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Shear Rate Dependence of Ultrasound Backscattering from Blood Samples Characterized by Different Levels of Erythrocyte Aggregation

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Abstract—The objectives were (1) to determine the effect of the erythrocyte aggregation level (wide range of aggregation) and shear rate (which also affects aggregation) on the ultrasound backscattered power, and (2) to evaluate the reproducibility of the ultrasound method. Experiments were performed under steady flow (100–1250 ml/min) in a 12.7 mm diameter vertical tube. Doppler ultrasound at 10 MHz was used to measure simultaneously the velocity and the backscattered power across the tube. For each radial position, the shear rate was computed from the derivative of the velocity profile. The backscattered power decayed exponentially as a function of the shear rate, and for a given shear rate, the power increased monotonically with the level of aggregation measured by laser reflectometry. Using blood samples simulating hypo-, normal, and hyperaggregating erythrocytes, the power of the ultrasound signal varied respectively by −7.8, −13.2, and −16.1 dB as a function of the shear rate (from 0.4 to 50 s^{-1}). The reproducibility of the backscattered power was 5.5 dB, which is less than the variations observed as a function of the shear rate. In conclusion, ultrasound backscattering is sensitive to the level of erythrocyte aggregation. At a first glance, ultrasound seems less accurate when compared to laser reflectometry but it is suggested that this is because ultrasound backscattering may be sensitive to structural aggregate changes that are not detected by the laser method. © *2000 Biomedical Engineering Society.* [S0090-6964(00)00804-3]

Keywords—Echography, Acoustic backscattering, Power Doppler ultrasound, Biorheology.

INTRODUCTION

Physicians and researchers using several methods have evaluated erythrocyte aggregation. The erythrocyte sedimentation rate is the most widely used approach in laboratory medicine. However, it is difficult to assess the effect of the shear rate on erythrocyte aggregation with this method. Other *in vitro* techniques developed to characterize erythrocyte aggregation involve direct microscopic observations under shear flow,[23] viscosity measurements at low shear rates,[3] light reflection,[1] and transmission[24] measurements, and ultrasound backscattering.[15,26] Intravital microscopy is also widely used to study erythrocyte aggregation *in vivo* in microvessels.[2,11,19] However, ultrasound backscattering is the only method that can be used to measure erythrocyte aggregation noninvasively in large human vessels.[9,17] The purpose of this study was to further validate the ultrasound method to measure erythrocyte aggregation.

Sigel *et al.*[28] were the first authors to demonstrate, in a flow model, that the intensity of the echoes backscattered by normal human blood was velocity (shear rate) dependent. Yuan and Shung[34] showed that the echo intensity of porcine whole blood increased as the velocity was reduced in a horizontal steady flow model, whereas that of porcine erythrocytes suspended in a saline solution was velocity independent. The presence of erythrocyte aggregates for porcine whole blood and the absence of aggregates in the erythrocyte suspension could explain these results. Shehada *et al.*[25] studied the echogenicity of porcine whole blood across a tube and its shear rate dependence. Foster *et al.*[13] and Van Der Heiden *et al.*[31] quantified, in a Couette flow arrangement, the effect of the shear rate on the echo intensity of human blood at high ultrasound frequencies (30–70 MHz). At 10 MHz in a vertical steady flow model,[8] we showed that the power backscattered by porcine whole blood is characterized by a rapid reduction at shear rates between 1 and 5 s^{-1}, a transition between 5 and 10 s^{-1}, and a region above 10 s^{-1} with little variations of the power.

According to the literature, the shear rate is a major determinant of the echogenicity of blood and of the level of erythrocyte aggregation. However, the ultrasound backscattered power has not been studied with blood samples presenting a wide range of aggregation. The reproducibility of the ultrasound method has also not been specifically addressed. The specific objectives of the present study were to provide answers to these questions.

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MATERIALS AND METHODS

Steady Flow Model

The vertical steady flow model and the methodology that was used can be found in Cloutier *et al.*[8] and Qin *et al.*[22] Briefly, the model was composed of a peristaltic pump that circulated blood from a bottom reservoir to a top reservoir that contained dampers to minimize the pulsatile oscillations of the pump. Doppler measurements were performed through a thin-wall Kynar tube having an inside diameter of 12.7 mm. The tube was fixed vertically (parallel to gravity) to eliminate the effect of blood sedimentation. A valve controlled the flow rate in the model and a cannulating type flow probe was inserted into the flow tubing to measure the flow rate with an electromagnetic flowmeter (Carolina Medical Electronics, Cliniflow II, model FM701D). A magnetic stirer was used to continuously mix the blood in the bottom reservoir. An overflow conduit was connected between the top and bottom reservoirs to keep a constant gravity driven blood pressure. At this constant pressure determined by the height difference of the blood levels between both reservoirs, adjusting the opening of the valve controlled the flow rate.

