

# Development of a Micro-ESR System with Potential for In-home Use

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**OBJECTIVE:** To develop a micro-erythrocyte sedimentation rate (ESR) system with potential to be self-administered by patients at home using capillary blood.

**DESIGN:** For each subject, three tubes of blood were collected in ethylenediaminetetraacetic acid (EDTA), centrifuged, and the cells separated from the plasma. Plasma was pooled and divided into three aliquots, two of which were spiked with defined amounts of fibrinogen creating a normal ESR and two distinct degrees of ESR acceleration. Fifteen hundred microliters of pooled autologous cells were resuspended in 1500 $\mu$ L of the three prepared autologous plasmas to standardize hematocrit values. ESRs were performed using the Westergren method and four potential micro-ESR systems, utilizing a micro-hematocrit tube, S/P capillary blood gas tube, Natelson blood collecting tube, and Caraway micro blood collecting tube.

**SETTING:** Saint Louis University, St. Louis MO.

**PATIENTS/SAMPLES:** Twenty-eight volunteers between the ages of 18 and 60 years with no underlying conditions participated in the study.

**INTERVENTIONS:** Hematocrit was standardized to approximately 40% for all samples and fibrinogen concentrations of approximately 200mg/dL, 382mg/dL, and 563mg/dL were achieved for each subject.

**MAIN OUTCOME MEASURES:** ESRs were measured in mm/hour and in percentage. Micro-ESR values were plotted versus the paired Westergren ESR value and the data were analyzed using Pearson's r correlation.

**RESULTS:** When compared to the Westergren ESR method, the following correlation coefficients were achieved: S/P tube ( $r = 0.808$ ), Caraway tube ( $r = 0.797$ ), Natelson tube ( $r = 0.719$ ), and micro-hematocrit tube ( $r = 0.655$ ).

**CONCLUSION:** Three of the four micro-ESR methods achieved correlation coefficients acceptable to the authors, with the potential of being converted into a capillary ESR system for in-home use.

**ABBREVIATIONS:** EDTA = ethylenediaminetetraacetic acid; ESR = erythrocyte sedimentation rate; RBC = red blood cells.

**INDEX TERMS:** blood sedimentation; capillary-erythrocyte sedimentation rate; erythrocyte sedimentation rate; micro-erythrocyte sedimentation rate.

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The erythrocyte sedimentation rate (ESR) was originated in 1921 by Robin Fahraeus.<sup>1</sup> Historically, the ESR was routinely performed as either a screening test to detect various inflammatory diseases such as infections, arthritis, myocardial infarction, cirrhosis, diabetes, malignancy, and renal disease or to validate suspected disease. When used as a screening test, an accelerated ESR would prompt physicians to investigate occult disease. Inflammation is the physiological process that links these diverse diseases and is responsible for elevating the ESR. In some patients, an increased ESR could suggest

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disease prior to the onset of symptoms, providing opportunity for earlier interventions. However, a high false positive and false negative rate made the results difficult to interpret without patient history and additional clinical data. This, in conjunction with cost containment issues, prompted physicians to discontinue the use of the ESR as a screening tool.<sup>2,3</sup> While the ESR is no longer considered appropriate for screening, it is still performed to monitor the status of select inflammatory diseases and to predict responsiveness to treatment. It has been shown that the ESR is often accelerated in disorders such as polymyalgia rheumatica, temporal arteritis, rheumatoid arthritis, systemic lupus erythematosus and some cancers.<sup>3-6</sup> In a pilot study conducted by Randolph, a relationship between tumor burden and the acceleration of the ESR was demonstrated. The data suggested that the ESR holds potential in monitoring treatment, remission, and relapse in patients with certain malignancies.<sup>6</sup>

The ESR is a non-specific test that measures the rate at which red blood cells settle in whole blood. The carboxyl group of sialic acid residues on the membrane surface of red blood cells produces a negative charge, which causes the cells to repel. Plasma proteins can adsorb onto the surface of the red blood cells (RBCs) and neutralize this natural negative charge, thus reducing the repulsion. The RBCs then attract one another and stack like coins, a formation known as rouleaux. RBCs in rouleaux possess an increased RBC density which accelerates the rate of RBC sedimentation, thus increasing the ESR. During inflammation, acute phase proteins are increased and adsorb onto RBC surfaces, initiating rouleaux formation. Several acute phase reactants, like fibrinogen and C-reactive protein (CRP), are known to accelerate the ESR, with fibrinogen having one of the strongest effects. Many inflammatory diseases cause an increase in fibrinogen and CRP levels and therefore accelerate the ESR.<sup>7-10</sup> Increased levels of other plasma proteins, such as albumin, decrease the ESR by increasing plasma viscosity.

