ORIGINAL RESEARCH PAPER

Volume-6 | Issue-10 | October-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ERYTHROCYTE SEDIMENTATION RATE: DIAGNOSTIC RELIABILITY, STABILITY AND STORAGE OF THE SAMPLES

Science

R. Mihailov Medical laboratory RAMUS LTD, Medical University Sofia, MK Filaretova, Sofia

B. Pencheva Medical laboratory RAMUS LTD

St. Zlateva Medical laboratory RAMUS LTD

ABSTRACT

Erythrocyte sedimentation rate (ESR) is a very old and widely used test in a number of infectious, neoplastic, autoimmune and other diseases. In the literature, the argue on the use of ESR and its replacement with more advanced biomarkers, such as the C-reactive protein (CRP), which are less affected by gender, age, protein and cellular blood factors, is still going on. In practice, the combination of both biomarkers is still in use. The question of sample stability during transportation and storage is also discussed. We studied 100 controls, each with 3 blood samples, stored in a refrigerator at 2-8°C, which were analyzed at 1, 2, 6, 12 and 24 hours. The obtained results show ESR sample stability by 24 hours.

KEYWORDS

ESR, CRP, sample stability

Introduction:

Erythrocyte sedimentation rate (ESR) is a very old, widely used, relatively simple, inexpensive, non-specific test that can help in the diagnosis of infections, neoplasms, autoimmune diseases and is still a subject of on-going research (1, 2, 3). There are many questions about this test, due to its relatively low specificity. Is it not the time for replacing the ESR with newer, automated and standardized tests, such as the CRP? The standardization of ESR (external and internal control) and the extent to which the test result is influenced by the temperature, sample storage time, tube inclination, anticoagulant type, anticoagulant/blood ratio, result interpretation within 60+1 minutes, etc., are also discussed. The effect of plasma and cellular blood components on ESR results is still under monitoring (2, 3, 4, 5).

Material and methods:

100 controls, 50 men and 50 women, aged 18-65 years, were studied. Three parallel samples of 2 ml venous blood were obtained from each individual by using vacutainer blood collection tubes. Each sample was studied at 1, 2, 6, 12, and 24 hours. After 2 hours of sampling, the samples were stored in a refrigerator at 2-8°C. The mean value of the three measurements in each of the periods was determined. A total of 1,500 analyzes were performed. The estimation of the samples was performed within 60+1 minutes by two independent investigators. The reference sample was obtained manually by using the Westergren method.

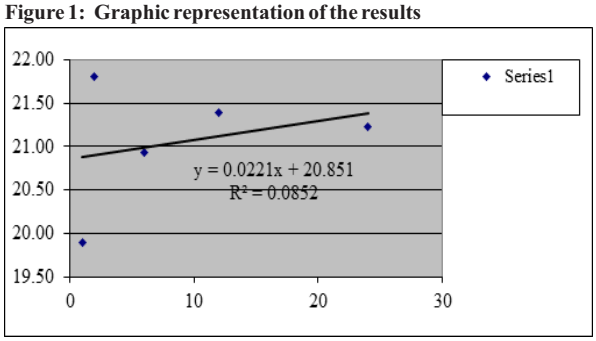
Results:

The results are presented in Table 1 and on Figure 1. It can be seen that the obtained results do not differ significantly between the different periods. According to our data, the ESR sample stability is retained by 24 hours.

Table 1:

Period/Hours	1 hour	2 hours	6 hours	12 hours	24 hours
Mean value of all measurements in this period	19.90	21.81	20.94	21.39	21.22
Number of tests	300	300	300	300	300

Figure 1: Graphic representation of the results



The calculated total mean value is 20.46.

The mean standard deviation for all measurements is 5.96.

Discussion:

Erythrocyte sedimentation rate (ESR) is the oldest laboratory test, suggested in 1897 by the Polish scientist E. Biernacki. In 1918, the Swedish pathologist Robert Fåhræus demonstrated a similar test and, together with A. Westergren, suggested it for diagnostic use mainly in England (1-6). Later in 1921, Westergren suggested sodium citrate as an anticoagulant and the test was introduced in many countries in Europe and the world as the Westergren method (1-6). This method is influenced by a number of factors, such as age, gender, blood/citrate ratio, vibration, temperature, light, and tube inclination. The ESR increases in inflammatory conditions, pregnancy, anemia, autoimmune diseases (rheumatoid arthritis and lupus erythematosus), infections, certain kidney diseases and some neoplasms (e.g. lymphoma and multiple myeloma), mild inflammation of the bones (1, 6, 7, 11).

