

SEED HAEMATOLOGY



The 'erythrocyte sedimentation rate': a well-timed indicator of clinical value

The determination of the 'erythrocyte sedimentation rate' (ESR) is a commonly performed laboratory test with a traditional role. It is a sensitive, non-specific marker of inflammation. The ESR is used as a marker of the 'general physical condition' [1], in combination with the patient's clinical history and physical examination, and can serve to aid diagnosis, management and follow-up of different autoimmune diseases, acute and chronic infections and tumours [2].

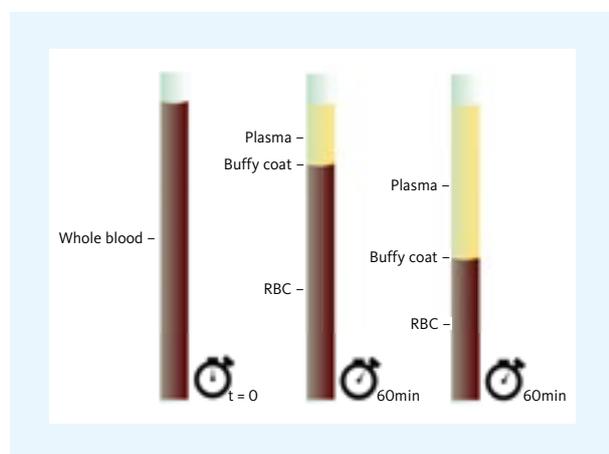


Fig. 1 Determining the ESR [1]:
a. The diluted sample is aspirated and transferred to the Westergren tube.
b. A normal ESR reading after 60 minutes; < 20 mm plasma.
c. An elevated ESR result after 60 minutes; > 25 mm plasma.

The ESR measures the distance that red blood cells have dropped, in a defined period of time, in a vertical column of diluted anticoagulated blood under the influence of gravity [2], as shown in Fig. 1.

The first discovery of the ESR

The discoverer of the ESR was a Polish physician, Edmund Faustyn Biernacki, and he announced the discovery in 1897 in two articles simultaneously (in Polish in the 'Gazeta Lekarska' and in German in the 'Deutsche Medizinische Wochenschrift'). In June 1897 (during a meeting of the Warsaw Medical Society) Biernacki presented the five most important conclusions from his observations, which clearly showed the clinical significance of the ESR. These five conclusions were [3]:

1. The blood sedimentation rate is different in different individuals.
2. Blood with small amounts of blood cells sediments faster.
3. The blood sedimentation rate depends on the level of plasma fibrinogen.
4. In febrile diseases (including rheumatic fever) with high levels of plasma fibrinogen the ESR is increased.
5. In defibrinated blood the sedimentation process is slower.

In 1906, Biernacki modified his method using a capillary pipette of his own design, called 'microsedimentator', instead of the 20-mm-high cylinder originally used. This technique allowed the determination of the ESR after sampling capillary blood from a fingertip. As an anticoagulant, he used a solution of sodium oxalate. Seven years after Biernacki's death, Fåhræus used the ESR as a possible pregnancy test since he analysed the time differences of red blood cell sedimentation in two groups of women: pregnant and not pregnant.

Another scientist involved in the ESR was the Swedish internist Alf Vilhelm Albertsson Westergren. Based on observations of the sedimentation of blood obtained from patients with pulmonary tuberculosis, Westergren presented a similar description of the phenomenon of red blood cell sedimentation to those given by Biernacki and Fåhræus. Westergren applied a blood sampling method to the ESR test using sodium citrate as anticoagulant. Westergren also defined standards for the ESR test.

The mechanism of red blood cell aggregation and sedimentation [1, 4]

Red blood cell sedimentation is governed by factors that stimulate or inhibit red blood cell aggregation and sedimentation. Normal red blood cells are negatively charged as shown in Fig. 2 and repel each other, which limits the sedimentation rate. Large clumps fall faster than small ones, so factors that increase aggregation will increase sedimentation. Red blood cells usually aggregate into clumps that resemble a stack of coins, which is called 'rouleaux formation'.

