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Rheological and flow properties of blood investigated by ultrasound

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Ultrasonic waves of 1-15 MHz frequencies easily propagate through soft biological tissues, thus providing qualitative and quantitative information on mechanical and flow properties of blood and red blood cell (RBC) suspensions. Two types of techniques allow to investigate blood behaviors: echographic devices via amplitude detection and Doppler effect based devices via frequency detection of the ultrasonic signal. When ever B mode serves to construct images of tissue slabs from the ultrasonic backscattering coefficient and can give qualitative information on the mechanical properties of blood, A-mode allows to quantify the ultrasonic backscattering coefficient. Ultrasonic Doppler modes also provide both qualitative and quantitative information on blood flow velocity: continuous and pulsed Doppler modes provide curves of blood flow versus time when color Doppler and power Doppler imaging visualize blood flowing in human vessels. Association of echographic and Doppler modes to investigate simultaneously structure and velocity of blood is commercially available. Some examples of results given by such ultrasonic techniques that contribute to characterize, both *in vitro* and *in vivo*, structure and flow properties of blood or red blood cell (RBC) suspensions are presented.

Keywords: Blood, Flow properties, Rheological properties, Ultrasound

Medical ultrasonic techniques such as echography, continuous or pulsed wave Doppler scanning or color Doppler imaging are nowadays the most useful medical non-invasive techniques used in routine to explore in first intention, both mechanical properties of soft human organs except lung and velocity of blood flow in human vessels. Indeed, interaction between ultrasound of 1 to 15 MHz frequencies and biological tissues provide qualitative and quantitative information on rheological and flow properties of blood. Therefore such ultrasonic techniques become more importance to characterize mechanical properties of biological cells such as red blood cells (RBCs) and particularly the structure of blood suspensions, and to explore in routine blood vessels and hemodynamics. For example, ultrasonic scattering depending on the dimension of the scattering centres encountered by ultrasonic waves provides information on the size of red blood cell or platelet aggregates present in the medium by amplitude analysis or on RBC aggregation kinetics by frequency analysis of the echographic signal. Measurement of blood velocity can also be assessed in medical investigation to characterize by ultrasonic Doppler effect, the velocity profile (parabolic or plug) or the type (laminar or turbulent) of blood flow. Different means allow to present echographic or velocity information to the physician: the echographic A or B mode signals (1D signal), the Doppler (or velocity) signal, or color echo-Doppler (2D) imaging.

All the ultrasound echographic and Doppler methods are based on reflection or scattering of ultrasonic waves. In general way, an emitting-receiving ultrasonic transducer emits wave bursts propagating in tissues, just in front of the ultrasonic transducer, and interact with them. Discontinuities of acoustical impedance schematically produce reflection of waves in presence of large interfaces such as vessel walls or scattering in presence of obstacles of small size with respect to the ultrasonic wavelength such as red blood cells. Amplitudes of waves that return back to the transducer are then presented versus flight time (or penetration depth). This representation of the ultrasonic signal is named A-mode echography. B-mode, defined after transformation of amplitude of the ultrasonic signal into brightness, is also presented versus flight time. A low or fast scanning of the ultrasonic field in a direction perpendicular to the propagation direction gives finally a 2D-echographic image of a slab of tissue. For a dense suspension of Rayleigh aggregates, the ultrasonic backscattering coefficient scales as the volume of the scattering centres[1-3] :

$$\chi_r = \chi_a / \chi \approx \left(\frac{R}{a}\right)^3 \qquad \ldots(1)$$

where χ_r is the dimensionless backscattering coefficient defined as the ratio of the backscattering coefficient for an aggregated suspension, χ_a, to the backscattering coefficient for the same completely disaggregated suspension, χ. R and a are the mean radii of respectively aggregates and individual RBCs both considered as spherical.

