Elisa Piva, Alice Stoppa, Michela Pelloso and Mario Plebani*

The VES-Matic 5 system: performance of a novel instrument for measuring erythrocyte sedimentation rate

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Abstract

Objectives: The VES-Matic 5 is an automated analyzer that assesses erythrocyte sedimentation rate based on a modified Westergren sedimentation technique. Instrument performance was established by addressing the recommendations of the International Council for Standardization in Haematology.

Methods: Comparison against the reference Westergren method was performed for all samples, and further for the low, middle, and upper third of the analytical range. Intra-run precision, inter-run precision, and interference studies were further assessed. This study included the evaluation of reference ranges.

Results: The comparison of methods by Passing–Bablok analysis has shown a good agreement without systematic or proportional differences. The regression equation was $y = -0.646 + 0.979x$. The mean bias of $-0.542$ was obtained by Bland–Altman analysis and the upper limit of $8.03$ with the lower limit of $-9.11$ can be considered clinically acceptable. Intra-run and inter-run precision were good for each parameter and interference studies did not show any significant bias with exception of anemia samples, which showed a proportional difference when comparing high erythrocyte sedimentation rate values. Using the local adult reference population, we verified the reference ranges in comparison to those available in the literature, and according to the Clinical Laboratory Standards Institute (CLSI) EP28-A3C document. We determined the upper limit partitioned by gender and the following age groups: from 18 to 50, from 50 to 70, and over 70.

Conclusions: The VES-Matic 5 analyzer presented good comparability with the reference method. As there are commercial quality control and suitable external quality assessment (EQA) material and programs, the VES-Matic 5 can be employed appropriately for routine purposes.

Keywords: automated ESR; erythrocyte sedimentation rate (ESR); ICSH recommendations; modified Westergren method.

Introduction

The erythrocyte sedimentation rate (ESR) was applied in clinical practice following the studies by Edmund Biernecki in the 19th century, even though the test was standardized many years later by another scientist, Alf Vilhelm Albertsson Westergren [1]. Since its discovery, ESR is the laboratory test that quantifies a complex biological phenomenon, i.e., the behavior of a suspension of red blood cells in plasma under specified conditions. ESR is not linear and plotted against time; the sedimentation shows a sigmoid curve. Three phases are known. The first is the “lag phase” in which erythrocytes form rouleaux and aggregates and the sedimentation is very slow. The second, or “decantation phase,” is the true sedimentation phase. Erythrocytes fall more rapidly because the plasma interface and the sedimentation can occur more rapidly. In the final phase, erythrocyte aggregates pile up on the bottom of the tube [2–4]. New technologies and technical advances allow automated instruments to perform ESR testing, in consolidation with the modern organization of clinical laboratories. Automated instruments have great advantages like safety for operators, reduced turn-around time, and better analytic precision. Some of these instruments use the sedimentation principle by selecting an appropriate time interval, while others use other innovative techniques, which are different from the sedimentation rate [5].

For a long time, the International Council for Standardization in Haematology (ICSH) advocated ESR standardization and harmonization. The last document, published in
2017, was an obvious need to evaluate the different automated methodologies and all the working methods against that of the reference, that is the Westergren method. The recommendations were established to document the reliability, accuracy, and robustness of the results against the Westergren method for all new instruments, both for those based on the modified sedimentation principle and those that use different techniques [6].

Recently, a novel fully automated system, the VES-Matic 5 (DIESSE Diagnostica Senese, Monteriggioni Siena, Italy), has been proposed for ESR determination. Using closed ethylenediamine tetraacetic acid (EDTA) tubes and with a complete automation process, the instrument evaluates the sedimentation of the red cells and using a mathematical algorithm converts the raw data obtained into ESR results. The instrument also uses artificial intelligence (AI) to detect pre-analytical errors, for example, coagulated or unfilled samples. The aim of the present study was to perform the analytical validation of this new automated analyzer for ESR testing, in agreement with ICSH recommendations.

**Materials and methods**

**Study design**

The automated analyzer for ESR determination, VES-Matic 5 was evaluated at the Department of Laboratory Medicine, University-Hospital of Padua according to the ICSH recommendations and included method comparison, precision study (intra-run and inter-run), hemolysis, triglycerides (TGs), and anemia interference studies, sensitivity to fibrinogen, and verification of reference ranges [6].

