Comparative Analysis of Erythrocyte Sedimentation Rate Measured by Automated and Manual Methods in Anaemic Patients

Vikram Narang[1] Sumit Grover[1] Amandeep Kaur Kang[1] Avantika Garg[1] Neena Sood[1]

[1]Department of Pathology, Dayanand Medical College & Hospital, Ludhiana, Punjab, India

Address for correspondence Sumit Grover, MD, Department of Pathology, Dayanand Medical College & Hospital, Tagore Nagar, Ludhiana, Punjab, 141001, India (e-mail: sumitgrover1204@gmail.com).

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Abstract

Purpose Erythrocyte sedimentation rate (ESR) is a widely used indicator of inflammation and a routinely done hematology investigation to monitor patients of autoimmune and infectious diseases. We aimed to compare the ESR results obtained by Roller 20LC automated instrument and standard reference Westergren method and analyzed the effect of anemia (hematocrit) on ESR measurements through the automated method.

Methods We analyzed 1377 random anemic OPD patients (hematocrit [HCT] < 35%) for ESR levels measured by Roller 20LC using EDTA blood and Westergren method using citrated blood for a one and half year period from January 1, 2018 to June 30, 2019. Fabry's formula was used to correct the Westergren ESR.

Results The total number of samples after evaluation were divided into low (n = 232), intermediate (n = 417), high (n = 406), and very high range of ESR (≥100 mm/hr; n = 422). Mean difference between values of corrected and automated ESR for the low, intermediate, high and very high ESR range was 2.33 ± 5.03, 10.95 ± 8.04, 28.22 ± 19.11 and 43.3 ± 19.22 mm/hr, respectively. The 95% limit of agreement calculated by the Bland–Altmann analysis between the two methods for low-ESR range was −7.53 to 12.2 (highest correlation coefficient −0.65), while for very high ESR, range was −5.1 to 81.5 (least coefficient of 0.18) (p < 0.001).

Conclusion In laboratories with high-sample load and where manual measurement may be tedious, the automated method of ESR measurement can safely replace the Westergren method for low-ESR values in patients with low hematocrit. While for high-ESR values, validation by the standard Westergren method may be needed.

Keywords
- corrected ESR
- Hematocrit
- Fabry's formula
- Anemia

Background

The erythrocyte sedimentation rate (ESR) is widely used in clinical practice as an indicator of inflammation, infection, trauma, or malignant disease.[1] Many methods can be used for measuring the ESR such as Westergren method, Wintrobe's method, Zeta sedimentation ratio, and micro-ESR. The most satisfactory method of performing the test was introduced by Westergren in 1921.[2] The Westergren method is recommended for measuring the ESR by the International Council for Standardization in Hematology (ICSH).[3,4] ESR ranges in adults from 2 to 20 mm/hour.[5]

Various factors affect ESR value such as ratio of red blood cells to plasma, and cellular factors like cell size and cell

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surface area and their intrinsic capability to aggregate and sediment. Zeta potential plays an important role in this. Increase in rouleaux formation is due to increased plasma proteins such as haptoglobin, ceruloplasmin, α1-acid glycoprotein, α1-antitrypsin, and C-reactive protein (CRP), whereas globulin contributes the least. Fibrinogen is the most abundant acute phase protein with the greatest impact on ESR.[6,7] ESR is retarded by albumin. ESR measured by the Westergren method is affected by many factors such as room temperature and length and angle of placement of the tube.[8]

Since ESR performed by the manual standard Westergren method is also affected by hematocrit, Fabry's formula (Westergren ESR X 15/55-HCT) can be used to correct ESR values obtained by the manual method.[9] Also, to overcome the confounding factors, recently many new automated techniques for measuring ESR have been developed and introduced in clinical laboratories. They also provide many advantages like safety of operators, reducing biohazards risks, quicker results, speedy processing time, and ease of performance of other hematological tests (erythrocyte, leukocyte and reticulocyte concentrations) in a single specimen.[10] In 2010 and 2011, ICSH and Clinical and Laboratory Standards Institute (CLSI) released new recommendations.[11,12] They kept the Westergren method as reference procedure and stated that all new technologies, instruments, or methodologies have to be evaluated against the Westergren reference method before being introduced into clinical use. Also, it was recommended that the "systems which give the results same as the Westergren method with diluted blood at 60 minutes or normalized to 60 minutes are the only ones of clinical value."[10]