The 10 MHz Doppler probe was positioned at an angle of 45° with respect to the tube axis to measure the backscattered ultrasound echoes. The 3 mm by 3 mm crystal was aligned with the central axis of the tube for all measurements. A micrometer was used to axially move the Doppler probe by steps of 0.5 mm, thus allowing measurements at different radial positions within the tube. Because of the divergence of the ultrasound beam, the position of the probe was changed instead of that of the gated echoes to maintain a constant sample volume size of 3.7 mm^3 (at -3 dB) for all measurements. To maintain a constant sound attenuation when moving the probe and to allow acoustic coupling, the Doppler transducer was immersed in a small blood tank. By gating the echoes backscattered by blood, velocity and backscattered power profiles across the tube were obtained. Experiments were performed at room temperature and flow rates of 100, 180, 250, 500, 750, 1000, and 1250 ml/min. Several minutes were allowed between measurements at a different flow rate.

Blood Sample Preparation

Because the *in vitro* model required a large quantity of blood and to cover a wide range of aggregation level, i.e., from normal to hypo- and to hyperaggregating erythrocytes, horse blood was chosen for all nine experiments reported in this study. A blood sample from a different animal was used for each experiment. To simulate a wide range of aggregation level, different proportions of the total volume of plasma were replaced by an isotonic saline solution, as described by Weng *et al.*[33] Different levels were obtained because of the reduction of the concentration of plasma proteins responsible for the aggregation. The horse blood was collected from a local abattoir using ethylenediamine tetraacetic acid (EDTA, 3 g/l) as the anticoagulant. Blood was brought to the laboratory and stored at 4 °C. All experiments were performed within 48 h after blood collection. Blood was circulated into the flow model for at least half an hour before beginning measurements. This procedure eliminated air bubbles that interfere with the transmission of ultrasound and allowed the temperature of blood to reach that of the ambient air. All experiments were performed at a hematocrit of 40%. The hematocrit was measured by microcentrifugation (Haemofuge, Heraeus Instruments) at 14 980 g_n (12 000 rpm) for 10 min.

Level of Aggregation Measured with the Erythroaggregameter

For each experiment, a blood sample of 1.5 ml was taken from the flow model to measure the aggregation. The erythrocyte aggregation level was determined with a previously validated erythroaggregameter based on a Couette flow arrangement (Regulest, France).[16] The instrument provides measurement of an aggregation index (S_{10}, no unit) that is obtained from the analysis of the variation in light intensity of the signal scattered by blood. The blood sample was sheared for 10 s at 550 s^{-1} to provide rouleaux disruption. After abrupt cessation of the rotation, the variation of the scattered light intensity (780 nm) was recorded during the rouleaux formation process. The index S_{10} was calculated as the ratio of the area above the light intensity curve during the first 10 s following flow stoppage to the total area within the same period of time. A low value of this index indicates a low level of aggregation and *vice versa*. For normal human blood, we reported mean values of S_{10} of 23 ± 3 (mean $\pm$ one standard deviation).[33] Reported intra-assay reproducibility for S_{10} ranged from 2% to 2.3%.[21]

Velocity, Shear Rate, and Backscattered Power Across the Tube

The Doppler ultrasound signal was measured with a pulsed-wave Doppler system developed at the Baylor College of Medicine, Houston, TX. The mean velocity and backscattered power were measured from the frequency spectrum of the Doppler signal at 25 positions across the tube for each flow rate. The Doppler equation was used to relate the Doppler mean frequency shift of the spectrum (f_d) to the mean velocity of erythrocytes (v) within the sample volume ($v = f_d c / 2 f_t \cos\theta$, where c is the speed of sound in blood, f_t is the ultrasound transmitted frequency, and θ is the Doppler angle). The signal processing that was performed to obtain the mean velocity and backscattered power can be found in