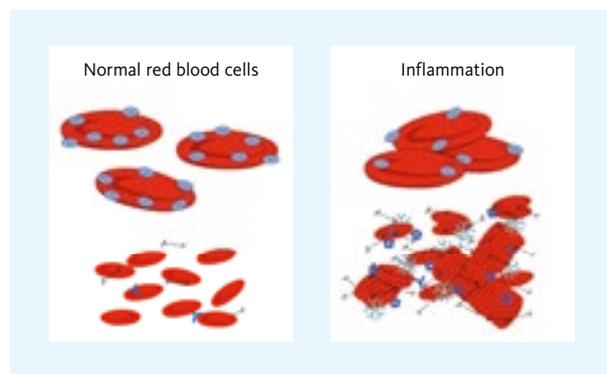


Fig. 2 Red blood cell behaviour
Normal: Negatively charged red blood cells; low sedimentation rate.
Inflammation: Less negatively charged red blood cells; sedimentation occurs, stimulated by all the different factors that increase rouleaux formation (fibrinogen, CRP, immunoglobulin).

The sedimentation process can be divided into three stages as shown in Fig. 3 :

A. Lag stage – rouleaux formation (0 – 20 min)

Red blood cells start to aggregate and form rouleaux. The presence of acute phase proteins encourages rouleaux formation. During this phase, no sedimentation occurs.

B. Decantation stage – sedimentation (15 – 30 min)

Red blood cell aggregates sink to the bottom under the influence of gravity at a constant rate. Large aggregates drop faster than small aggregates or single cells. Falling aggregates induce an upward plasma current that slows down sedimentation.

C. Packing stage (25 – 60 min)

The rate of sedimentation slows down to zero and cells start to pack in the bottom of the tube.



Fig. 3 Rouleaux formation: at low or no flow condition, red blood cells adhere side to side and form stacks called 'rouleaux', followed by end-to-end connections creating 3D aggregates.

The ESR test requires a standardised procedure [5]

■ Blood collection

Obtain a non-haemolysed blood specimen by venepuncture. Immediately mix thoroughly with EDTA anticoagulant. Examine the specimen for the presence of small clots that could invalidate the test results and cause specimen rejection.

■ When to test

Stable ESR test results after collection and storage of up to 24 hours at 4 °C have been reported. Where possible, testing should be started within four hours after collection if the specimen was held at room temperature. If longer storage is required, testing can be started up to 24 hours later if specimens are kept refrigerated and returned again to room temperature for 15 minutes before testing.

■ Specimen preparation

Mixing the blood specimen is critically important for reproducible results. For standard tubes (10 – 13 x 75 mm containing 5 mL of blood and with an air bubble comprising at least 20% of the tube volume), there should be a minimum of 12 complete inversions with the air bubble travelling from end to end of the tube. Non-standard tubes,

particularly when narrower, may require more than 12 inversions; the required number of inversions should be determined. Mixing should be completed just before the ESR pipette is filled to start the test. Specimens stored under refrigerated conditions should be allowed to return to room temperature for at least 15 minutes before mixing and starting the test.

■ Preparing the blood cell suspension

Before transferring the blood to the Westergren pipette, dilute an aliquot of the EDTA sample at a ratio of 4:1 with sterile trisodium citrate dehydrate (blood diluent) that has a concentration of 100 to 136 mmol/L. Physiological saline is also an acceptable diluent, using the same 4:1 ratio.

■ Handling of the pipette

Using a mechanical suction device, aspirate the diluted, bubble-free sample into a clean and dry Westergren pipette, filling it exactly to the '0' mark. Place the filled pipette in a vertical position, at 18 to 25 °C, in an area free from vibration, draught and direct sunlight.

■ Reading of the test

At 60 ± 1 minute, read the distance, in millimetres, from the bottom of the plasma meniscus to the top of the column of sedimented red blood cells. Be careful not to include any white blood cells (buffy coat) in the red cell column. Record the numerical value. (Infrequently, the plasma / red cell interface is so blurred that it is unreadable. The cause for this is unknown).

