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Kinetic Measurement of the Erythrocyte Sedimentation Rate

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ABSTRACT

A method for estimation of the erythrocyte sedimentation from repeated measurements during the initial part of the sedimentation phase is described. Based on the measurements between 18 and 24 minutes after the beginning of the reaction, the sedimentation at 60 minutes is estimated. The estimate is highly correlated to the measured ESR in the range which the conventional ESR permits (up to about 90 mm). Higher ESR will be estimated from the kinetic data and thus give reproducible and meaningful results of samples which will be reported as "falsely low" by conventional methods. Sedimentations of several hundred mm can thus be estimated suggesting a new use for the ESR, particularly in monitoring patients with severe inflammatory reactions.

INTRODUCTION

Measuring the sedimentation of erythrocytes has long traditions and is still much used in spite of many new and specific markers for "activity" i.e. inflammation and cell destruction. Recently the ICSH (International Council for Standardization in Haematology) (2) and WHO (1) published recommendations for measuring the sedimentation reaction.

Under otherwise constant conditions (e.g. plasma protein concentrations), the erythrocyte sedimentation (erythrocyte sedimentation rate, ESR) will be limited by the length of the column and influenced by the erythrocyte volume fraction (haematocrit). The reaction itself can be subdivided into three distinct phases, the aggregation, the sedimentation and the packing phases. The ESR, as traditionally reported, is the total sedimentation during 60 minutes. At this time the aggregation phase has been concluded (5-10 min) and most often the sedimentation is proceeding.

However, in some cases the sedimentation is also concluded and the packing has begun. In these cases the reported sedimentation is entirely unsatisfactory and misleading, giving erroneous results. Some modifications have been made to improve the performance, thus, already in the 30-ies an "open system" was suggested (6) which allowed the blood corpuscles to leave the sedimentation tube. This approach would have allowed an almost undisturbed sedimentation for the full length of the tube but does not seem to have been used in the practice of medicine. Estimation of the ESR from measurements made at 30 minutes would be an improvement since many samples which otherwise would have reached the packing phase will in fact be measured during the sedimentation phase. An evaluation of this modification has recently been published (4). Access to modern electronics makes it possible to estimate the sedimentation from kinetic data. In the present report we describe a method to calculate the sedimentation from kinetic data collected during different time-periods of the sedimentation reaction.

METHODS

Commercially available vacuum tubes (Seditainer, Becton-Dickinson) were used throughout the present study. Blood was drawn from the antecubal vein to fill the tubes. The filling of the tubes was checked and only those which were filled to the mark $\pm 0,5$ cm were accepted. After adequate mixing of the blood and citrate in the tubes the sedimentation was measured every second minute for 60 minutes in a modified Sedimatic 100 instrument (Analysinstrument AB, Bromma, Sweden).

This instrument has been described in detail in a previous report (4). In summary, the tubes are placed in racks, each with ten positions. There are ten slots for racks, thus allowing simultaneous processing of one to 100 samples. The measuring unit consists of an arm carrying ten diodes and light sensors. This arm is moved horizontally and vertically by step motors. The vertical resolution of the procedure is about 0,3 mm. One rack is measured by one movement of the arm. Initially, the height of the individual blood columns is measured. At subsequent readings, the light beam will measure the height of the column of blood corpuscles. The readings are recorded and stored for each position in the array. The difference between the readings forms the basis for estimation of the sedimentation.

In the regular mode the instrument can be set for readings at either thirty or sixty minutes. The

readings are then converted to traditional "Westergren mm" by algorithms. Since tubes from different manufacturers perform slightly different, the instrument is equipped with algorithms which allow the reading of tubes from several major manufacturers. In the modified instrument readings were made every second minute and continuously reported in arbitrary units proportional to the movement of the step motor. Data were collected in serial mode by a personal computer and all data eventually became accessible for analysis. Thus, a true sedimentation rate could be plotted and the data extrapolated to any given time to give the sedimentation S, at that time.