**Specimens collection**

Blood samples, used for method evaluation, precision determination, sensitivity to fibrinogen, and assessment of the interferences as well as verification of reference ranges, were selected from the daily routine. Samples were leftovers that had an initial request for cell blood counter (CBC) and/or ESR of both hospitalized patients and outpatients admitted to the Padua University Hospital. All samples were collected in 3.0 mL bi-potassium EDTA (K2-EDTA) BD Vacutainer® blood collection tubes (Becton Dickinson, BD-Plymouth, UK), processed according to manufacturers’ specifications, and analyzed within 5 h from venipuncture. The study followed the ethical principles for medical research involving human subjects, according to the World Medical Association (WMA) Declaration of Helsinki, and under the terms of all relevant local legislation.

**VES-Matic 5**

The new automatic instrument for the determination of the ESR, the VES-Matic 5 is based on a modified Westergren sedimentation technique. Briefly, all samples are collected in an EDTA anticoagulated tube, loaded into the instrument on the same rack of the CBC instruments, samples are mixed, and under controlled temperature evaluated for sedimentation measurement using an optoelectronic light source. The sedimentation rate is evaluated by a large number of optical recordings during a 20 min period and the differences are evaluated. Raw data are corrected for temperature variations and the obtained results are converted to Westergren values using a mathematical algorithm. The first result is performed after 28 min. Technologically innovative, the new analyzer applies an AI system in recognition of lipemic, hemolyzed, coagulated, or mislabeled samples, while the Internet of things, through an internal camera, is used for advanced remote assistance. In this way, the analyzer is connected directly to diagnostic devices, reporting any malfunction directly and receiving instructions for self-repair, where possible. The throughput is 190 samples per hour, and walk away mode and continuous loading are supported.

**Westergren method**

According to ICSH recommendations, ESR by the Westergren method was performed using a diluted sample and circular glass Westergren tube with an inner diameter of 2.55 mm. Briefly, the EDTA-anticoagulated blood was thoroughly mixed by complete inversion of the tube 20 times and diluted 4:1 using a trisodium citrate dihydrate solution (3.8%). The Westergren tube was allowed to stand for 60 min in an appropriate supporting rack in a stable, vertical position in a fume hood, at constant temperature (20–25 °C) and free from external influences such as vibrations, heat, and direct sunlight. The sedimentation rate was read by visual determination after 60 min as the distance from the top of the plasma level to the top of the Red Blood Cell (RBC) layer and recorded in mm/h. All Westergren analyses were performed by a single analyst to minimize pipetting and reading variations. The comparison study against the gold standard method was made on the same working day within 5 h of blood sampling.

**Method comparison study**

Samples of 132 females and 139 males (a total of 271) were selected among those over the age of 18 years (median age 58, interquartile ranges (IQR): 42.25–70.75 years), spanning the entire ESR analytical range (from 2 to 120 mm), and with hematocrit value ≥ 0.35 L/L [0.61 ± 0.03 L/L, mean ± SD; 95% confidence interval (CI): 0.606–0.614]. Hematocrit was determined as part of the complete blood count on Sysmex XN-10 automated hematology analyzer (Sysmex Corporation, Kobe, Japan).
Undiluted EDTA samples were used first for the determination of ESR by VES-Matic 5, then the EDTA-anticoagulated sample was diluted manually with 3.8% citrate solution in a ratio of 4:1, according to the ICSH recommendations for the Westergren method. Following the ICSH recommendations, method comparisons were further assessed in three subgroups, according to the ESR values obtained with the Westergren method, that is, low (<20 mm), middle (20–60 mm), and upper third (>60 mm) of the analytical range.

**Precision study**

Intra-run precision was estimated using 10 routine patient samples with ESR values covering the analytical range from 2 to 140 mm. All samples were analyzed on VES-Matic 5 for five replicates. Inter-run precision was performed using commercial quality control (QC) samples in the normal and abnormal range. Specifically, inter-run precision on VES-Matic 5 was assessed using two lots of the control material, the ESR Control Cube, composed of stabilized human blood, the precision was performed using commercial quality control (QC) samples.