There are many variables in addition to plasma proteins that affect the ESR, including hematocrit, temperature, and storage time. The ESR should be performed within six hours of specimen collection and optimally between 23°C-25°C. A delay in testing may slow sedimentation, significantly decreasing ESR. Storage at 4°C may prevent this retardation of the ESR, however the specimen should be returned to room temperature prior to testing.<sup>11</sup> A low hematocrit may cause a falsely increased ESR due to the increased ratio of plasma to red blood cells.

Although the Westergren ESR method has been standardized and is considered the reference method, many other ESR techniques exist. The original Westergren procedure used a 200mm open-ended pipette with an internal diameter of at least 2.55mm. The pipette was filled with undiluted blood collected in EDTA, having a packed cell volume (hematocrit) of 35% or less, and the RBCs were allowed to settle for one hour. The method developed by Wintrobe in 1943 used a 100mm glass tube with an internal diameter of 8mm. Since then several ESR methods have been introduced that vary in technique. For example, some use a 4:1 ratio of blood to diluent while others use undiluted blood. Most methods are read at one hour, some at 20 or 30 minutes, and others are read every five minutes.<sup>12</sup> However, as recommended by the International Committee for Standardization in Hematology, the Westergren method, using a 4:1 dilution of EDTA anticoagulated blood, remains the standard for ESR testing.<sup>13</sup>

There have been several automated ESR methods developed which have become popular in clinical laboratories because they are more rapid and the results are comparable to the standard method. Some methods require that the results be read in as little as 15 minutes, as opposed to one hour for the Westergren method. The Dienes Mini-Ves is a four-place point-of-care instrument that reads the ESR at 20 minutes and is the second most popular method according to the College of American Pathologists survey.<sup>12</sup> Some other automated ESR methods currently available are the MicroSed SR system, TEST1, and Microtest 1. TEST1 and Microtest 1 use a capillary photometric-kinetic technology, which reads the sedimentation and aggregation of erythrocytes 1000 times over 20 seconds with an infrared microphotometer operating at 650nm wavelength.<sup>14</sup> There is also a new method of estimating the ESR by viewing erythrocyte aggregation on a slide using an image analysis system operating at 200x magnification called the INFLAMET™ inflammation meter. This can be performed bedside within a few minutes.<sup>15</sup>

A major concern regarding traditional ESR testing involves the large blood volume needed. The Westergren ESR and many other techniques require at least one mL of blood, presenting problems if only a small volume of blood is available or when only capillary blood can be obtained. This is of particular concern in pediatric hematology because it is common to obtain capillary blood from a heel or finger stick and there is often a very small volume available. There have been several methods developed which use smaller volumes of blood. Barrett and Hill conducted a study comparing the Micro-Dispette method (Guest Medical) to the Westergren

method. While the Micro-Dispette method showed higher values using venous blood than those obtained by the reference method, there was good correlation between the micro-dispette method using capillary blood and the reference method using venous blood. The Micro-Dispette requires 240 $\mu$ L of blood, but it must be collected in a capillary blood collection tube and then transferred to the ESR tube.<sup>16</sup> Another study conducted by Hackett, Hinchliffe, and others indicated that capillary blood still resulted in significantly higher values than the reference method. The method used in the study was the Sterilin micro technique, which requires 0.4mL of blood to 0.1 mL citrate and uses 230mm disposable tubes made of polystyrene with a 1.25mm bore. The sample is drawn up the tube using a suction pump. Venous EDTA samples were used to compare the micro-ESR method to the reference method, and capillary EDTA samples assessed the reproducibility of the micro-ESR. The study found that the elevated results only occurred with high ESRs and were not likely to be clinically significant.<sup>17</sup> One of the first micro-ESR methods using capillary blood, developed by Stuart and others, set the stage for the more recent studies with micro-ESR methods. However, Stuart's method, using an Accu-Tech ESR system, is not commercially available. The study also showed that capillary blood produced higher ESR values with the micro method than did venous blood using the reference method.<sup>18</sup>