RESULTS

The sedimentation rate was measured in blood from 262 patients. The sedimentation after 60 minutes was also measured in the same samples and patients were chosen with a variety of diagnoses to ensure a wide range of results. A selection of temporal developments of the ESR is shown in figure 1.

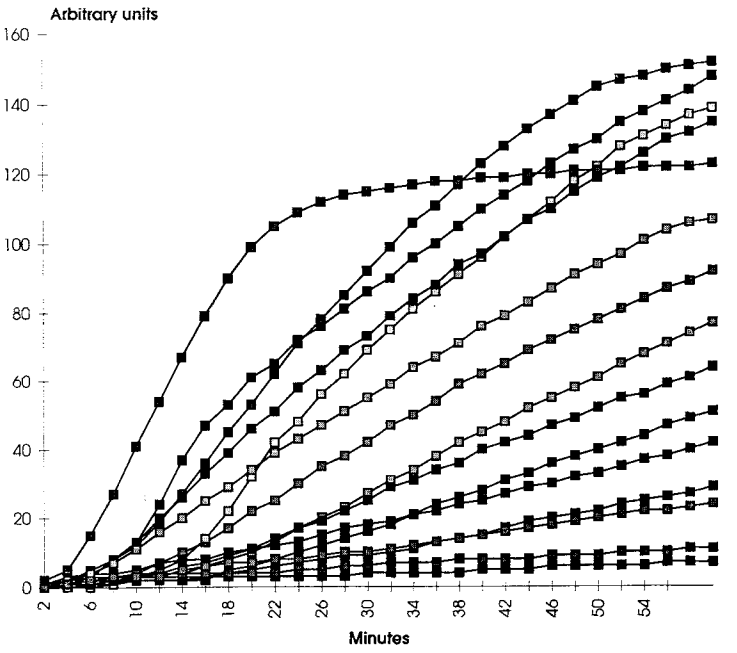

Fig 1. Temporal development of ESR from samples selected to illustrate some different patterns and the three phases, aggregation, sedimentation and packing.

Data from all patients were collected in four series of six minutes, beginning from the 14., 16., 18. and 20. minute after the beginning of the sedimentation reaction. Thus, in each series, four measurements were obtained. The correlation coefficient (r) was calculated for each of the lines fitted to the data (four points). One-way ANOVA showed no significant difference between the calculated r-values of the four series (mean= 0.970, $sd_{tot} = \pm$ 0.064, P= 0.558, df= 1 047).

The time-series 18 - 24 minutes was chosen for further analysis (r_{mean}= 0.971, sd= $\pm$ 0.074). This choice is a suitable compromise between short measuring times and a desire to incorporate as many samples as possible. In some cases the first measuring point will give results below the detection limit. Therefore, shifting the reading interval upwards will create a comfortable intercept.

The four observations from each individual sample were used to calculate algorithms from which the sedimentation at 60 minutes could be estimated. For the extrapolation it was assumed that the sedimentation continued linearly. The calculated sedimentation of samples with an ESR of less than about 90 mm corresponding to about 170 arbitrary units were compared to the measured ESR at 60 minutes, figure 2.

The estimated sedimentation was compared to the ESR measured at 60 minutes. There is a very good correlation (r^2=0.967, N=222) between the two estimates (figure 2). In the low end, however, the calibration function will be different from that of readings above about 30 arbitrary units or about ESR 30 mm. This is taken care of by the instrument. The two 'outliers' at about ESR 80 - 85 mm refer to samples which had reached the packing phase (figure 1) and are thus underestimated and erroneously low as determined by the conventional method.