**Sensitivity to fibrinogen**

According to ICSH recommendations, the sensitivity of response to added fibrinogen was assessed in six healthy volunteers (3 females and 3 males) recruited from the laboratory staff. Human fibrinogen (Sigma-Aldrich, St Louis, MO, USA), expected concentration 20 g/L was dissolved at 37 °C in 0.9% saline solution, and sterile-filtered using a 0.22 μm filter (Merck Millipore Ltd., County Cork, Ireland), as recommended by the manufacturer. Fibrinogen concentration obtained in stock solution was 14.8 g/L, measured using the automated Clauss method (Sysmex CS-5000, Sysmex Corporation, Kobe, Japan). Samples were prepared, with the addition of saline solution alone, or saline spiked with stock fibrinogen to reach concentrations of: 1.5, 3, 4.5, 6, and 8 g/L and added to each tube. ESR was determined first on the VES-Matic 5 analyzer and then by the original Westergren method. The baseline fibrinogen concentration (normal range 1.5–4.5 g/L) of six volunteers (shown as series in Figure 2), measured by the Clauss method, was the following: series 1: 1.9 g/L; series 2: 2.7 g/L; series 3: 2.7 g/L; series 4: 4.1 g/L; series 5: 2.4 g/L; series 6: 2.2 g/L. The sensitivity for fibrinogen in four of the volunteers was assessed to reach the concentration of 8 g/L.

**Hemolysis interference**

Twenty-eight routine patient samples, covering the whole ESR range, were selected for a hemolysis interference study. At first, ESR was determined by VES-Matic 5 analyzer and later hemolysis was induced in vitro by the addition of 50 μL of lysing solution (Osmored, Eurospital Spa, Trieste, Italy). To eliminate the dilution effect, 50 μL of blood was withdrawn from each tube before the addition of lysing solution. Hemolyzed samples were analyzed using the VES-Matic 5 and then by the Westergren method. Hemolysis index (HI) was determined on Architect c8000 (Abbott Laboratories, Abbott Park, IL, USA), which uses a spectrophotometric method incorporating four wavelength pairs and predefined constants to report numeric H-index into plasma-free hemoglobin results.

**Triglycerides interference**

TGs interference was evaluated in three routine patient samples, each with the following different values of ESR: 7, 32, and 138 mm/h, respectively. To evaluate interferences, a solution of TGs from a human plasma TG fraction (Lee Biosolutions, Maryland Heights, MO, USA, lot number W105417) was made, reaching a final concentration of 360 mg/dL (4.06 mmol/L) (Cobas Modular 8000, Roche Diagnostics S.p.A, Monza, Italy). Using the VES-Matic 5, ESR was evaluated first in each sample; then, aliquots of 150 μL of TG solution (0.61 mmol/L) were added three times, reaching the final addition of 450 μL of TG solution (1.83 mmol/L).

**Anemia interference**

Anemia interferences were evaluated in 141 routine patient samples, spanning the entire ESR analytical range (from 4 to 140 mm) and selected based on hemoglobin values (<109 g/L) and hematocrit values (<0.34 L/L) (Sysmex XN-10, Sysmex Corporation, Kobe, Japan). ESR was first evaluated by the VES-Matic 5 analyzer and then using the Westergren reference method.

**Verification of reference ranges**

Reference ranges were verified according to the CLSI EP28-A3C document [7]. For transferability assessment ESR was determined both using VES-Matic 5 and the Westergren method in a total of 311 samples (161 healthy males and 148 healthy females, aged from 18 to 94 years). Samples were from routine outpatients that required a CBC, without the collection of additional test tubes for this study. Information such as food in the past 24 h, alcohol consumption, lifestyle, and health status was investigated at the time of collection. Results of the required laboratory tests were then verified. Samples were used for age and gender-related reference interval groups. Due to the difficulty of recruiting pediatric samples, only gender, and age-specific adult reference ranges were verified in this study, divided by age into three groups: from 18 to 50 years, 50–70 years, and older than 70 years.

The statistical significance was calculated at 95% of the reference interval. The following reference ranges were applied: under 50 years: 15 mm for males, and 20 for females; over 50 and below 70 years: <20 mm for males and <30 mm for females. Additional upper limits for people over 70 years old, were <30 mm for males and <35 for females. The ranges chosen for verification of the reference ranges obtained in this study are the most common given in the literature and the H02-A5 CLSI document [8–10].