■ Reporting of the test results

Express results as the distance – in millimetres – the red cell column has sunken / dropped from the top after one hour. Only results obtained for the time interval of 60 min are acceptable. Record the test results as the ESR, 1 hour = x mm. Note that reporting an ESR result in this manner emphasises the fact that this test measures a distance after a specific time interval has elapsed.

The ESR test: demands on materials [5]

The pipette (tube) should be colourless and of sufficient length of at least 200 mm of red cell sedimentation distance, as is afforded by the original 300-mm-Westergren-type glass pipettes. A sedimentation scale may be marked on the pipette or adjacently and should comprise clearly marked lines in divisions of 1 mm up to 200 mm from the bottom of the pipette. If the scale is separated from the pipette, the scale must be part of a pipette-holding device that ensures precise and reproducible alignment of the pipette and the scale. If the reading of the pipette is optoelectronic, rather than visual, a marked scale is not necessary.

The diameter of sedimentation pipettes for the Westergren ESR is recommended not to be less than 2.55 mm (no upper limit is specified, except that the volume of the blood required should be minimised). The diameter should be constant (within 5%) throughout the length of the pipette with its interior being cylindrical (absolute variation of diameter not exceeding 0.1 mm). Glass (reusable) or plastic pipettes may be used. Plastic pipettes should not show adhesive properties toward blood cells and should not release plasticisers that alter the sedimentation. After use, reusable pipettes must be washed free of blood in running cold water, soaked in disinfectant for one hour, rinsed thoroughly in distilled water and dried in an incubator at 37 °C for one hour.

During the test, the pipettes must be held motionless in a vertical position. This can be accomplished by the use of a rack or stand equipped with an accurate levelling device to ensure that the tubes remain within ± 2 degrees of the vertical.

The ESR test: selected routine method

At present, the working method recommended is the same as the standardised method [5]. However, other procedures including those based on automated analysers are acceptable as working methods.

Clinical interpretation of the ESR

The ESR is a sensitive, non-specific marker of inflammation and is, in combination with clinical history and physical examination, being used as a marker of the 'general physical condition' [6]. There is a linear correlation between fibrinogen levels in blood and ESR readings, so any condition that increases fibrinogen levels, increases the ESR.

Rheumatoid arthritis (RA) and other autoimmune conditions

Rheumatoid arthritis is a chronic inflammatory condition of unknown aetiology whereby autoimmune destruction of the joints occurs usually in a symmetric fashion. The American College of Rheumatology (ACR) has established criteria [7] for the diagnosis of RA. The ESR can aid in the diagnosis of RA, but it cannot be used solely for diagnosing RA. It is very useful when used with other parameters as outlined in the ACR guidelines, in the diagnosis and follow-up of RA patients. The ESR is also helpful in the follow-up of systemic lupus erythematosus (SLE), but of questionable value, if any, in inflammatory myopathy or spondyloarthropathy.

Temporal arteritis and polymyalgia rheumatica (PMR)

Traditionally, the ESR is almost always elevated in both temporal arteritis and polymyalgia rheumatica. In temporal arteritis it may exceed 100 mm/hr. However, it was emphasised that a normal ESR in patients with symptoms suggestive of either temporal arteritis or polymyalgia rheumatica or both should not rule out the diagnosis. Fortunately, only a minority of patients have a normal ESR. This does not diminish the value of the ESR in the diagnosis and follow-up of these patients. It is important, however, to emphasise that if clinical features of temporal arteritis are present, such as headache with jaw claudication, a temporal artery biopsy is highly recommended even if the ESR is not elevated.

Multiple myeloma and other paraproteinaemia conditions

The importance of the ESR parallels that of plasma viscosity in these conditions. While an increased ESR is helpful in suspecting these conditions, the diagnosis depends on criteria such as monoclonal spike or serum electrophoresis, marrow plasmacytosis, and lytic bone lesions. However, the ESR should not be the main distinguishing feature, but the proportion of plasma cells in the bone marrow. A bone marrow plasmacytosis of 20% or more is more predictive of multiple myeloma than the ESR.