The research designs described in the literature utilizing micro-ESR methods had three major deficiencies: 1) they used only capillary blood from children, 2) the micro-ESRs were performed in two steps, blood collection then set-up, and 3) they were performed by laboratory personnel. There is currently no ESR method available for in-home use. It is our goal to develop a micro-ESR method that can easily be performed by patients at home using a simple one-step process that is reliable for the monitoring of various inflammatory conditions as indicated by their physicians.

We believe that an in-home ESR system could be beneficial in monitoring the progression of various conditions like rheumatoid arthritis, lupus, temporal arteritis, and potentially certain malignancies, and in monitoring the effectiveness of treatment. The ESR is currently being used as a monitoring tool for many of these inflammatory conditions and previous studies have shown that the ESR rises and falls as these conditions worsen or remit. An in-home ESR method could have at least two major benefits for patients. First, patients could reduce the number of doctor visits thus minimizing cost and inconvenience. Second, an in-home ESR method

could be performed more frequently than those performed during office visits, potentially predicting disease relapse sooner and thus improving early intervention and clinical outcomes. Patients would send their ESR results to their physicians and be provided with a set of guidelines to determine when further consultation might be necessary.

We conducted a study to evaluate the potential of four micro-ESR methods developed in our laboratory that we hope can be adapted to be performed by patients at home. For the purposes of our study, venous blood was collected into EDTA tubes from 28 volunteer subjects. The cells were separated from the plasma and both were pooled. The plasma was divided into three aliquots and increasing concentrations of fibrinogen was added to the plasma to produce a normal, medium, and high ESR result for each volunteer. Fibrinogen spiking was used to ensure a wide range of ESR values to improve correlation across the testing range. Autologous cells and plasma were reintroduced, producing a standardized hematocrit of 40% to prevent this variable from affecting the results. Four micro-ESR methods were compared to the Westergren method.

## MATERIALS AND METHODS

### Specimen collection

Venipuncture was performed on a total of 28 healthy individuals between the ages of 18 and 60, with no underlying diseases. The venipuncture procedure was performed using a standard evacuated tube system and phlebotomy technique. Blood was collected from two to three individuals per day, 15 minutes apart to allow adequate time for blood collection while maintaining specimen freshness. Samples for each subject were collected into one ten mL EDTA tube and one five mL EDTA tube and centrifuged for ten minutes at 3000 RPM to separate cells from plasma.

### Plasma preparation

Plasma from the two EDTA tubes was pooled, and 1500 $\mu$ L was added to three separate 20mL sample cups containing 0mg, 5.4mg, and 10.9mg of fibrinogen powder (Sigma-Aldrich, St. Louis MO). The cups containing plasma and fibrinogen were mixed for 15 minutes at 125 RPM on a serological rotator to dissolve the fibrinogen in the plasma. Then 1500 $\mu$ L of autologous erythrocytes from each individual were added back to the three corresponding sample cups containing the manipulated plasmas. The cups were again mixed on a serological rotator for 15 minutes at 125 RPM, and then set up as sedimentation rates. This preparation standardized the hematocrit at approximately