**Statistical analysis**

The comparison of the VES-Matic 5 against the reference method was evaluated by calculating bias and limits of agreement using Bland–Altman analysis. Linear regression was carried out using Passing–Bablok regression to estimate constant and/or proportional difference, and Spearman’s rank correlation coefficient (ρ) was calculated to assess the strength and direction of the association between the compared data. Mean and median values, standard deviations (SD), and IQRs (25th and 75th quartiles) were also reported. For the precision study, coefficients of variation (CVs), mean values,
Results

Method comparison study

The mean ESR values obtained were 18.81 mm (95% CI: 16.27–21.36 mm) for the VES-Matic 5 and 18.25 mm (95% CI: 15.55–20.95 mm) for the gold standard Westergren method. The median ESR values were 11 mm (IQR: 5–23.75 mm) using VES-Matic 5 and 9 mm (IQR: 5–22.75 mm) with the Westergren method. The comparison of methods by Passing–Bablok analysis has shown a good agreement without systematic or proportional differences. The regression equation was $y = -0.646$ (95% CI: $-1.00$ to $0.11$) + $0.979$ (95% CI: $0.944$–$1.00)x (Figure 1A). The mean bias of $-0.542$ (95% CI: $-1.06$ to $-0.01$) was obtained by Bland–Altman analysis; the value of 8.03 for the upper limit and $-9.11$ for the lower limit can be considered acceptable limits, implicating a non-significant bias based on clinical criterion (Figure 1B). The Spearman’s coefficient of rank correlation ($\rho$) was 0.963, with its 95% CI from 0.953 to 0.971. The study comparison was also performed for the subgroups of results, based on the analytical interval, i.e., for the low (<20 mm), middle (20–60 mm), and upper range (>60 mm) of values and are presented in Table 1. Passing–Bablok regression analysis, Spearman’s rank correlation coefficient, and Bland–Altman analysis results were reported for each subgroup. For the low and middle range ESR values, the mean bias obtained by the Bland–Altman analysis can be considered acceptable from a clinical point of view.

Precision studies

In Table 2 the results of the intra-run precision study are presented, spanning a full range of values. ESR results are reported as mean value, 95% CI for the mean, SD, CV%, and the minimum and maximum values obtained. Five replicate measurements of the specimens showed a good level of precision that was below 10% (CV < 10%), except for two specimens, one with very low results, another one with a value of 11.2 mm, and a CV of 11.64%. In Table 3 the inter-run precision is reported. The evaluation was determined with two different lots of QC material, both in the normal and abnormal range, analyzed three times on five consecutive days. Inter-run precision was below 11% (CV 10.9%) for the normal level and <3% (CV 2.7%) for abnormal level. The Grubbs’ test did not reveal any outliers.
**Sensitivity to fibrinogen**

The sensitivity to increasing amounts of fibrinogen was determined and an excellent similar sensitivity was obtained both with the VES-Matic 5 and the Westergren reference method (Figure 2A and B). The assessment of the linearity of response between methods and the sensitivity was evaluated using the Bland–Altman statistical method, and no significant bias was observed (arithmetic mean: −0.7353; 95% CI from −1.547 to 0.0765; p=0.0743, NS). Calculation of the correlation coefficient (r=0.998, 95% CI from 0.996 to 0.999) and the slope (0.993, 95% CI from 0.971 to 1.014) gives an excellent assessment of response to fibrinogen of VES-Matic 5. The most significant rise of ESR related to increasing concentrations was observed for both methods at the final added fibrinogen concentration of 6 and 8 g/L. The highest ESR elevation was six and 9.47 times the baseline (mean value from 8.5 mm/h to 51.2 and 80.5 mm/h) for the Westergren method, while ESR elevation was 5.6 and 8.64 times the baseline (mean value from 9.3 mm/h to 51.67 and 80.5 mm/h) for the VES-Matic 5.

**Hemolysis interference**

The results of the assessment of hemolysis interference were evaluated by the Bland–Altman method, and the mean of the differences was not significant (bias: 0.036, 95% CI from −0.80 to −0.22). According to ICSH recommendations results are reported for the low (<20 mm), middle (40–60 mm), and upper range (>60 mm) of the analytical interval.
Triglycerides interference

For the VES-Matic 5, the assessment of TGs interference on ESR determination was evaluated by ANOVA test, and the values obtained for the three samples did not differ significantly (p=1.00, not significant, NS), demonstrating that there are no significant effects on the ESR results due to the increase of TG concentration. The baseline values compared to those obtained in samples with the final concentration of 162 mg/dL of TGs were respectively: from 6 to 8 mm/h; from 41 to 30 mm/h; from 120 to 140 mm/h.

