Leo R. Zacharski: The Erythrocyte Sedimentation Rate

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Judging by the volume of literature on the phenomenon of erythrocyte sedimentation (ESR) and the frequency of inuqiries from students and housestaff concerning interpretation of the ESR, interest in this subject is very nuch alive.

Attempts to assign significance to the spontaneous partitioning of shed blood into its component parts date from antiquity. While application of the scientific method to the study of red cell sedimentation during the last 50 years has added a degree of refinement to our methods and interpretations, the appearance of inconclusive and seemingly contradictory data has not been prevented. Indeed, such studies have all too often raised more questions than they have answered, which has resulted in a voluminous literature on the subject. To compound the problem investigators in many disciplines, such as cell membrane biochemistry and rheology, have studied ESR from various points of view.

The Mechanism of Red Cell Sedimentation

The rate of sedimentation of spherical particles in a simple physical system is dependent upon both the difference in density between the particle and the fluid medium and the radius of the particle (Stokes' law). While erythrocytes are capable of settling (their density exceeds that of plasma), the ESR of red cells from normal subjects is much slower than such a simple formula would lead one to predict. In addition, the wide fluctuations in the rate of settling that occur in subjects with various diseases are not necessarily related to density changes. Clearly red cell sedimentation is influenced by a myriad of factors, some of which are not well understood.

It must be kept in mind that the clinical laboratory test, the ESR determination, by which red cell sedimentation is measured introduces profound differences from conditions obtaining in vivo. These include the container into which the blood is transferred, the fact that the blood is no longer flowing, the presence of an anticoagulant, and the fact that the test is usually performed at room temperature. At this point it is reasonable to enquire whether the altered properties of blood that are manifested by an abnormal ESR have in vivo significance. The answer to this question is largely speculative.

Nonetheless, the ESR is susceptible to objective study and will be our primary concern. Basically the test is a measure of the rate at which the red cell meniscus descends when a sample of anticoagulated blood is placed in a calibrated cylindrical tube. In performing the tests the endpoint (the level of the meniscus) is read one hour after the test is set up. As with any clinical test scrupulous attention must be given to technical detail. For example, tipping the tube as little as 5˚ from the vertical may double the ESR. The Westergren method for determining the ESR has been widely adopted. Recommendations for performing this test in a standardized manner have been formulated by the International Committee for Standardization in Haematology (1973).

Sedimentation occurs in three phases: red cell aggregation, rapid red cell descent, and red cell packing. Events occurring during the first and second phases are paramount in determining the ESR in the vast majority of cases. The haematocrit (packed cell volume) influences the results only when the ESR is very rapid or when the haematocrit is either very high or very low.

The ESR is strongly correlated with the ability of red cells to aggregate into orderly stacks or rouleaux. Red cell aggregation into rouleaux should be contrasted with red cell agglutination. The latter occurs when red cells are linked together by antibodies which combine with red cell membrane antigens. Agglutinated red cells are irreversibly aggregated in all planes. In comparison, the aggregation of red cells into rouleaux is an orderly reversible electrochemical process in which red cells are arranged in flexible stacks, comparable to stacks of coins, with maximal opposing surfaces. Under conditions conducive to rouleaux formation, alignment of cells gradually occurs over a 10-40 second interval and rouleaux may continue to grow over a 30-mintue period. Talstad observed aggregation of red cells into rouleaux that formed a fine meshwork in normal blood, and a coarse meshwork in blood from patients with an elevated ESR or in normal blood with an experimentally induced increase of erythrocyte sedimentation. The ESR is directly related to rouleaux length.

Due to their negative surface charge adjacent red cells normally repel one another. This electrical force, or zeta potential, tends to inhibit rouleaux formation but may be overcome by conditions present in the surrounding plasma. Rouleaux formation may be enhanced by elimination of negatively charged substances such as sialic acid from the red cell surface, or by the addition of positively charged substances such as polylysine to the plasma.