The automated Roller 20 LC method is based on the measurement of change in blood impedance after the red cell aggregation-sedimentation phenomenon has occurred. Roller 20 LC works on the principle of photometrical capillary stopped flow kinetic analysis.[10] Roller 20 LC recreates the physiological body conditions as it is thermostat at 37°C. The inbuilt microcapillary mimics the blood vessel. The blood sample in the capillary is accelerated and immediately stopped in the flow, which is known as stopped flow system. This simulates the blood pressure given by the cardiac muscle, which pumps the blood in the body. Roller 20 LC instrument can measure ESR of 18 samples in 10 minutes with minimal blood volume of 800 µL, whereas the Westergren method takes 60 minutes to interpret the result.

As per the ICSH guidelines,[10,11] for comparison of automated method to the Westergren method, correlations and bias should be calculated for the entire analytical range as well as the low, middle, and upper third of the analytical range separately. Correlation coefficients for the three parts of the analytical range should be compared with each other and to the total correlation coefficient. The statistical methods recommended for validations of alternate ESR methods are the coefficient of correlation, Passing–Bablok regression, and the Bland–Altman method. If these criteria are met, results can be mathematically transformed to the corresponding Westergren values. ICSH has also recommended to perform interference studies for anemia, hemolysis and lipemia, and indicate the level where interfering factors begins to affect the ESR results.[10,11]

According to our knowledge, very few studies have been conducted following these guidelines properly to statistically compare automated ESR with the Westergren method for the entire range of ESR values. We also evaluated the interference by anemia (hematocrit [HCT]) in an automated method. Hence, we compared ESR values in anemic patients using automated method with the corrected manual Westergren ESR.

In this study, automated ESR values of patients having HCT < 35% were measured on Roller 20 LC instrument using EDTA anticoagulated blood samples and compared with the corrected manual ESR performed on blood samples of the same patients by the Westergren method using citrated blood.

Materials and Methods

Collection of Data

In this study conducted over one and half year period from January 1, 2018 to June 30, 2019, in a tertiary referral institute of north India, ESR of random anemic patients measured by automated method was compared with manual method. Permission was obtained from the ethical committee to conduct the study. ESR measurement was done on random blood samples of anemic indoor patients (HCT < 35%) by the standard Westergren method (citrated blood) and automated method (EDTA blood) using Roller 20 LC. The manual Westergren values were corrected using Fabry's formula (Westergren ESR X 15/55-HCT). During the study period, a total of 1800 samples of anemic patients were tested for ESR using both the methods. The duplication or triplication of tests was avoided using the hospital information system (HIS) and patient unique identification number or medical record department number (MRD). The ESR tests with the same MRD if repeated within a week of admission or during the same visit were excluded by authors (S.G., V.N., and A.K.). The cases were further categorized into four groups, including ESR up to 20 mm/hour and elevated ESR (> 20 mm/hour), which were further categorized into mildly, moderately and markedly elevated ESR.

Exclusion Criteria

Patient samples whose HCT > 35% and sample was sent for re-evaluation of ESR within a week during same admission or same visit were excluded from the study

Statistical Analysis

Data were described in terms of range, mean ± standard deviation (SD), frequencies (number of cases), and relative frequencies (percentages) as appropriate. All the entries were entered in a Microsoft Excel sheet and the duplication or triplication was further rechecked by AG. Evaluation of Roller 20 LC method was done as described by Bland and Altman. The 95% limits of agreement were calculated as d ± 1.96 SD, where d = mean difference between the two measurements and SD = standard deviation of differences. Pearson correlation was used to find

the correlation among various parameters. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science) 21 version statistical program for Microsoft Windows.

Results

A total of 1800 random samples having HCT value < 35% were evaluated during the study period of one and a half years. Of these, duplicates and triplicates ordered during the same visit within a week were omitted, as described in materials and methods. Finally, 1377 samples were evaluated after excluding repeats. ESR was first measured by the Westergren method and corrected using Fabry's formula (Westergren ESR X 15/55-HCT), followed by the automated method. On dividing the cases on the basis of corrected ESR and automated ESR values into low range, intermediate, high and very high range, the cases falling in each range were 232 (16.8%), 317 (23.1%), 406 (29.5%) and 422 (30.6%), respectively.