Anemia interference

To evaluate potential anemia interference, samples selected had hemoglobin values from 72 to 109 g/L (mean value: 96.82 ± 8.25 g/L, 95% CI from 95.72 to 97.93) and hematocrit values from 0.21 to 0.34 L/L (mean value: 0.297 ± 0.27 L/L, 95% CI from 0.29 to 0.30). The mean ESR value obtained by VES-Matic 5 was 57.11 ± 44.21 mm (95% CI: from 49.75 to 64.47 mm) while the mean ESR with the gold standard Westergren method was 49.53 ± 41.11 mm/h (95% CI: from 42.69 to 56.38 mm).

Figure 2: Sensitivity of ESR to added fibrinogen concentrations, measured with (A) the VES-Matic analyzer and (B) the Westergren method.
The regression equation \( y = 1.80 \times 1.12 \) calculated by the Passing–Bablok regression analysis showed a good comparison of methods, with no statistically significant systematic differences while a minor constant and proportional difference was found, when comparing high values (Figure 3). The cumulative test (Cusum linearity test, p-value=0.17) indicated linearity between methods, while the Spearman’s coefficient of rank correlation (\( \rho \)) was 0.966 (95% CI: from 0.953 to 0.975).

**Discussion**

ESR is still considered an overall test to assess the acute phase reactants, as a “sickness index” that clinicians have used for decision-making for over 50 years, in conjunction with the physical examination and clinical history of the patient [11, 12].

The diagnostic accuracy of ESR and C-reactive protein in acute inflammation diseases has recently been reassessed and has shown that ESR can provide valuable clinical information. For example, its determination may contribute to the management of COVID-19 patients in the determination of disease progression [13, 14]. ESR continues to demonstrate its unquestioned clinical usefulness in diagnosis, monitoring, and progression of response to therapy of certain diseases associated with immune response, such as rheumatoid arthritis or systemic lupus erythematosus [15–17]. In other arthritic disorders, ESR is one of the main laboratory tests in investigating patients presenting signs and symptoms of temporal arteritis or polymyalgia rheumatica [18, 19].

Automation of ESR testing, based on technological improvements in studies of the sedimentation phenomenon, has brought about the development of many automated instruments which are now available for routine use. It should be underlined that automation allows for workflow optimization, increases personnel safety by using closed systems, and shortens turnaround times. Consequently, updated recommendations published by ICSH addressed standardization and harmonization of the novel methods to decrease variation in the interpretation of ESR results [6].

**Table 4:** Upper reference limit for the erythrocyte sedimentation rate obtained from the VES-Matic 5 and the Westergren method.

| Gender and age, years | n | VES-Matic 5, mm Mean ± SD | Upper limit | Westergren, mm Mean ± SD | Upper limit |
|-----------------------|---|---------------------------|-------------|--------------------------|-------------|
| Male < 50             | 52 | 6 ± 6                     | 15 (11–18)\(^a\) | 6 ± 6                    | 14 (9–19)\(^a\) |
| Male from 50 to 70    | 64 | 9 ± 7                     | 19 (16–22)\(^a\) | 8 ± 7                    | 18 (15–21)\(^a\) |
| Male >70              | 49 | 17 ± 10                   | 33 (30–37)\(^a\) | 14 ± 9                   | 30 (24–33)\(^a\) |
| Female <50            | 61 | 12 ± 8                    | 25 (21–28)\(^a\) | 11 ± 8                   | 22 (18–26)\(^a\) |
| Females from 50 to 70 | 43 | 14 ± 8                    | 27 (23–30)\(^a\) | 12 ± 7                   | 24 (19–28)\(^a\) |
| Female >70            | 44 | 19 ± 11                   | 37 (33–41)\(^a\) | 16 ± 9                   | 31 (26–35)\(^a\) |