The ESR decreases in polycythemia, hyperviscosity, sickle-cell anemia, leukemia, hypoproteinemia (due to hepatic or renal disease) and congestive heart failure (3, 6, 7, 11). Very high ESR usually occurs with severe infection, increased globulin levels (myeloma), rheumatic polymyalgia or temporal arteritis. People with multiple myeloma or Waldenstrom's macroglobulinemia usually have a three-digit ESR (6, 7, 8). Some medications, such as Dextran, Methyldopa, oral contraceptives, Penicillamine Procainamide, Theophylline and Vitamin A, may increase the ESR, while Aspirin, Cortisone and Quinine may reduce it (5, 8, 9). Nowadays, there are a large number of modifications of the Westergren method, both manual and automatic (7, 9-13). New studies continue to seek the optimum conditions for obtaining reliable results (1, 3, 5, 13). The role of plasma and cellular factors, the development of a control material (a fresh sample) and the optimal time for analysis are discussed. The method, recommended by the International Council for Standardization in Haematology (*ICSH*) and the National Committee for Clinical Laboratory Standards, is the Westergren's traditional method with EDTA as an anticoagulant, as well as non-dilution samples (15, 16, 17).

There are two main factors that may influence the aggregation process: high molecular weight components in the plasma and erythrocyte structure (shape, size, and number). Typically, the erythrocytes are negatively charged and repel each other. Many plasma proteins have positive charges and neutralize the superficial charges of the erythrocytes, thus stimulating aggregation. The relative impact of plasma proteins on aggregation in a 10-point scale is, as follows: 10 for fibrinogen, 5 for beta-globulins, 2 for alpha-globulins, 2 for gamma-globulins and 1 for albumin (12). On the other hand, the ESR is directly proportional to the mass of erythrocytes, but reversely correlated with their surface area and number. Macrocytes precipitate faster than normal cells and microcytes. It is known that the ESR reaction occurs in three stages, i.e. aggregation, precipitation and packing (7, 13, 14): a) the first stage occurring within the first 10 minutes is the formation of

cellular rouleaux by agglomeration, mainly of erythrocytes that precipitate faster due to their density; b) the second stage occurring in the next 40 minutes is the sedimentation of the formed erythrocyte rouleaux; and c/ the third stage occurring in the last 10 minutes is the packing of cellular rouleaux at the bottom of the tube.

In the literature, it is still argued on whether the ESR should be replaced by newer, automated and standardized biomarkers, such as the CRP, RF, antinuclear antibodies. Some authors prefer the CRP, due to its earlier positivation and non-inversion by gender, age, pregnancy, temperature, plasma protein concentration and erythrocyte factors (1, 3,14). The ESR is more useful than CRP for diagnosing and monitoring of low-grade bone and joint infections and for monitoring of systemic autoimmune diseases. There are also authors who advocate the use of the two biomarkers to achieve higher sensitivity (>90%), for example in septic arthritis, and because of the discrepancy in their variations, reaching 2.5-12.5% in certain conditions, such as some non-infectious diseases, pelvic infections, renal insufficiency, hypoalbuminemia and rheumatoid arthritis (1, 3, 8, 15, 16).

Feldman et al. (2) examined the two biomarkers in 1,731 adult patients and found inconsistent results in 1 out of 8 patients: high CRP with non-increased ESR, mainly in some infections and vice versa, high ESR and low CRP in mild infections of the bones, joints and lupus erythematosus. In 2011, Hariharan and Kabrhel (10) monitored the ESR and CRP in 167 adult patients with septic arthritis and found sensitivity of 98% and 92%, respectively. According to the report of Litao and Kamat in 2014 (14), ESR is more helpful in monitoring chronic inflammatory conditions, whereas CRP is more useful in diagnosing and monitoring acute inflammatory processes (14). In 2007, Greidanus et al. found 93% sensitivity and 83% specificity of ESR in 145 patients with knee arthroplasty. In 2011, Hariharan and Kabrhel recorded ESR sensitivity >90% in 167 patients with septic arthritis. In 2012, Kermani et al. (22) found 86.9% sensitivity in 764 patients with giant cell arteritis. Also in 2012, Costa et al. (23) found 89% sensitivity and 69% specificity of ESR in 77 patients with periprosthetic femoral infections.