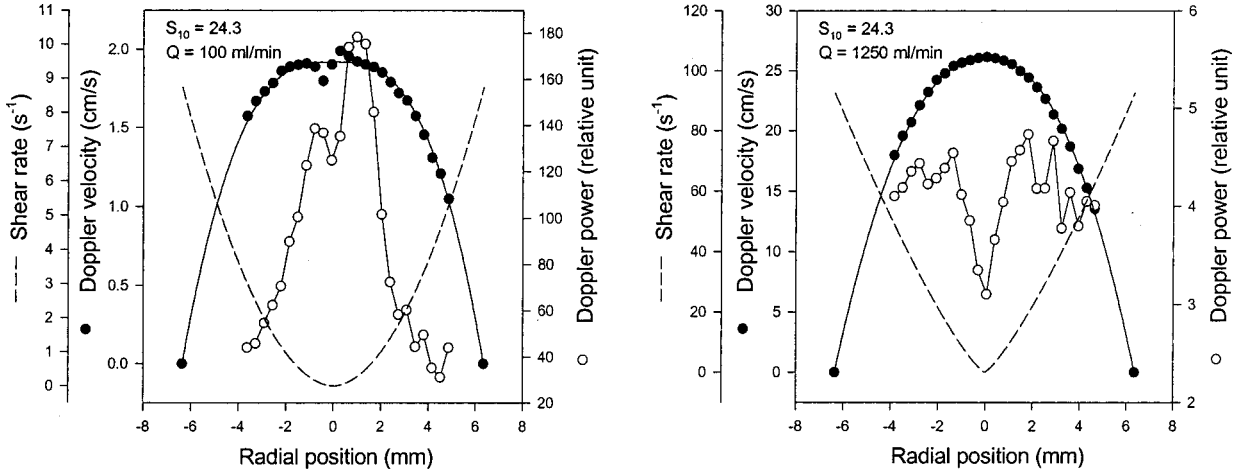

FIGURE 1. Examples of the experimental measurements of the Doppler velocity, the Doppler backscattered power, the fitted power law velocity model (full line), and the shear rate model (dashed line) for flow rates (*Q*) of 100 and 1250 ml/min, and an erythrocyte aggregation index S_{10} of 24.3. The parameter *n* [Eqs. (1) and (2)] was 2.7 at 100 ml/min, and 2.2 at 1250 ml/min.

Cloutier *et al.*[8] The power scales used in this manuscript are linear. All graphs can be compared to each other but they cannot be compared, in terms of absolute power values, to measurements obtained by others. No absolute power units are provided because the characteristics of the ultrasound transducer, instrumentation, and signal processing algorithms affect the intensity of the backscattered echoes.

By definition, the shear rate γ is the rate of change of the velocity for a given displacement. To determine the shear rate at each position of measurement, the velocity profile across the tube was fitted to the following power law model:

$$\nu(r) = \nu_{\max}[1-(r/R)^n], \tag{1}$$

where $\nu(r)$ represents the Doppler mean velocities across the tube, r is the distance from the center of the tube, $\nu_{\max}$ is the maximum centerline velocity, R is the radius of the tube, and n is the power law exponent. The 25 velocity measurements and the zero velocity values corresponding to the position of the wall were used to fit the model of Eq. (1). In a second step, the absolute magnitude of a shear rate profile, $\gamma(r)$, was obtained by calculating the derivative of $\nu(r)$, i.e., $\partial\nu(r)/\partial r$

$$\gamma(r) = n\nu_{\max}r^{(n-1)}/R^n. \tag{2}$$

The shear rate averaged across the tube was computed using

$$\overline{\gamma} = \frac{2v_{\max}}{R}\left(\frac{n}{n+1}\right). \tag{3}$$

The shear rate within the Doppler sample volume at the position $r, \gamma(r)_{sv}$, was estimated by weighting the shear rate $\gamma(r)$ with a theoretical function describing the radial power pattern of the acoustic field. More details about the computation of $\gamma(r)_{sv}$ can be found in Cloutier *et al.*[8] For all flow rates tested, the Doppler power was expressed as a function of the shear rate within the Doppler sample volume, γ_{sv}. To improve the visual presentation of these last results, the experimental Doppler power values were fitted to the following exponential model:

$$P(\gamma_{sv}) = A + Be^{(-\gamma_{sv}/C)}, \tag{4}$$

where P is the Doppler power at the shear rate γ_{sv}, and A, B, and C are the parameters of the model.

Blood Viscosity Measurements

For each experiment reported in this study, viscosity measurements were performed with a cone-plate rheometer (Brookfield, MA, model LVDVIII-CP-42, cone angle = 1.56°). One ml of blood was withdrawn from the flow model and sheared step by step from 288 to 1 s^{-1} in the rheometer. At each step shear rate, 30 s was allowed to obtain a steady state of viscosity. Measurements are reported in centipoise (cP).

RESULTS

Figure 1 shows examples, for $S_{10} = 24.3$, of the distribution across the tube of the Doppler velocity, the Doppler backscattered power, the fitted power law velocity model [Eq. (1)], and the shear rate model [Eq. (2)] for