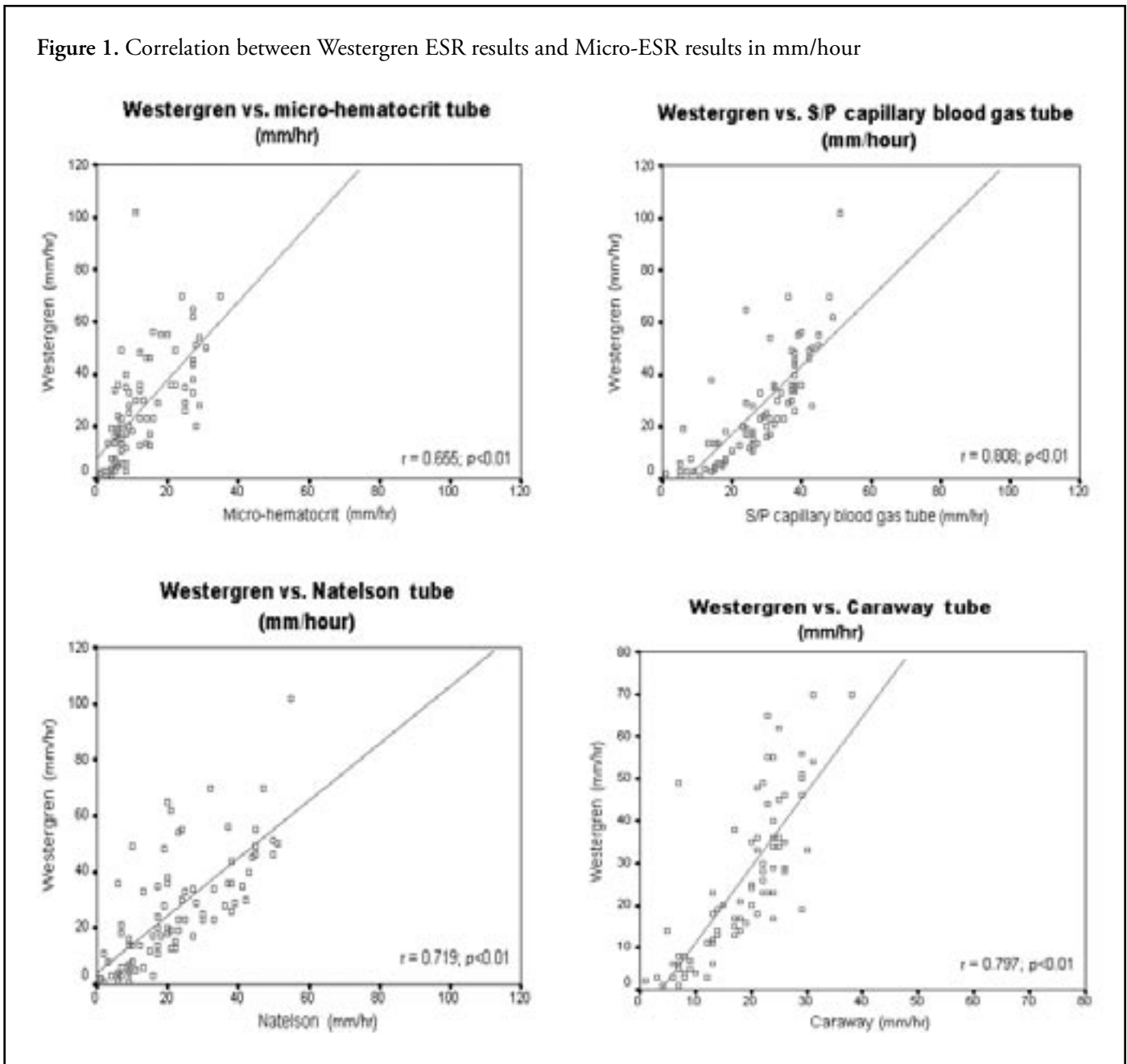
40% and the fibrinogen concentrations at approximately 200 mg/dL, 382 mg/dL, and 563 mg/dL for each subject.

**ESR procedure**

ESRs were performed on the three sample aliquots for each subject by the Westergren method and four potential micro-ESR methods within four hours of specimen collection. The four micro-ESR methods tested used the following pipettes: 1) micro-hematocrit tube with a 75mm length, 1.1-1.2mm bore, and 70µL maximum volume, 2) S/P capillary blood

gas tube with a 130mm length, 1.5mm bore, and 160µL maximum volume, 3) Natelson blood collecting tubes with a 150mm length, 1.5mm bore, and 250µL maximum volume, and 4) Caraway micro blood collecting tube with a 75mm length, 2.4-2.7mm bore, and 370µL total volume. All capillary tubes were heparinized from the manufacturer. Holders were developed and adapted to ESR racks to keep each capillary tube in a vertical position. Each micro-ESR tube was filled approximately two thirds full with the manipulated samples and the end was sealed with clay. The

Figure 1. Correlation between Westergren ESR results and Micro-ESR results in mm/hour





tubes were placed in their holders in a vertical position in either a Wintrobe rack or Universal ESR rack. After 60 minutes, the distance from the plasma meniscus to the top of the red blood cells was measured for each ESR method and recorded in both mm/hour and as a percentage of the plasma to total blood volume that we have termed the sedimentation hematocrit.