Other uses

Clinical studies, often small studies, have suggested possible relevance of ESR levels in different conditions, e.g. bacterial otitis media, acute haematogenous osteomyelitis in children, sickle cell disease, pelvic inflammatory disease, IV drug users with fever, prostate cancer, coronary artery disease and stroke.

An extreme elevation of the ESR, defined as >100 mm/hr, is indicative of a serious underlying disease, most notably infection, collagen vascular disease, metastatic malignant tumours or renal disease. In most cases, the underlying condition is clinically apparent. In < 2% of patients with an extremely elevated ESR, no obvious cause can be found, but the underlying cause can usually be found in combination with the clinical history, physical examination and other standard laboratory tests.

Reference values [5]

Reference values should be established locally in accordance with recommendations on reference values [8]. In view of the progressive rise in ESR with age, separate values should be established for each decade of adult life in men and women. Several other variables influence the ESR and may thus affect reference values, e.g. haemoglobin (HGB) level, medication, menstrual cycle, pregnancy and smoking. Table 1 lists reference values for the ESR, which can be used as a guide for establishing a local set of reference values.

Table 1 (Mean) reference values for the Westergren ESR method (mm/hr)

| Age (years) | Male | Female | Upper limit of male | Upper limit of female |
|-------------|------|--------|---------------------|-----------------------|
| 18–30 | 3.1 | 5.1 | <7.1 | <10.7 |
| 31–40 | 3.4 | 5.6 | <7.8 | <11.0 |
| 41–50 | 4.6 | 6.2 | <10.6 | <13.2 |
| 51–60 | 5.6 | 9.4 | <12.2 | <18.6 |
| 61–70 | 5.6 | 9.4 | <12.7 | <20.2 |
| Over 70 | 5.6 | 10.1 | <30 | <35 |

Physiological and clinical factors that decrease the ESR [1]

Polycythaemia is characterised by an increased proportion of red blood cells in the blood, which artificially lowers the ESR. Polycythaemia can be caused by increased numbers of red blood cells or by a decrease in plasma volume. Red blood cell abnormalities can affect aggregation, rouleaux formation and sedimentation rate. Red blood cells with irregular or small shapes tend to settle slower and decrease the ESR. A decrease in plasma proteins, especially of fibrinogen and paraproteins, decreases the ESR.

Physiological and clinical factors that increase the ESR

ESR values are higher for women than for men and increase progressively with age [1]. Pregnancy also increases the ESR. During acute phase reactions, macromolecular plasma proteins, particularly fibrinogen, are produced and decrease the negative charges of red blood cells and the repulsion between them, thereby encouraging rouleaux formation. Paraproteins are positively charged molecules that are abundantly present in patients with multiple myeloma and Waldenström's macroglobulinaemia. Like fibrinogen, paraproteins decrease the negative charges of red blood cells and the repulsion between them, which increases rouleaux formation.

High protein concentrations increase plasma viscosity, which slows down the sedimentation rate and thus the ESR. However, the effects of fibrinogen and paraproteins on the negative charges of red blood cells, reducing repulsion between them and promoting rouleaux formation, far outweigh the effect of increased plasma viscosity, resulting in a strong net increase of the ESR.

In anaemia, red blood cell counts are reduced, which increases rouleaux formation. In addition, the reduced haematocrit (HCT) affects the velocity of the upward plasma current so that red blood cell aggregates sediment faster. In macrocytosis, red blood cells are changed into a shape with a small surface-to-volume ratio, which leads to a higher sedimentation rate.

Mechanical sources of error

ESR values might falsely appear higher or lower also due to mechanical reasons [9]:

- If the concentration of EDTA is greater than recommended, the ESR will be falsely lowered.
- The anticoagulants sodium or potassium oxalate and heparin cause the red blood cells to shrink and the ESR will be falsely elevated.
- If the ESR is left to stand for more than 60 minutes, the results will be falsely elevated. If the test result is read after less than 60 minutes, invalidly low values will be obtained.
- A marked increase (or decrease) in room temperature leads to increased (or decreased) ESR results.
- Tilting of the ESR tube increases the ESR.
- Bubbles in the blood cause invalid results.
- Fibrin clots present in the blood invalidate the test results.