The mean difference between the ESR values of subjects measured by corrected manual and automated method was calculated. Mean difference between values of corrected and automated ESR for the low, intermediate, high and very high ESR range was 2.33 ± 5.03, 10.95 ± 8.04, 28.22 ± 19.11 and 43.3 ± 19.22 mm/hr, respectively. Ninety five percent limit of agreement and correlation coefficient was calculated between corrected manual ESR and automated ESR values using Bland and Altmann analysis (►**Table 1**).

We inferred that for low range of ESR, the ESR values measured by automated method for 95% of subjects would be 7.53 mm/hour, below the corrected ESR or 12.2 mm/hour above it (►**Fig. 1**). For intermediate range, the ESR measured by automated method for 95% of subjects would be 4.81 mm/hour, below the corrected ESR or 26.72 mm/hour above it and so on for high and very high ESR ranges. Thus, maximum variation and least correlation was found in very high ESR ranges, signifying that the ESR measured by two methods showed least correlation for very high ranges. The maximum correlation was seen in the low and intermediate ranges with coefficient of 0.65 and 0.69, respectively (►**Fig. 1**)

Discussion

ESR is commonly used as an indicator of inflammation and infection, although it is not a specific test. The Westergren method is a recommended method for measuring the ESR by the ICSH. However, many confounding factors, including decreased RBC concentration and fall in HCT in anemic patients, and plasma proteins like globulins and fibrinogen which affect the plasma viscosity and hence sedimentation of RBCs, may affect the results.[5,13-15] This method requires longer time and more specimen.[14,15]

Various automated ESR instruments like Roller 20 LC (Alifax S.p.A, Polverara, Italy), Ves-matic 60 (Menarini Diagnostics S.r.l. Milan, Italy), Sediscan (Becton Dickinson, Meylan Cedex, France), Sedimatic (Technicon international Inc, Tokyo, Japan), and others[10] claim to overcome all the confounding factors involved in manual method. Also, these methods use a very small amount of blood samples with higher throughput in less time.

But these instruments need to be validated against the standard Westergren method to enable their routine use in laboratories and hospitals.[10,11] Roller 20 LC is one such instrument which can measure ESR of 18 samples in 10 minutes with minimal blood volume of 800 µL, whereas the Westergren method takes 60 minutes to interpret the result. The Westergren method uses the principle of sedimentation ESR, while Roller 20 LC is based on capillary photometry and measures ESR by converting aggregation of RBCs by optical density, which is then converted into mm/hr.[16]

In this study, we compared the ESR of anemic patients measured by Roller 20 LC with the reference Westergren Method. While reviewing the literature, we found that our study is the biggest study on comparison of automated methods with standard methods of ESR measurement with maximum number of samples (*n* = 1377), while other authors like Dhruva et al[17] included just 209 cases, Patil et al[18] included just 162 cases, Subramanian et al[19] studied 200 cases, and Alfadhli et al[20] had 150 cases.

While evaluating the influence of HCT levels on ESR measurement by Roller 20 LC, we found that the difference between corrected Westergren ESR and automated ESR increased with increase in ESR values. Thus, the more the ESR value, the more the variability between corrected Westergren ESR value and the automated value.

Sonmez et al and Romero et al, similar to our study, evaluated the effect of low HCT (< 35%) on ESR levels. Sonmez et al[9] used samples of 755 patients and divided the corrected ESR values into low (≤20 mm/hour) and high (> 20 mm/hour) range and according to HCT (≥35%, < 35%) levels. Like our study, Sonmez et al[9] also found that in anemic patients,

Table 1 Mean difference between corrected manual ESR and automated ESR value, 95% limit of agreement and correlation coefficient

ESR range (mm/hr)	Mean difference between corrected ESR and automated ESR value (mm/hr)	95% limit of agreement	Correlation coefficient	*p* value
Low (*n* = 232)	2.33± 5.03	− 7.53 to 12.2	0.65	< 0.001
Intermediate (*n* = 317)	10.95 ± 8.04	− 4.81 to 26.72	0.69	< 0.001
High (*n* = 406)	28.22 ± 19.11	− 9.24 to 65.7	0.63	< 0.001
Very high (*n* = 422)	43.3 ± 19.22	− 5.1 to 81.5	0.18	< 0.001

Abbreviation: ESR, erythrocyte sedimentation rate.