When an ultrasonic wave of frequency F_0 encounters obstacles such as red blood cells moving in vessels with velocity, V, the frequency of the scattered wave, F_{sc}, is different from F_0, smaller or larger, depending on V and the angle, θ, between the velocity direction and the ultrasonic field direction. This difference between frequencies of the incident and the scattered waves, $(F_0 - F_{sc})$, is the Doppler frequency, F_D, related to V by the well known Doppler relationship available for an emitting-receiving ultrasonic device:

$$F_D/F_0 = (F_{sc} - F_0)/F_0 = (2V/c) \cos \theta \qquad \ldots(2)$$

Thus, measurement of the Doppler frequency, F_D, and knowledge of the angle, θ, allow to calculate blood velocity and to display either velocity curve versus time or 2D color Doppler images.

Information provided by ultrasound from blood or red blood cell suspensions, and detected by echographic or Doppler devices to characterize mechanical and flow properties of blood can be quantitative, semi-quantitative or qualitative. Several authors have already been interested during the last two or three decades to investigate red blood cell suspensions by ultrasound. Much of contributions have been directed towards a better understanding of ultrasonic scattering processes between ultrasonic waves and blood and its componants[4-9]. Ultrasound scattering technique provides indeed a way to explore quantitatively the aggregation process of red cells by using the Rayleigh scattering theory. The ultrasonic backscattering coefficient (BSC) from suspensions at rest or in controlled shear rate, when quantified, can be considered as an index of aggregation. Moreover, frequency analysis of BSC versus time can help to characterize aggregation kinetics[10]. The backscattering coefficient can also be used to built images of blood and provides qualitative or semi-quantitative information on its echogenicity, either *in vitro* or *in vivo*[11–15]. On the other hand, many investigations have been conducted in connection with Doppler effect and ultrasound signal processing to determine medical indications in routine for exploration of blood flow and tissue motion[16–18]. Intra vascular ultrasonic probes have also been developed to explore blood behaviors directly inside vessels[19]. Noninvasive evaluation of intracardiac or intravascular pressure gradients are also assessed by using the Bernouilli equation[20]. Power Doppler ultrasound scanning, pulsed Doppler scanning and color Doppler flow imaging can determine *in vivo* conditions of blood cell aggregation[21].

The aim of the present article is to propose, among all the echographic techniques employed for biomedical applications, those specifically employed to explore the cardio-vascular system, and to present some results given by different methods providing quantitative and semi-quantitative information on mechanical and flow properties of blood and red blood cells, both *in vitro* and *in vivo*. The first technique concerns *in vitro* ultrasonic characterization of RBC suspensions by scattering and shows how the ultrasonic technique is able to quantitate physical and geometrical parameters of aggregates[1,11]. The second technique explains how both echography and Doppler velocity *in vivo* measurements can contribute to characterize spontaneous echocardiographic contrast (SPEC), a phenomenon encountered on trans-oesophageal echographic images, in patients with non-valvular atrial fibrillation, and considered by the physician as a factor of thrombo-embolic risk[22,23].

Methods

In vitro rheo-acoustical determinations were carried out to characterize the aggregation state of RBC suspensions both at rest and in controlled shear stress. At rest, the ultrasonic backscattering coefficient was measured on RBC suspensions sedimenting inside a vertical cavity and insonified by ultrasonic wave bursts of 6 MHz, using a technique described previously[13]. For measurements in the controlled shear stress[1–3], the flow field was generated between a stationary plate and an upper rotating altuglas plane disk, separated from the plate by a distance *h*, and driven by a stepper motor. The lower compartment was filled with water to ensure a good ultrasonic coupling, the transmitter-receiver ultrasonic transducer emits short ultrasonic wave bursts of frequency 8 MHz in the direction perpendicular to the