Technical innovations for erythrocyte sedimentation rate testing

In recent years, a variety of sedimentation rate test methods were developed that improve the practicability of the original Westergren method and reduce its biohazard. Automated instruments are now available that shorten the testing time and improve the analytical turnaround time [1].

In the original Westergren method, the ESR is read after 60 minutes, which puts practical limitations on the workflow in clinical laboratories. A laboratory investigation comparing the Westergren ESR method readings of a wide range of blood samples at 30 and 60 minutes showed that 30-minute

ESR readings correlate highly with the corresponding 60-minute ESR readings over a wide range of blood samples (correlation coefficient = 0.984). This means an ESR reading after 30 minutes can reliably be extrapolated to the corresponding ESR reading at 60 minutes.

The Starrsed ESR analysers from RR Mechatronics are automated ESR analysers that use the reference Westergren method as recommended by the CLSI. Starrsed analysers perform fully automated ESR measurements in 30 or 60 minutes.

Pre-mixing, sampling and dilution of standard whole blood EDTA samples in sodium citrate is fully automated, which ensures accuracy and frees up time for the operator, who only needs to load the samples into the analyser. The analyser contains a built-in barcode reader that automatically identifies and registers the correct blood samples. Starrsed analysers use a specifically designed needle for sampling that minimises damage to the septum and ensures that blood vials can be sampled reliably multiple times.

A correct placement of a Starrsed analyser guarantees a vertical position, a vibration-free environment and shielding from sunlight and draught. The Starrsed analysers use infrared light to read the ESR results and the optical reader is, in combination with built-in algorithms, even capable of detecting the relevant plasma – blood cell interface in hazy samples. The results are temperature-corrected to 18.3 °C and enable reliable clinical interpretation.

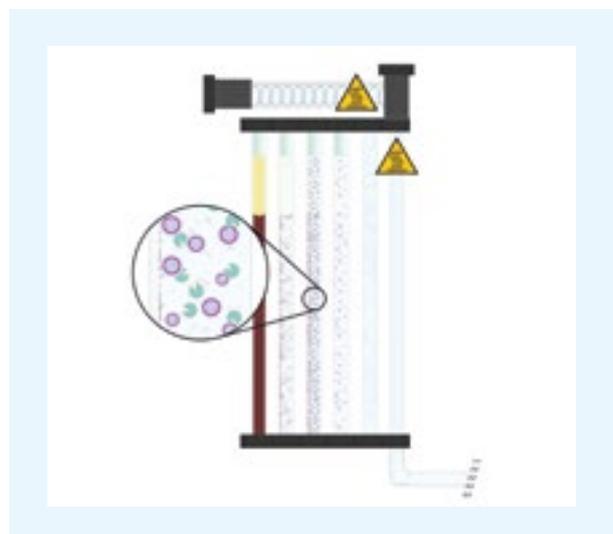


Fig. 4 The tubes are cleaned using detergent and protease enzymes. The inside of the tube is dried and disinfected by air that has passed through a heating element.

Starrsed analysers use standardised, reusable glass tubes that are specially made and tested. The tubes are cleaned using detergent and protease enzymes (as shown in Fig. 4), rinsed and dried after each cycle, ensuring that the tubes are clean before use. This reduces waste and minimises bio-hazard risks and the cost of operation.

Conclusion

The erythrocyte sedimentation rate following the gold standard of Westergren is a useful indicator of the general condition and an inflammation marker. Modern and fully automated instruments have made the ESR test even more accurate and safe in comparison to the manual Westergren version.

All new instruments and methodologies, though, should be verified by comparing their results with the standardised procedure, and are required to report results in accordance with the traditional Westergren reference ranges.

References

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