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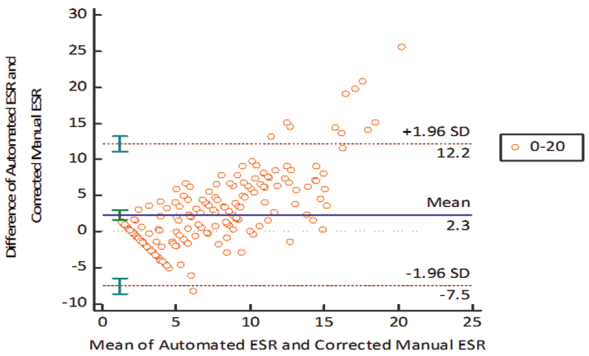

Fig. 1 Bland and Altman analysis of the comparison between automated method erythrocyte sedimentation rate (ESR) and corrected ESR with mean difference 2.3; 95% limits of agreement from −7.5 to 12.2.

ESR values measured by the direct Westergren method were higher than automated method. Hence, Fabry's formula was applied to correct the overestimation. After applying Fabry's formula, the mean of difference between the Westergren ESR and automated ESR values dropped down in low and intermediate range; however, still a significant difference was observed for high and very high ESR ranges.

In our study, at high and very high ESR values, more variation was found between the two methods. For high ESR values, mean difference was 28.22 ± 19.11 (95% limit of agreement − 9.24 to 65.7; correlation coefficient 0.63; $p < 0.001$), and for very high ESR values, mean difference was 43.3 ± 19.22 (95% limit of agreement − 5.1 to 81.5 with least correlation coefficient 0.18; $p < 0.001$). Sonmez et al also found a poor correlation between the two methods at high ESR values ($p > 0.10$).[9] Dhruva et al also had similar observations in patients with HCT between 30 to 35%. They realized that samples with high ESR values vary considerably around the mean difference compared with samples which had normal ESR readings. They found mean difference of 2.58 ± 9.17 (95% limits of agreement − 15.39 to 20.55) for samples with higher ESR values (> 25 mm/hour) compared with mean difference of 1.22 ± 1.90 (95% limits of agreement –2.50 to 4.94) for ESR values less than 25 mm/hour.[17]

Subramaniam et al used automated instrument MONITOR 100 from Electa Laboratory Italy and divided ESR of 200 patients having HCT between 30 to 36% into 0–25 mm/hr (n = 79) and > 25 mm/hr (n = 121) ranges. They found mean difference of just–7.7 and 95% limit of agreement between –18.9 to 3.5 for ESR values less than 25 mm/hour compared with mean difference of 13.4 and 95% limits of agreement between –57.3 to 30.5 for high values.[19] Similarly, on analyzing SEDI system (Becton Dickinson, MeylanCedex, France), Alfadhli et al[20] showed low agreement between the automated and Westergren methods at the higher ESR values as compared with normal ranges. They also reported that for samples with ESR readings > 25 mm/hr (n = 81), the mean of difference (–21.4) and the 95% limits of agreement (–45.2 and 2.26) were markedly different from the corresponding values (–3.9,–13.5 and 5.7, respectively) for samples with ESR values < 25 mm/h (n = 69).

Thus, all these studies highlighted the importance of the effect of HCT on ESR values of different automated instruments, but according to our information, ours is the largest study to date, with the maximum number of samples, which is conducted based on ICSH guidelines, and where the comparison has been made for all the ranges of ESR using Bland and Altmann analysis and effect of interference of HCT has been evaluated.

Conclusion

Hence, we can conclude that in laboratories where the workload is high, Roller 20 LC automated method of ESR measurement can be conveniently used as a replacement of the standard Westergren method in anemic patients (HCT < 35%) at low and intermediate ESR values < 50 mm/hr; however, in patients having ESR > 100 mm/hour, the automated values cannot be relied upon because of statistical discrepancy, and validation by Westergren method may be needed.

Source(s) of Support
Nil.

Presentation at a Meeting
Nil.

Conflicts of Interest
None declared.

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