#### Data analysis

The results for each micro-ESR method were compared to the paired result for the Westergren ESR method using Pearson's *r* correlation. Based on standard correlation criteria established in the social and biological sciences a correlation  $>0.7$  is considered quite high,  $0.3-0.7$  is considered moderate, and  $<0.3$  is considered weak.<sup>19</sup> To establish the correlation stringency for this study the authors set the following *r* values:  $r >0.9$  (excellent correlation),  $r = 0.8-0.9$  (very good correlation),  $r = 0.7-0.79$  (good correlation), and  $r <0.7$  (undesirable correlation).

#### RESULTS

Each of the 28 normal blood samples collected was standardized to a hematocrit of 40% and manipulated to create three aliquots with increasing fibrinogen concentrations. Each aliquot was of sufficient volume to set up five ESRs; one Westergren and four micro-ESR methods. The ESR results obtained by the Westergren method were compared to each of the four micro-ESR methods at all three fibrinogen concentrations. The results of all five ESR methods were measured in both millimeters per hour and in percentage. Each micro-ESR result was compared to the corresponding Westergren result for each paired sample and analyzed by the Pearson's *r* correlation test.

When we compared the results of the four micro-ESR methods in mm/hour to the results of the Westergren method in mm/hour, we obtained the following correlation coefficients: the micro-hematocrit tube was 0.655, the S/P capillary blood gas tube was 0.808, the Natelson blood collecting tube was 0.719, and the Caraway micro blood collecting tube was 0.797. Distributions of the data points around the line of best fit for each method are shown in the scatterplots in Figure 1. As we had expected, the results of the micro-ESR methods produced a maximum ESR result much lower (30-50mm) than the Westergren method (60-70mm) when measured in mm/hr. All four correlations produced *p* values of  $< 0.01$ .

When the results of the micro-ESR methods measured in sedimentation hematocrit were compared to the Westergren

results also measured in sedimentation hematocrit, the following correlation coefficients were obtained: the micro-hematocrit tube was 0.653, the S/P capillary blood gas tube was 0.806, the Natelson blood collecting tube was 0.719, and the Caraway micro blood collecting tube was 0.797. The scatterplots of the data points obtained by measuring the sedimentation hematocrit with each ESR method are shown in Figure 2. In this case the micro-ESRs produced higher maximum values (40% to 60%) compared to the Westergren ESR (30% to 35%). As before, all four correlations produced a *p* value of  $<0.01$ .

#### DISCUSSION

We decided to measure ESR results in both millimeters/hour and in percentage to overcome an expected complication regarding tube length in the micro methods. The micro-ESR methods have at least one thing in common; they all use a tube that is significantly shorter than the tube used in the Westergren method. Due to the short tube length we expected these micro-ESR methods to produce a maximum ESR significantly lower than that attainable by the Westergren method when measured in mm/hr. Given sufficient time, RBCs in any ESR system will eventually settle to a maximum point, essentially becoming a hematocrit. If the RBCs settled to this level during the hour allotted for the test, the ESR would reach a maximum value and sediment no further. The distance in millimeters of this maximum value would be expected to be significantly lower in a micro-ESR system than even a moderately accelerated Westergren ESR given tube lengths of 75 mm to 150mm and 200mm, respectively. We attempted to overcome this expectation by also measuring the ESRs in percentage, which we have termed sedimentation hematocrit. Since a Westergren ESR would also produce a maximum ESR value similar to a hematocrit given sufficient time and hematocrit values should be the same regardless of tube size, we suspected that expressing the ESR as a sedimentation hematocrit might produce comparable values between the systems. However, this does not appear to be the case. In all cases where a micro-ESR was compared to the Westergren, the mm/hr measurement was either similar or superior to the sedimentation hematocrit measurement.