Another important question is the duration of the time interval after obtaining the blood within which the sample should be examined, because the protein and cell components involved in the ESR reaction may undergo changes at room temperature and in the refrigerator. Literature data on this issue are not unidirectional (16, 17). In 2004, Hamid (16) examined ESR in 50 healthy adult controls (28 males and 22 females) using EDTA and sodium citrate. Samples were tested immediately and at 24 hours stored at 4°C, and the resulting differences in the results were negligible. In 2002, Plebani and Piva (17) examined fresh samples at 24 hours stored at 4°C and found no significant differences. It is assumed that the sooner the blood is taken, the more optimal the results will be. In many cases, however, this has to be done after a certain period of time, even hours. Existing standards, including that for a clinical laboratory, suggest sample storage at 2-8°C, usually from 2 to 72 hours. The literature data obtained so far has shown that optimal results are obtained (18) a) when the sample is stored at room temperature (18-30°C), when the analysis is performed up to 2, 4, 6 and 8 hours, if the blood is taken with a vacutainer tube with a purple cap and up to 12 hours with a vacutainer tube with a black cap; b) upon storing the sample in a refrigerator (2-10°C), good results are obtained up to 12, 24, 48, 72, 124 and 144 hours (18). erence values also show some differences depending on the method used (4, 5, 13). In 1996, Wetteland et al. (4) studied 3,910 healthy controls. The mean ESR values for men were about 3 mm/h at 20 years, 6 mm/h at 55 years and 10 mm/h at 90 years and 6, 9 and 11 mm/h for women, respectively. The upper reference values were 12, 14 and 19 mm for men and 18, 21 and 23 mm/l for women. In 2016, Giri (13) suggested reference values of 0-9 mm/h for men and 0-20 mm/h for women. The most frequently referenced values are (19-24) <15 mm/h for men, aged up to 50 years and <20 mm/h for men over 50 years and <20 mm/h and <30 mm/h for women, respectively (22, 23, 24). We monitored the ESR stability while storing the samples in vacutainer tubes in a refrigerator (2-8°C) by 24 hours. The results show stability by 24 hours with a negligible difference ($p>0.05$) between the hours. Similar results are reported by other authors (16, 17, 18). Our results are in support of the argument that ESR samples can be transported to central units, without allowing errors in the good results. Undoubtedly, in cases of strongly altered plasma and cellular factors, the ESR monitoring over the time may show other results.

Conclusions:

Literature and our studies show that very good ESR results are obtained by using the Westergren method, when storing the sample in a refrigerator (2-8°C) for 24 hours.

References:

1. Harrison M. Erythrocyte sedimentation rate and C-reactive protein. Aust Prescr. 2015 Jun, 38, N 3, 93–94. Published online 2015 Jun 1. doi: 10.18773/austprescr.2015.034PMCID: PMC4653962.
2. Feldman M1, Aziz B, Kang GN, et al. C-reactive protein and erythrocyte sedimentation rate discordance: frequency and causes in adults. Transl Res. 2013 Jan,161, N 1, 37-43. doi: 10.1016/j.trsl.2012.07.006. Epub 2012 Aug 23.
3. Singh G. C-reactive protein and erythrocyte sedimentation rate: Continuing role for erythrocyte sedimentation rate. Advances in Biological Chemistry, 2014, 4, No.1, 5-9. Article ID: 42706, 5 pages DOI: 10.4236/abc.2014.41002.
4. Wetteland P, Røger M, Solberg HE. Population-based erythrocyte sedimentation rates in 3910 subjectively healthy Norwegian adults. A statistical study based on men and women from the Oslo area. J. Intern. Med. 240 (3): 125–31. doi: 10.1046/j.1365-2796.1996.30295851000.x. PMID 8862121.
5. AACC Lab Tests Online . ESR. October 29, 2015.
6. McCabe BH. Brief History of theErythrocyte Sedimentation Rate. LAB MEDICINE, 16, 1985, N 3, 177-179.
7. Zhbanov A, Yang C. Effects of Aggregation on Blood Sedimentation and Conductivity. PLoS ONE 10, 2015, N 6, https://doi.org/10.1371/ journal.pone.0129337
8. Reitzenstein JE, Yamamoto LG, Mavoori H. Similar erythrocyte sedimentation rate and C-reactive protein sensitivities at the onset of septic arthritis, osteomyelitis, acute rheumatic fever. Pediatr Rep, 2, 2010, N 1, e10. Published online 2010 Jun 18.doi: 10.4081/pr.2010.e10
9. Erythrocyte Sedimentation Rate (ESR) - Health Facts, www.ndhealthfacts.org/wiki/Erythrocyte_Sedimentation_Rate_(ESR), Mar 24, 2014.
10. Hariharan P, Kabrhel C. Sensitivity of erythrocyte sedimentation rate and C-reactive protein for the exclusion of septic arthritis in emergency department patients. J Emerg Med. 2011 Apr, 40, N 4, 428-31. doi: 10.1016/j.jemermed.2010.05.029. Epub 2010 Jul 22.
11. Subramanian A, Rangarajan K, Pandey R, et al. Evaluation of an automated erythrocyte sedimentation rate analyzer as compared to the Westergren manual method in measurement of erythrocyte sedimentation rate. Indian J Pathol MIcrobiol, 54, 2011, N 1, 70-74.
12. Hashemi R, Majidi A,Motamed H, et al. Erythrocyte Sedimentation Rate Measurement Using as a Rapid Alternative to the Westergren Method. Emerg (Tehran), 3, 2015, N 2, 50–53.
13. Giri D. Erythrocyte Sedimentation Rate (ESR): Principle, Methods of Determination and Clinical Significance. Hematology, Immunology, January 11, 2016.
14. Litao MKS, Kamat D. Erythrocyte Sedimentation Rate and C-reactive Protein: How Best to Use Them in Clinical Practice. Pediatric Annals, 43, 2014, N 10, 417-420. Posted October 1, 2014. DOI: 10.3928/00904481-20140924-10.
15. Bray C, Lauren N. Bell LN, et al. Sedimentation Rate and C-reactive Protein Measurements and Their Relevance in Clinical Medicine, 15, 2016, N 6, 317-322.
16. Hamid GA. Effect of Blood Storage on ESR by using EDTA and Citrate Anticoagulant J Natural Applied Sciences, 8,2004, N 3, 565-568.
17. Plebani M, Piva E. Erythrocyte sedimentation rate: use of fresh blood for quality control.Am J Clin Pathol, 117, 2002 N 40, 621-626.
18. Dorion RP. Sedimentation Rate. Geisinger Medical Laboratories Review Date:01/04/2017.
19. Markanday A. Acute Phase Reactants in Infections: Evidence-Based Review and a Guide for Clinicians. Open Forum Infect Dis, 2, 2015 N 3, ofv098.
20. Kellner CP, Wheeler TM. Erythrocyte Sedimentation Rate. Medscape. Updated: Aug 01, 2014.
21. Greidanus NV, Masri BA, Garbuz DS, et al. Use of erythrocyte sedimentation rate and C-reactive protein level to diagnose infection before revision total knee arthroplasty. A prospective evaluation. J Bone Joint Surg Am, 89, 2007, N 7, 1409-1416.
22. Kermani TA, Schmidt J, Cynthia S. Utility of Erythrocyte Sedimentation Rate and C-reactive Protein for the Diagnosis of Giant Cell Arteritis. Semin Arthritis Rheum, 41, 2012, N 6, 866–871.
23. Costa CR, Johnson AJ, Naziri Q, et al. Efficacy of erythrocyte sedimentation rate and C-reactive protein level in determining periprosthetic hip infections. Am J Orthop (Belle Mead NJ), 41, 2012 N 4, 160-165.
24. Pisetsky DS. Laboratory testing in the rheumatic diseases. In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine. 25th ed. Philadelphia, PA: Elsevier Saunders; 2016:chap 257.