–7.
14. Bull BS. Quality assurance strategies. In: Koepke JA. Ed. *Practical Laboratory Hematology*. New York, NY: Churchill Livingstone; 1991.
15. Piva E, Pajola R, Temporin V, Plebani M. A new turbidimetric standard to improve the quality assurance of the erythrocyte sedimentation rate measurement. *Clin Biochem* 2007; 40: 491–5.
16. Plebani M, Piva E. Erythrocyte sedimentation rate: use of fresh blood for quality control. *Am J Clin Pathol* 2002 Apr; 117: 621–6.
17. Asher E, Parag Y, Zeller L, Yerushalmi R, Reuven H. Unconscious defensive medicine: the case of erythrocyte sedimentation rate. *E J Int Med* 2007; 18: 35–8.
18. Plebani M. Erythrocyte sedimentation rate: innovative techniques for an obsolete test? *Clin Chem Lab Med* 2003; 41: 115–16.
19. Hunder GG, Bloch DA, Michel BA. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum* 1990; 33: 1122–8.
20. Salvarani C, Cantini F, Boiardi L, Hunder GG. Poly-myalgia reumatica and giant-cell arteritis. *N Engl J Med* 2002; 347: 261–71.
21. Salvarani C, Hunder GG. Giant cell arteritis with low erythrocyte sedimentation rate. *Arthritis Care Res* 2001; 45: 140–5.
22. Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. *Arthritis Rheum* 2001; 44: 1736–43.
23. Andresdottir MB, Sigfusson N, Sigvaldason H, Gudnason V. Erythrocyte sedimentation rate, an independent predictor of coronary heart disease in men and women: the Reykjavik Study. *Am J Epidemiol* 2003; 158: 844–51.
24. Steinvil A, Shapira I, Arbel Y, Justo D, Berliner S, Rogowski O. Determinants of the erythrocyte sedimentation rate in the era of microinflammation excluding subjects with elevated C-reactive protein levels. *Am J Clin Pathol* 2008; 129: 486–91.

Received: July 6, 2010

Accepted: July 14, 2010