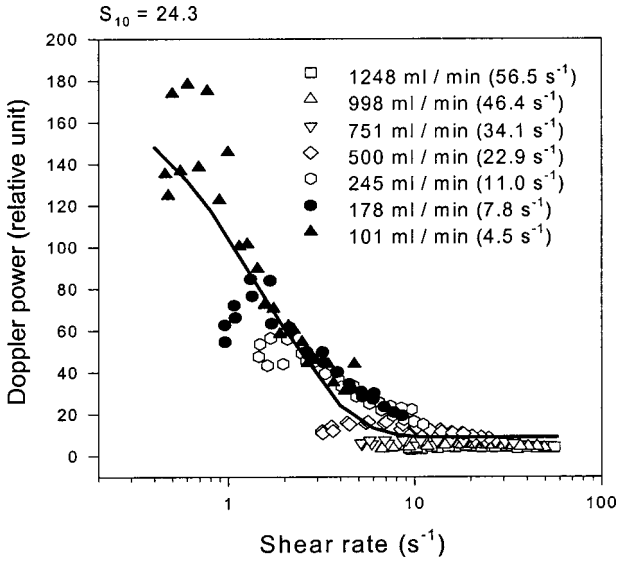

FIGURE 2. An example of the shear rate dependence of the Doppler backscattered power for an erythrocyte aggregation index S_{10} of 24.3. The fitted exponential model was computed from Eq. (4) with $A=8.9$, $B=178.1$, and $C=1.6$ (correlation $r=0.94$). The legend gives the flow rates measured with the electromagnetic flowmeter and the mean shear rate across the tube (number in parenthesis) computed from Eq. (3).

flow rates of 100 and 1250 ml/min. From this figure, it is observed that the Doppler power is much higher at 100 than 1250 ml/min; that the power is maximum between the wall and the center of the tube, and decreases at the tube center; and that the velocity profile is relatively blunt at 100 ml/min and is close to a parabola at 1250 ml/min.

By expressing the Doppler power as a function of the shear rate affecting erythrocytes within the Doppler sample volume $[\gamma(r)_{sv}]$, the shear rate dependence of equine erythrocyte aggregation was obtained. Figure 2 shows an example of the raw data and the fitted exponential model of Eq. (4) for $S_{10}=24.3$. The power was maximum at low shear rates and decreased as the shear rate was increased. At shear rates higher than approximately 10 s^{-1}, little power variations were observed.

Reproducibility of the Doppler Method

A series of five experiments was performed using horse blood models with erythrocyte aggregation levels close to that observed for normal human blood.[33] The parameter S_{10} for those measurements ranged from 23.6 to 24.4 (24.1 ± 0.3). The reproducibility of the velocity and shear rate measurements across the tube was first tested. For this purpose, the power law velocity model of Eq. (1) was fitted to the Doppler velocities measured across the tube and the shear rate model of Eq. (2) was obtained as in Fig. 1. From these results, the reproducibility of the parameters v_{max}, n, and $\bar{\gamma}$ [Eq. (3)] was assessed for all flow rates tested (Table 1). The coefficients of variation shown in Table 1 were all below 12%. The reproducibility of the shear rate dependence of the backscattered power was also determined, as shown in Fig. 3. For each shear rate, the reproducibility was computed as the ratio of the maximum power, obtained from a given experiment, to the minimum power measured from another experiment. The reproducibility was 3.6 ± 0.1 (range of 2.7–3.9), or in decibels, 5.5 ± 0.2 dB (range of 4.3–5.9 dB).

Shear Rate Dependence of the Backscattered Power for Different Levels of Erythrocyte Aggregation

As shown in Fig. 4 for shear rates below 30 s^{-1}, the power increased monotonically with the level of aggregation. For shear rates higher than approximately 50 s^{-1}, little Doppler power variations were observed for all experiments. For the highest level of aggregation ($S_{10}=38.6$), the range of variation of the Doppler power from 0.4 to 50 s^{-1} was approximately 16.1 dB (41 times). For that experiment, the shear rate needed to disrupt erythrocyte aggregates was much higher than that

TABLE 1. Reproducibility of the parameters v_{max}, n, and $\bar{\gamma}$.

Mean flow rate (ml/min)	v_{max} (cm/s)	n	$\bar{\gamma}$ (s^{-1})
1254 ± 8	25.3 ± 0.7 (3%)	2.31 ± 0.09 (4%)	55.7 ± 1.1 (2%)
1006 ± 10	20.2 ± 0.5 (3%)	2.35 ± 0.09 (4%)	44.8 ± 0.9 (2%)
752 ± 8	15.0 ± 0.4 (3%)	2.35 ± 0.09 (4%)	33.3 ± 0.8 (2%)
500 ± 5	9.8 ± 0.4 (4%)	2.37 ± 0.06 (3%)	21.9 ± 0.5 (2%)
250 ± 6	4.6 ± 0.3 (7%)	2.63 ± 0.12 (5%)	10.6 ± 0.6 (6%)
176 ± 3	3.2 ± 0.3 (9%)	2.68 ± 0.08 (3%)	7.3 ± 0.6 (8%)
100 ± 7	1.8 ± 0.2 (11%)	2.95 ± 0.17 (6%)	4.1 ± 0.5 (12%)

Values are means $\pm$one standard deviation; $n=5$. v_{max}, maximum centerline velocity [Eqs. (1) and (2)]; n, power law exponent [Eqs. (1) and (2)]; $\bar{\gamma}$, mean shear rate across the tube [Eq. (3)]. These results were obtained from five experiments with an erythrocyte aggregation index S_{10} of 24.1 ±0.3. The numbers in parenthesis are the coefficients of variation of each measurement in percent.