DISCUSSION

The ESR has a reputation for not being particularly sensitive or specific in a diagnostic sense. Nevertheless, it continues to be used as a general indicator of disease and is much used in primary health care and in the monitoring of chronic inflammatory diseases (1). Because of physical restraints the sedimentation cannot proceed indefinitely. Further, the ESR is not a linear or simple measurement of sedimentation rate but rather the result of a series of complex reactions in which three distinct phases can be distinguished. The practical use of the ESR rests on the

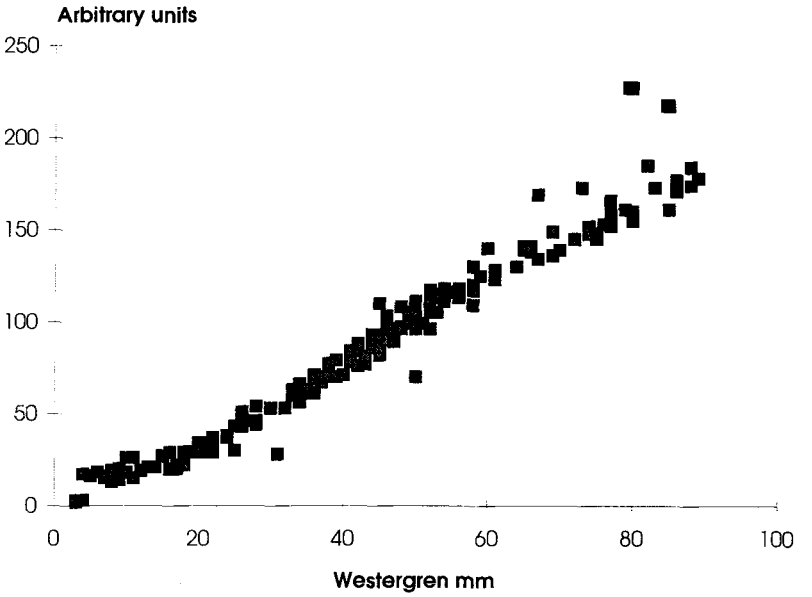

Fig. 2. Relation between the sedimentation (in arbitrary units) estimated from mesurements between 18 and 24 minutes and the ESR measured at 60 minutes and converted to Westegren mm.

assumption that the sedimentation has not reached the third (packing) phase before the reading takes place, conventionally after 60 minutes. As previously shown (4) there are advantages to make readings already after 30 minutes, the main being that a number of samples in which the sedimentation has been completed before 60 minutes will be caught and properly measured. Also, the measurement is quicker which is a great advantage to patients and the health care system. However, a sensitive and precise instrument is required to make it possible to measure the sedimentation accurately after short reaction times.

As illustrated in figure 1 there will still be a number of misleading results if the ESR is estimated from one reading at 30 minutes only. In the present study the ESR was therefore estimated from several measurements during a period of time when the first phase could reasonably be assumed to be terminated and the sedimentation rate linear. The first phase is terminated in most cases already after about 10 minutes whereas the third phase rarely begins before the 30. minute (figure 1). Within this time interval most samples sediment linearly. The 18-24 minutes interval gives a correlation coefficient which indicates that as much as about 94 per cent of the mean variance is explained by the model. In addition, a substantial intercept will be obtained in most

cases which adds to the sensitivity of the measurement procedure.

As illustrated in figure 1 the suggested method is not capable to accurately measure all samples. In cases when the packing phase has already begun, the extrapolation will give too low a value. A regression coefficient (a in $Y=aX + b$) equal to 0 indicates a horizontal line. This can occur in two circumstances: the packing is in progress or sedimentation has not yet started (delayed aggregation). The first case is recognized by an extrapolated value >90 mm (the intercept) whereas the other is generally below the upper limit of the reference interval (30 mm). In the latter case the value may be reported. In the first case the estimated S is not only pathological but also most likely indicates that the packing phase has been reached and the result is thus an erroneous estimate.

Practical experiences of the described method to estimate S in the monitoring of leukaemic patients (3) indicate that also very high S values have a diagnostic meaning. Thus, S-values far above 100 mm vary with the activity of the inflammation.

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