\( n \), sample size; SD, standard Deviation. \(^a\)Bootstrap confidence interval (500 iterations) at 90%. Reference values are established locally in accordance with the CLSI EP28-A3C document (see text).
In this study, following ICSH recommendations we evaluated the analytical performance of a new automated ESR analyzer, the VES-Matic 5, that uses the sedimentation principle and undiluted EDTA samples. This is an important issue because the most suitable specimen for ESR testing is that employed for CBC, i.e., samples anticoagulated with EDTA that avoid the collection of a second specimen if clinicians require both tests in the same patients. The use of undiluted or diluted specimens represents one of the most important testing variables. For automated ESR determination with diluted sodium citrate blood, test tubes are dedicated and the anticoagulated blood ratio is different from that of the citrate tube used for coagulation testing, which must never be used. To improve standardization, the anticoagulant type for specimens to use in ESR testing should be discussed again at an international level to achieve comparable results. The VES-Matic 5 comparison against the reference Westergren method, assessed in the diluted specimen, has shown excellent performance and agreement, with better data than other previous comparison studies [20, 21]. For ESR very low results, equal to 3.2 mm, the intra-run precision showed a CV% equal to 26.15; data should be judged on clinical interpretation because the minimum and maximum values vary from 2 to 4 mm. For other ranges of results, the imprecision was <10%, except for only one case with an ESR value of 11.2 mm, with a CV of 11.64%. In terms of inter-run precision, the system provided a satisfactory and acceptable performance that was better than those published in previous studies [22, 23]. As far as interferences are concerned, the VES-Matic 5 response to each level of fibrinogen concentration was in the agreement with Westergren, indicating that the new instrument is interchangeable with the gold standard method.

In hemolysis evaluation, the VES-Matic 5 did not show significant susceptibility to hemolysis, contrary to previous data observed using the instrument VES-Matic Cube 200, manufactured by the same company. For hemolysis interference we operated similarly to the study reported for the VES-Matic Cube 200, i.e., using spiking of native samples with a lysing solution. No significant decrease was observed for ESR values before and after lysis of the samples and no negative effects, similar to the studies on instruments that utilize photometric rheology principle, achieving harmonization in this issue [24, 25].

As compared to other studies, no significant decrease in ESR values was observed in TGs interference evaluation, but this study has some limitations because of the small number of samples assessed, and poor recovery of concentration of TGs [26–28].

Anemia can affect the erythrocytes sedimentation, causing an acceleration of the global phenomenon. We evaluated the effect of anemia by employing samples of 141 patients, with a hemoglobin level ranging from 72 to 109 g/L, minimizing variables that can affect the manual technique for the Westergren method. In this study, VES-Matic 5 showed a good comparison against Westergren by statistic Passing–Bablok regression and no significant systematic difference was found, except for ESR high values, when constant and proportional differences were found, clinically not significant. Our data are in agreement with the previous study [29].

ESR varies greatly with age and sex and can be influenced by lifestyle factors (physical activity, smoking, and alcohol consumption), and common metabolic abnormalities (obesity and related metabolic syndrome) [30, 31].

Although reference values should be established locally, many laboratories are adopting references published in papers or books or stated by the manufacturers [9, 32, 33]. In this regard, some consideration should be made: reference ranges were published years ago when there were variations in methodology such as different pipettes and materials used in the Westergren method. In an effort, to achieve maximum diagnostic efficiency when interpreting the results of the ESR test, this issue deserves special attention.

In this study, VES-Matic 5 showed reference ranges that meet those obtained utilizing the Westergren method. The preliminary upper range of the present study should be interpreted considering undiluted samples and a non-parametric approach, statistical method recommended by CLSI. The highest ESR was found in healthy subjects over 70, different in men and women, and therefore separate values should be established for people in this age bracket.

This study assessed the analytical validity of the VES-Matic 5 analyzer and the comparability with the gold standard method. According to our findings, and considering that there are useful commercial QC and suitable EQA materials and programs, the VES-Matic 5 can be employed appropriately for routine purposes. Finally, the VES-Matic 5 works using AI-based software for the reduction of pre-analytical errors including mislabeled, hemolyzed, or coagulated samples. Laboratory medicine has a central role in the diagnostic workflow of many diseases and the application of AI can improve diagnostics through more accurate detection of pathology. Determination of erythrocyte sedimentation parameters utilizing AI has already been found to allow the monitoring of rare
hereditary neurodegenerative diseases and further studies may reveal other possible clinical applications [34]. In conclusion, ESR is by no means an obsolete test.

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