The expected differences in values between the micro-ESR methods and the Westergren method prompted us to select the Pearson's *r* correlation coefficient statistical test to analyze our data. Since it was anticipated that the shorter tube length of the micro-ESR methods would produce lower raw values as compared to the Westergren method, selection of a statisti-

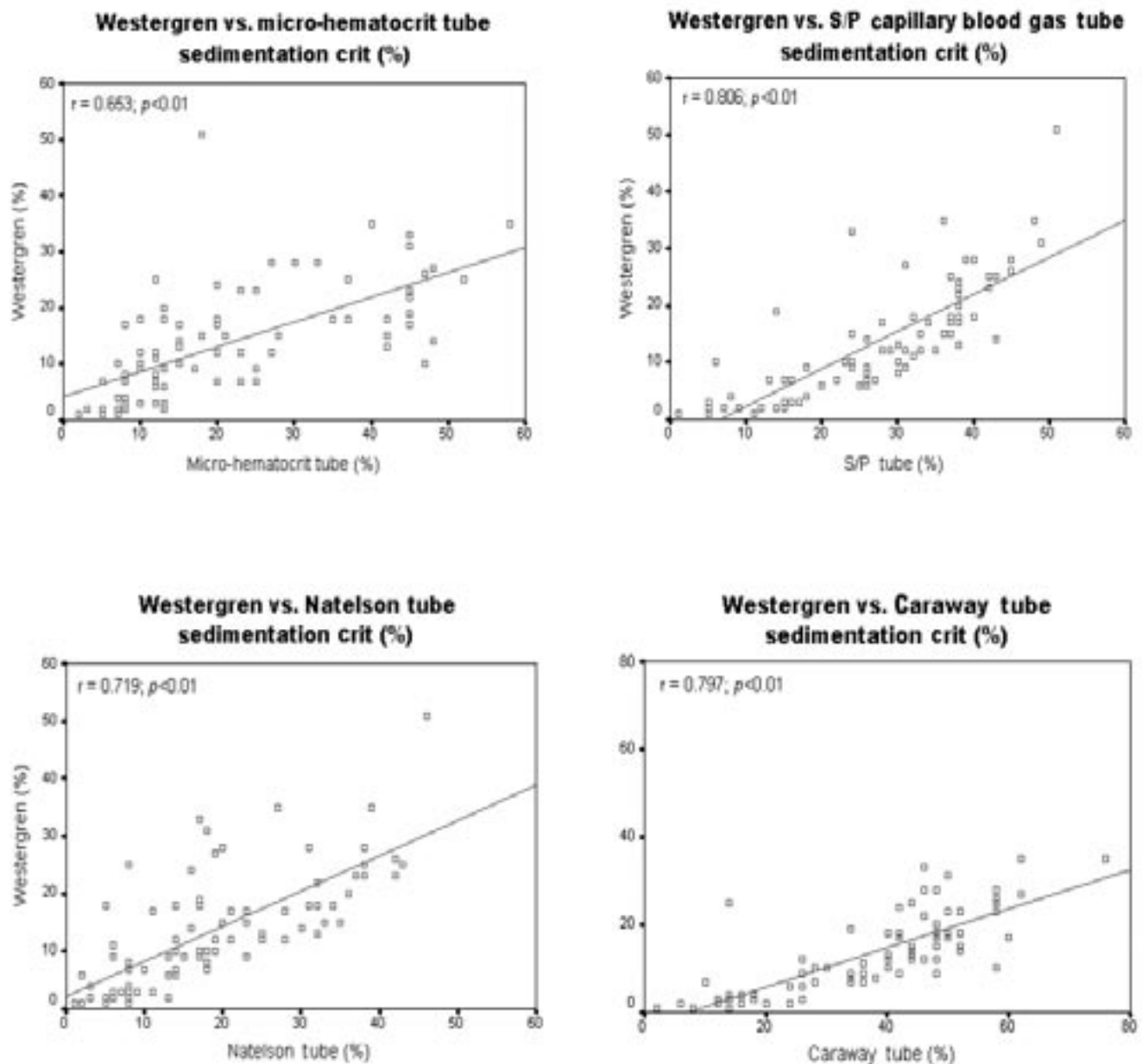
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cal test designed to determine differences in means would be inappropriate. In addition, we were more interested in how well the results of the micro-ESR methods correlated to the Westergren results as opposed to how closely the micro-ESR results duplicated the Westergren results.