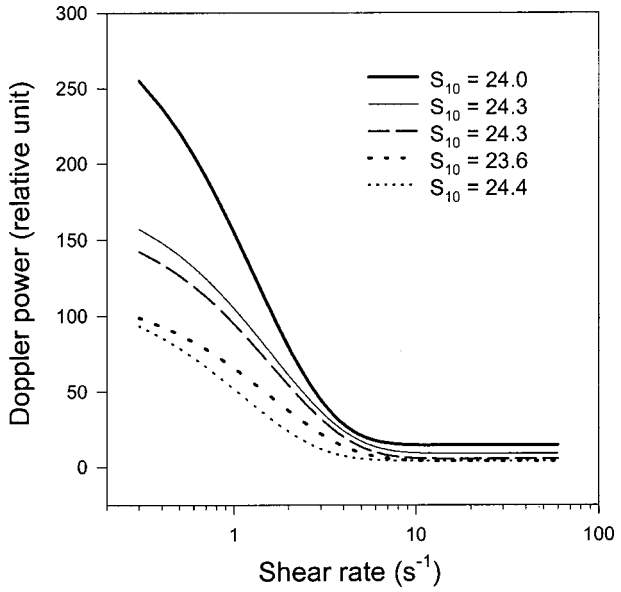

FIGURE 3. Shear rate dependence of the Doppler backscattered power for five experiments characterized by similar erythrocyte aggregation levels ($S_{10}=24.1\pm0.3$). The model of Eq. (4) was used to fit the experimental data points (correlation r ranged from 0.88 to 0.96, $A=7\pm4$, $B=175\pm77$, and $C=1.5\pm0.2$).

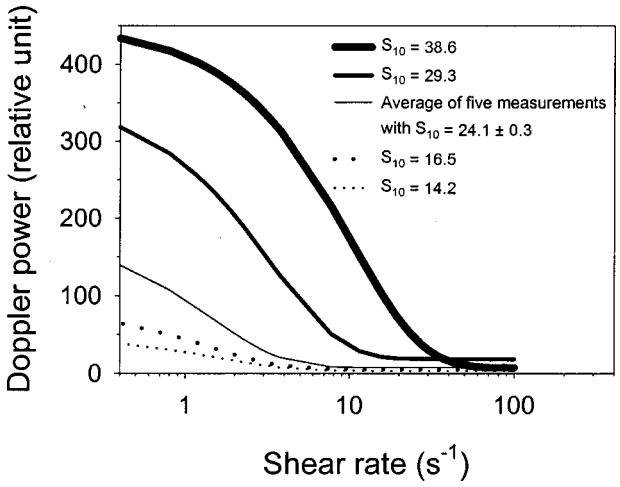

FIGURE 4. Shear rate dependence of the Doppler backscattered power for horse blood models characterized by different erythrocyte aggregability. The parameter S_{10} reflects the level of aggregation measured with the laser reflectometry method. The model of Eq. (4) was used to fit the experimental data points (correlation r varied from 0.74 to 0.96). Values of A, B, and C for S_{10} of 14.2, 16.5, 29.3, and 38.6 were, respectively, 3.1, 44.8, and 1.6; 5.0, 79.2, and 1.4; 18.4, 338.4, and 3.29; and 7.1, 443.4, and 10.2.

needed for any other experiments. For S_{10} close to the values measured for normal human blood (the five experiments at $S_{10}=24.1\pm0.3$), a rapid reduction of the power was observed between 0.4 and 3 s^{-1}, a transition region was noted between 3 and approximately 7 s^{-1}, and a very low reduction of the power occurred beyond 7 s^{-1}. The power variation as a function of the shear rate was 13.2 dB (21 times), approximately. For hypoaggregating erythrocytes ($S_{10}=16.5$ and 14.2), the Doppler power variations occurred only at very low shear rates. The range of variation of the Doppler power for $S_{10}=14.2$ was around 7.8 dB (six times).