The ability of red cells to form rouleaux is closely related to their normal biconcave shape and is, therefore, dependent upon their metabolic integrity and the composition and structure of their membranes. Numerous studies have demonstrated that the ESR is reduced under conditions in which red cell size and shape are abnormal. For example, it has been shown that the ESR is maximal at 37 °C and that further increases in temperature result in an abrupt reduction in ESR coincident with formation of spherocytes. On the other hand the low ESR, characteristic of hereditary acanthocytosis, rises during experimental manipulations that cause the red cells to assume a more normal shape.

It has been shown that the ABO blood group substances are not directly related to the ESR but that Rh-positive subjects have a significantly higher ESR than Rh-negative subjects. Less well characterized red cell surface receptors for lectins and enzyme-labile substances also contribute to the degree of erythrocyte sedimentation. Furthermore, red cell association may be modified by treating the cells with periodate ions or by adsorption of bacterial products to the cell surface, as well as by the elimination of sialic acid by neuraminidase. Repeated washing of the red cells results in a reduction of their ability to sediment. The importance of the metabolic and structural integrity of the red cells in determining the ESR is illustrated by the fact that glutaraldehyde-fixed red cells sediment faster than unfixed cells despite the fact that they are less dense than unfixed cells.

Red cell descent requires upward displacement of plasma and the resulting friction tends to retard sedimentation (analogous to the resistance noted when forcing a sieve into a pail of water). Therefore, the higher the haematocrit the smaller will be the distance between red cells and the slower will be the ESR. The ESR is characteristically very low in the presence of polycythaemia.

Rouleaux formation results in a reduced ration of surface area to volume and greater distances between descending particles, and hence in acceleration of the ESR. Elevation of the ESR in normal blood can be produced in vitro by increasing the distance between adjacent cells simply by diluting a blood sample with its own plasma. The rise in ESR is proportionate to the fall in haematocrit.

Variation of cell size and shape in iron deficiency anaemia interferes with rouleaux formation and thus tends to retard the ESR. An ESR of 40 mm in such a patient may therefore be highly significant. Correction for anaemia may move that value into the normal range and thus obscure an important clue to an occult gastrointestinal malignancy responsible for the anaemia. In sickle cell disease the ESR is typically very low (due to inability of cells to form rouleaux) despite the presence of profound anaemia.

Plasma constituents exert a more profound influence on the ESR than do conditions found in the red cells. This has been demonstrated in experiments in which plasma from a subject with a high ESR is added to red cells from a subject with a low ESR. Upon testing, the ESR of the reconstituted plasma and red cell mixture is seen to very similar to that of the blood from which the plasma was obtained. Furthermore, the addition of dextran to normal blood enhances rouleaux formation and markedly accelerates the ESR in proportion to the amount and the molecular weight of the added dextran.

In electron micrograph studies it was shown that adjacent red cell surfaces in dextran-induced rouleaux were parallel to each other and had a uniform intercellular distance. The size of the intercellular gaps varied in the same direction as the molecular weight of the dextran used and were actually slightly smaller than the chain length of the corresponding dextran molecule. It was proposed that the red cells were linked by a mono-molecular layer of dextran. The degree of rouleaux formation was proportional to the concentration of dextran and undoubtedly, therefore, to the number of intercellular bridges.

Plasma Proteins and the ESR

The large variety of proteins present in plasma might modify the ESR in different ways. In patients with multiple myeloma the association of a high ESR with an elevated immunoglobulin level is well known. On occasion, however, the immunoglobulin level may be so high that the plasma density approaches the red cell density and the ESR is paradoxically low. On the other hand, the serum proteins in most patients with an elevated ESR are normal or show minor or nonspecific abnormalities. In the majority of such patients determination of the fibrinogen level provides the explanation for the high ESR.

Fibrinogen is the single protein that most influences the ESR. The ESR can be raised simply by adding fibrinogen to blood. This effect can also be demonstrated by adding fibrinogen to red cells suspended in serum but is not seen if the red cells are suspended in buffer or buffered albumin. Endogenously increased fibrinogen levels are associated with an elevated ESR, and defibrination by enzymatic methods or by means of glass beads reduces the ESR virtually to zero.

Elevation of the ESR is related to reduction in the albumin:fibrinogen and the globulin:fibrinogen ratios.