Using the correlation criteria previously discussed, the results suggest that two of the micro-ESR methods correlate very

well, one method correlates adequately, and one method is unacceptable. The micro-hematocrit tube was rejected since the correlation coefficient obtained by this method was only 0.655 when measured in mm/hr and 0.653 when measured in sedimentation hematocrit even though the  $p$  value was  $<0.01$ . In addition, the micro-hematocrit tube system produced low maximum ESR values of approximately 30mm. Lastly, the slope of the correlation line produced an

Figure 2. Correlation between Westergren ESR results and Micro-ESR results in sedimentation hematocrit



obtuse angle relative to the X-axis. Since the X and Y axes were plotted symmetrically, an obtuse best fit line indicates a relationship where the absolute values do not match.

All three of the remaining micro-ESR methods, S/P, Natelson, and Caraway produced acceptable correlation coefficients of 0.808, 0.719, and 0.797 respectively, when measured in mm/hr. The S/P and Natelson exhibited larger maximum ESR values of approximately 50mm and produced a line of best fit with a slope of approximately 45°. This slope indicates micro-ESR values that closely match those produced by the Westergren method. Although the Caraway micro-ESR produced an r value between the S/P and Natelson methods, the maximum ESR values were approximately 35mm and the slope of the best fit line was obtuse relative to the X-axis. Since the S/P and Natelson tubes are the longest among the micro-ESR methods tested, 130mm and 150mm respectively, tube length is the best explanation for the larger maximum ESR values and the close match in absolute values with the Westergren method.

The purpose in also measuring the ESRs in percent sedimentation (sedimentation hematocrit) was to correct for expected shorter sedimentation values in mm/hr due to the shorter tube lengths of the micro-ESR methods. When measured in percent, the S/P, Natelson, and Caraway methods also produced acceptable r values of 0.806, 0.719 and 0.797, respectively. Unlike the values measured in mm/hr, the maximum ESR values for the micro-ESR methods were greater (45-60%) than the Westergren method (35-40%). This produced a slope of the best fit line that formed an acute angle relative to the X-axis indicating that values did not match between each micro-ESR method and the Westergren method.

The ideal micro-ESR method would correlate closely with the Westergren method, produce an r value close to 1.0, and generate a best fit line with a slope of 45° when plotted on a linear/linear graph where both axes are equal. When evaluating the micro-ESR methods by these criteria, the S/P produced the best results when measured in mm/hr. However, since the r value for the Natelson and Caraway methods were acceptable when measured in both mm/hr and percent, these techniques should not be excluded from future studies.

We recommend that future studies be performed on a larger number of samples using capillary blood and the S/P, Natelson and Caraway micro-ESR methods. A Westergren

ESR should also be performed using venous blood from the same subjects so a comparison can be performed between the capillary methods and the standard Westergren ESR method. The hope is to test subjects with and without inflammatory illnesses to obtain ESR results that span the gamut of possible ESR values. This will provide a data set to test the correlation between the micro-ESR methods using capillary blood with the standard Westergren ESR using venous blood.

If one or more of the methods performed with capillary blood correlates with the Westergren method, the next step would be to determine clinical applicability. As an aside, we collected a capillary sample directly into both the S/P and Natelson tubes to primarily evaluate ease of blood collection and test performance. We found that the collection of capillary blood by finger stick directly into the S/P and Natelson tubes was relatively simple to perform, and therefore feasible for patients to perform on themselves at home. However, if the values obtained from the micro-ESR methods are reproducible and correlate with the Westergren ESR but do not produce similar numbers, a correction factor may be needed to align micro-ESR values with Westergren values to improve clinical interpretation.

The potential exists for an in-home micro-ESR method using capillary blood to serve as an alternative to the Westergren method using venous blood for the purpose of monitoring certain chronic inflammatory conditions. Such a method will save patients time and the inconvenience of frequent doctor visits, and in turn reduce healthcare costs. Patients could test themselves at regular intervals and report the results to their physicians via phone, email, fax or through the postal service. The increased testing frequency that in-home methods allow could result in earlier detection of changes in a patient's disease progression or response to therapy, thus improving interventions and patient outcomes.

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