Relationship Between the Viscosity of Blood and the Doppler Backscattered Power

Erythrocyte aggregation is the major determinant of blood viscosity at low shear rates.[3] Consequently, a relationship should exist between the viscosity and the ultrasound backscattered power. Figure 5 shows the viscosity as a function of the shear rate, for different levels of erythrocyte aggregation. As noted earlier for the Doppler power (Fig. 4), the viscosity monotonically decreased as the shear rate was increased for most S_{10} values. With the exception of some overlap for $S_{10}=14.2$ and 16.5, the viscosity was proportional to S_{10} for shear rates lower than 10 s^{-1}, approximately. A multiple linear regression analysis (SigmaStat®, SPSS Science, Chicago, IL, version 2.03 for Windows®) was performed to relate the viscosity of blood to the Doppler power (for shear rates between 1 and 50 s^{-1}). As seen in Fig. 6, the viscosity was predicted ($r=0.80$, standard error of the estimate = 63.6 cP) by the linear combination of the constant ($p<0.001$), Doppler power ($p<0.001$), and logarithm of the shear rate ($p<0.001$). The variance of the estimate increased markedly with increasing viscosity.

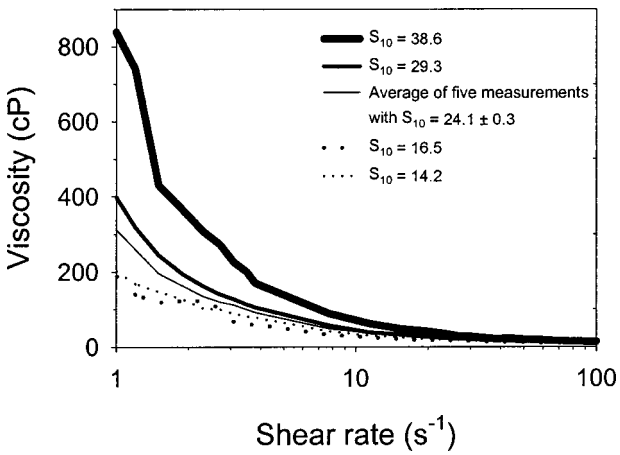

FIGURE 5. Shear rate dependence of the viscosity for horse blood models characterized by different erythrocyte aggregability. The parameter S_{10} reflects the level of aggregation measured with the laser reflectometry method.

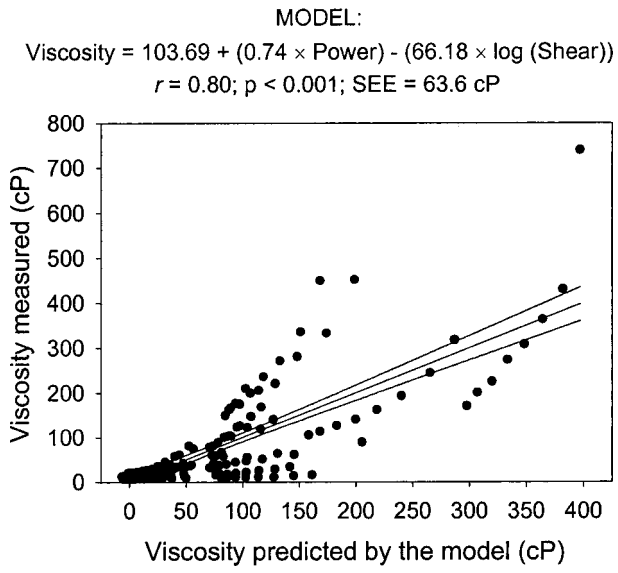

FIGURE 6. Relationship between the viscosity of blood measured with the cone-plate rheometer (*y* axis) and that predicted by the multiple linear regression model (*x* axis) for shear rates varying between 1 and 50 s^{-1}. The regression line and the 95% confidence interval are plotted on the graph. The variable ''power'' represents the Doppler backscattered power (relative unit), ''shear'' is the shear rate in s^{-1}, ''*r*'' is the correlation coefficient, and SEE is the standard error of the estimate in cP.

DISCUSSION

The present study showed the dependence of the Doppler power at 10 MHz on the shear rate and the level of aggregation (S_{10}) of equine blood models. For the range of shear rate considered (0.4–50 s^{-1}), the exponentially decaying relationship reported in Figs. 2–4 are in agreement with results obtained by others for human and porcine blood.[8,13,28,31] Very few studies investigated the shear rate dependence of the ultrasound backscattered power as a function of the level of aggregation. When performed, comparisons were generally made between whole blood and erythrocyte saline suspensions (no aggregation).[31,34] In a study by Van Der Heiden *et al.*,[31] human whole blood mixed with 4.5 mg/ml of dextran 200 was used to enhance the aggregation but the power expressed as a function of the shear rate was similar to that obtained for whole blood without dextran (less aggregation). The present study clearly showed, however, that the Doppler power as a function of the shear rate depends on the erythrocyte aggregation level (measured with S_{10}).