The ABO blood group is not directly related to the ESR in normal subjects. However, the ABO blood group is related to the effect of fibrinogen on the ESR. The correlation between concentration of fibrinogen and elevation in ESR is striking for group A, less so for group O, and almost non-existant for group B.

The ESR appears to be even more profoundly influenced by the fibrin monomer than by fibrinogen. An increase in the ESR may, therefore, be indirectly indicative of intravascular coagulation or increased fibrinogen turnover. It is intriguing that glass contact or contact with tissue may trigger the sedimentation of red cells and that inhibition of fibrinolysis may also inhibit the ESR. A variety of non-steroidal anti-inflammatory drugs (aspirin is a notable exception) directly inhibit the ESR when added to blood in vitro. The fact that many of these agents are also platelet inhibitors has led to the hypothesis that a relationship exists between the ESR and platelet reactions. The question of a link between the ESR and activation of the coagulation mechanism is most thought-provoking.

There is a highly significant negative correlation between the ESR and the level of lysolecithin which, incidentally, is also a platelet inhibitor. Upon increasing the concentration of lysolecithin a point is reached at which the ESR abruptly falls. This steep dose response reflects saturation of albumin-binding sites for lysolecithin and the existence of unbound lysolecithin.

Clinical Significance of the ESR

Subjects with elevated plasma cholesterol levels certainly have higher ESRs than those with low plasma cholesterol levels, but the differences are small and both values lie within the normal range. The higher ESR observed in females than in males has been attributed to the lower lysolecithin and haemoglobin levels in females, but again the differences are small. The usually accepted normal values are 0-30 mm for females and 0-20 mm for males. However, the effects of subclinical disease, physiological variants (such as age, menstruation, and pregnancy), and drug ingestion must be taken into account and mildly elevated values (for example in the 20-40 mm range) are often difficult to interpret.

The popularity of the ESR as a clinical laboratory test results from its usefulness in following the course of certain diseases such as tuberculosis, rheumatic fever, and connective tissue disorders which may not easily be followed by more direct means, and from the fact that an abnormal result may be a tip-off to the presence of organic disease when differential diagnosis

is difficult.

While elevation of the ESR has attracted the most attention, a number of conditions are thought of as being associated with an abnormally low ESR. These conditions include polycythaemia, haemoglobinopathies, hypofibrinogenaemia, hyperproteinaemia associated with hyperviscosity, hereditary spherocytosis, hypochromic microcytic anaemia, pyruvate kinase deficiency, cyanotic congenital heart disease, serum sickness, congestive heart failure, cachexia, therapy with anti-inflammatory drugs, increased serum bile salt concentration, and Coombs'-test-positive haemolyutic anaemia.

The Elevated ESR

Attempts to assign differential diagnostic significance to the elevated ESR have been in vain. The ESR is of little use in deciding whether a gastric ulcer or a pelvic lesion is benign or malignant, and is often unreliable as a screening test or as an index of the extent and severity of malignant disease. In addition, the ESR is not a dependable sign of the presence or absence of metastatic lesions not a sure guide to the diagnosis or prognosis of carcinoma in the lung.

Lawrence (1961) stated that the most common causes of an ESR of more than 100 mm were myeloma and carcinoma metastatic to bone. However, Peyman (1962) found that only 4 per cent of 300 patients with malignancy had an ESR of more than 100 mm, and several of these has skeletal metastases.

The patient with a high ESR for which there is no obvious explanation after routine examination and laboratory testing presents a particularly tantalizing clinical problem.

Diagnoses ultimately established in some cases included multiple myeloma, cranial arteritis, renal disease, metastatic carcinoma of the prostate, macroglobulinaemia, acute leukaemia, and histiocytic lymphoma. Tests that established these diagnoses included serum protein electrophoresis, bone marrow examination, lumbosacral spine X-rays, temporal artery biopsy, and blood urea determination.

Summary

There is little benefit in measuring the ESR if a definite diagnosis, such as malignancy, pneumonia, or rheumatoid arthritis, has already been made. However, the test may have several uses, for example in following the progress of certain diseases and their response to treatment, as a screening test during the course of a general examination, where the diagnosis is in doubt, or when the distinction between functional and organic disease is difficult.