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

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 argument 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, etc., 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:

<table>
<thead>
<tr>
<th>Period/Hours</th>
<th>1 hour</th>
<th>6 hours</th>
<th>12 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value of all measurements in this period</td>
<td>19.90</td>
<td>21.81</td>
<td>20.94</td>
<td>21.39</td>
</tr>
<tr>
<td>Number of tests</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
</tbody>
</table>

Figure 1: Graphic representation of the results

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 Fähræus 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, hypoproteineinemia (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 polyarthritis or temporal arteritis. People with multiple myeloma or Waldenström'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. Macrocies 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 recognition 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% in certain conditions, such as some non-infectious diseases, pelvic infections, renal insufficiency, hypalbuminemia 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 Kabrehl (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 2006, Greidanus et al. found 93% sensitivity and 83% specificity of ESR in 145 patients with knee arthroplasty. In 2011, Hariharan and Kabrehl 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 arthritis. 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. 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 reaching 2.5-12% in certain conditions, such as some non-infectious diseases, pelvic infections, renal insufficiency, hypalbuminemia and rheumatoid arthritis (1, 3, 8, 15, 16).

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:
16. 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.