According to Fig. 1, the flow rate affected not only the ultrasound backscattered power but also the velocity profile across the tube. As seen in Table 1, more blunted velocity profiles ($n>2$) were found when the flow rate was reduced from 1254 ml/min ($n=2.31$) to 100 ml/min ($n=2.95$). In Fig. 1, a reduction of the Doppler power was found at the center of the tube for both flow rates. Similar observations were previously reported with equine and porcine whole blood.[22,25,35] This power drop, known as the ''black hole'' phenomenon, is explained by the reduction of the interactions between erythrocytes and thus of the level of aggregation at low shear rates.[5,10] It has been suggested[35] that a nonuniform hematocrit distribution might explain the black hole. However, Mo *et al.*,[20] using magnetic resonance transverse relaxation rates over the tube cross section showed no significant hematocrit variation between the hypoechoic central zone and surrounding regions. Additional theories regarding the black hole phenomenon which consider the effect of the entrance length of the tube[25] and anisotropy in the shape of the aggregates[22] have also been reported. Besides these theories, the effect of destructive wave interference attributed to the structure and spatial arrangement of the aggregates can be considered, but this has to be confirmed.

Based on the Rayleigh scattering theory,[27] the power backscattered by one particle (backscattering cross section) depends on the fourth power of the ultrasound transmitted frequency, and the square of the particle volume. At a constant volume concentration (hematocrit), the ultrasound backscattered power is proportional to the scatterer's mean volume.[7,12] Provided that they are similarly distributed in space and small enough to satisfy the Rayleigh scattering condition. This condition requires that the dimension (diameter) of the scatterers in the direction of propagation of the ultrasonic waves is less than one tenth of the wavelength.[27] At 10 MHz, for a speed of sound in blood at 1570 m/s, the dimension of the scatterers should be less than 15.7 μm for this condition to be met.

According to this theory, doubling the mean volume of erythrocyte aggregates, at a constant hematocrit of 40%, should produce a similar impact on the backscattered power. In reality, this may not be the case. The packing organization of the aggregates in space may also affect the backscattered power. In the present study, the variations in Doppler power may thus be attributed to changes in volume, shape, and spatial arrangement of erythrocyte aggregates for the different equine blood models tested.

According to simulation results performed by our group at 10 MHz,[30] monodispersed rouleaux or clumps should contain less then ten erythrocytes, approximately, to be considered as Rayleigh scatterers. For non-Rayleigh scattering, the simulation results by Teh[30] suggest that the backscattered power may reach a plateau or decrease in magnitude when the aggregates further increase in size. By assuming Rayleigh scattering and a linear increase of the Doppler power with the mean scatterer's volume, this would limit possible power variations as a function of the shear rate to a ten fold increase

(aggregates of ten erythrocytes maximum). In the present study, up to 41 fold increase was observed which suggests an effect, on the Doppler power, of other factors such as the shape and packing arrangement of the scatterers.

According to this discussion, it is clear that the results of Fig. 4 cannot simply be interpreted in term of linear changes in the mean erythrocyte aggregate volume. In other studies,[13,28,31] the variation of the ultrasound backscattered power as a function of the shear rate for normal human blood ranged from 13 to 15.6 dB, at ultrasound frequencies varying between 10 and 35 MHz. In the present study, the range of variation of the Doppler power at 10 MHz was 13.2 dB for the equine blood models simulating normal human blood ($S_{10}=24.1 \pm 0.3$), whereas it was 16.1 dB for $S_{10}=38.6$ and 7.8 dB for $S_{10}=14.2$ (see Fig. 4). By comparing these variations to those reported in the literature,[13,28,31] the equine blood models seemed to adequately simulate hypo-, normal, and hyperaggregating human erythrocytes.

The correlation with the model of Eq. (4) was 0.94 for the data presented in Fig. 2. In Fig. 4, $r=0.74$ for $S_{10}=38.6$, whereas it was higher than 0.88 for all other measurements (range of 0.88–0.96). The lower correlation for $S_{10}=38.6$ can be explained by the higher magnitude of the black hole for erythrocytes with a high kinetics of aggregation.[22] For instance, at a given flow rate, one consequence of the black hole is to produce scattering of the backscattered power in the central region of the tube where the shear rate is minimum. In Fig. 2, the scattering at low shear rates around the fitted exponential model can in part be attributed to this effect. In addition, another explanation can be given for the range of correlations measured (0.74–0.96). According to Fig. 1, the position within the tube where a given shear rate was measured could differ as a function of the flow rate. For example, a shear rate of 10 s^{-1} was measured when the probe was close to the wall at a flow rate of 100 ml/min, whereas this shear rate was detected around the tube center at 1250 ml/min. Thus, for a given shear rate, each data point in Fig. 2 could be obtained from power Doppler measurements performed at a different tube position. Around the tube center, the spatial variation of the shear rate is much smaller than that observed closer to the wall. This may affect the way erythrocyte aggregates interact with each other within a given volume of blood insonified by the ultrasound transducer, and thus the backscattered power.

As shown in Fig. 3, the reproducibility of the ultrasound backscattered power was 5.5 dB for blood samples with similar $S_{10}=24.1 \pm 0.3$. At a first glance, the backscattered power seems less accurate in comparison to laser reflectometry. This can be attributed to the fact that ultrasound backscattering may be sensitive to structural aggregate changes that are not detected by laser reflectometry. For instance, light scattering depends on the mean distance between scattering events, which scales as the inverse of the scattering area per unit volume, the probability of photon absorption between successive scatterings (multiple scattering), and the anisotropy (orientation) of scatterers.[29] As discussed earlier, ultrasound scattering is a function of the volume and spatial organization of scatterers. Multiple scattering between erythrocytes is negligible with ultrasound, and the anisotropy in the shape of particles should influence the intensity of the signal only when they are no longer Rayleigh scatterers.[27] Thus, one major difference between both approaches that may explain the variance of Fig. 3 is the fact that light scattering is a function of the area of erythrocyte aggregates, whereas ultrasound scattering depends on their volume. Because ultrasound echoes are scattered by the three-dimensional structure of the aggregates, this method may be more sensitive to changes in their shape and spatial arrangement.

A fair association was found between the shear rate dependence of blood viscosity and the ultrasound backscattered power (see Figs. 4, 5, and 6). For instance, the prediction of the viscosity from the Doppler power and the logarithm of the shear rate was less accurate for high viscosity values, as seen in Fig. 6. The lower accuracy of cone-plate rheometer at low shear rates (high viscosities) may have contributed to this variance. There is also the possibility that the shear dependence of blood viscosity in the cone-plate rheometer may not be a good determinant of the spatially shear-varying viscosity in tube flow. The different mechanisms affecting the viscosity and the ultrasound backscattered power, although similar, can also be an explanation. At a constant hematocrit, the viscosity of blood is determined by the size and fractal dimension of the aggregates, the maximum packing fraction of the aggregates, and the critical shear stress that is representative of the particle surface adhesive energy.[29] As noted earlier, the factors influencing ultrasound backscattering are slightly different.

Physiological Relevance

The detection of high viscosity and/or erythrocyte aggregation with ultrasound would be of major importance to identify vessel areas prone to the development of thrombosis (flow stasis). Under conditions for which erythrocyte aggregation is increased (hyperlipidemia, hypertension, diabetes, atherosclerosis, thrombosis, some cancers, aids, etc...), the blood becomes more viscous and the adhesive strength between erythrocytes forming aggregates is increased.[4] In large vessels, a major consequence of the increase in erythrocyte aggregation is the occurrence of stagnation in areas of flow separation and recirculation at low shear rate. The presence of flow stasis seems to play an important role in the mechanism

of thrombosis in veins, possibly arteries, and heart chambers.[18,32] The higher residence time in areas of flow stagnation increases the interaction of cellular and plasmatic elements with the endothelium. The presence of compact erythrocyte aggregates and particle crowding may further promote this process by displacing platelets and leukocytes toward the vascular wall.[14] In the microcirculation, erythrocyte aggregation affects the flow resistance and the perfusion of tissues.[2,11,19] The increase in flow resistance can be attributed to the higher viscosity and higher energy needed to break compact aggregates upon entry into capillaries.

Conclusion

With the exception of ultrasound backscattering and intravital microscopy, no other method can assess the level of aggregation *in situ* in animal or human vessels. The visualization of erythrocyte aggregates with intravital microscope is limited to vessels below 30 μm, approximately, and this technique was mainly used for animal studies.[2,11,19] Ultrasound backscattering was utilized to measure erythrocyte aggregation in human veins and arteries.[9,17] In Cloutier *et al.*,[9] the attenuation of tissues was compensated by knowing the depth of each measurement. Recent developments suggest that this technique may be applicable to study erythrocyte aggregation in vessels as small as 50 μm.[6] Ultrasound backscattering can be used to measure erythrocyte aggregation *in vivo* noninvasively because ultrasonic waves propagate through soft tissues.

All accepted methods to study erythrocyte aggregation in human require the withdrawal of blood, the use of anticoagulant, and the analysis in a laboratory instrument.[1,3,23,24] With some exceptions, these instruments can only be found in research laboratories. On the other hand, ultrasound instruments are available in most obstetric, radiology, and cardiology hospital departments. The development of ultrasound backscattering would certainly stimulate clinical studies aimed at elucidating the role of erythrocyte aggregation in cardiovascular diseases. This noninvasive technique has also the potential to improve our basic understanding of the relationship between the hemodynamic of the circulation and erythrocyte aggregation in animal and human vessels.

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