plane-plane flow device. The average distance *r* from the explored volume to the axis of the plane-plane flow device and the angular velocity ω of the rotating disk determine the average local shear rate $\gamma \approx r\omega/h$ experienced by the insonified region across the gap width $h << r$. Measurements were performed in the range $0.1\ s^{-1} < \gamma < 50\ s^{-1}$. The suspension was first dispersed in an intense flow ($\gamma = 128\ s^{-1}$) before imposing the relevant shear rate γ at time $t = 0$. The flow was then quickly stopped to suppress any orientation of particles and anisotropy of the suspension microstructure. After flow stoppage, the BSC reaches a representative dynamical aggregation equilibrium for the aggregated suspension. The signal was then measured during the steady state following each relevant shear rate. With both experimental devices, the scattered waves coming back to the probe were transformed into an electrical signal (A-mode) first amplified and filtered before direct sampling, using a Tektronix 520 digitizer. The digitized signals are downloaded to a microcomputer (Macintosh, Power Mac 7200) where calculations of χ_a are performed. To determine the shear stress, the viscosity of the suspensions was measured in a Couette viscometer (RCHAIX-MCCA, France) with ± 3% accuracy from the steady-state torque reading.

For the spontaneous echo contrast *in vivo* study, semi-quantification of blood echogenicity, determined on echographic images of heart and associated to a quantification of blood velocity measured on color Doppler imaging of the heart cavities, were performed with a commercially avalaible echograph (ATL, USA) by using both the transthoracic and transoesophageal accesses. Transoesophageal echocardiography provides images for semi-quantification of SPEC and quantification of blood velocity. Velocity determinations were performed at different locations inside the left atrial and the left appendage on flow color Doppler images. Transthoracic echocardiography provides diameter, surface and volume measurements of the left atrial. Determinations were conducted in two groups of patients, 19 with prosthetic or stenotic mitral valve disease with non- valvular atrial fibrillation, all of them susceptible of presenting SPEC and 24 controls in sinusal rhythm without significant valve disease. Semi-quantitative evaluations of spontaneous echocontrast were determined by two independent observers at two different ultrasonic frequencies (5 MHz and 7 MHz), on 2D transoesophageal echocardiographic images. Fibrinogen concentration and the ultrasonic backscattering coefficient were determined *in vitro* respectively on plasma and blood samples after adjustment of hematocrit to 30%. Statistical analysis was done by using, first, the kappa test to evaluate the intra and inter observer variability for determination of presence or absence of SPEC on images analysed and, second, the Mann and Whiney statistical test to evaluate the level of significance of the different hemodynamical and hemorheological parameters measured previously in patients and controls[24].

For *in vitro* ultrasound characterization, blood samples are withdrawn, from healthy human donors or patients and examined on the day of withdrawal. After centrifugation and removal of the plasma and the white cell-platelet layer, red blood cells were washed twice in PBS (10 min at 3000 rpm). Finally, red cells were suspended in dextran 70 (MW: 70000 Dalton) saline solutions (ionic strength 150 mM) to artificially induce RBC aggregation, and at different hematocrit H (10% ≤ H ≤ 30%).

Results

In vitro measurements at rest and in controlled shear stress

For aggregated RBC suspensions studied at rest (i.e., during sedimentation), the ultrasonic backscattering coefficient was low just after emptying the measurement cell. Then fluctuations occur in the signal and χ_a increases to a plateau during which it was measured to characterize the RBC aggregation state of the medium. Variations of the BSC versus hematocrit of the suspension show large differences between non-aggregated suspensions of RBC in buffer and normal aggregated blood samples[13].

For aggregated RBC suspensions studied in controlled shear, the BSC versus the shear rate is measured. For example, Fig. 1 shows the shear rate dependence of $\chi_{a(\gamma)}$ upon the particle volume fraction (*H*) for normal red blood cells suspended in 3 g% dextran 70 – PBS ($0.16 \le H \le 0.35$). When increasing both the particle volume fraction and the shear rate, the steady ultrasonic backscattering coefficient, $\chi_a(\gamma)$, decrease until a value close to $\chi \approx 5\times10^{-5}\ cm^{-1}$, the value given by non-aggregated suspensions. We further measured the backscattering coefficient versus the shear stress $\tau = \mu_a(\gamma)\gamma$ (where μ_a is the viscosity of the aggregated suspension

determined by viscometry) to estimate the critical shear stress inducing a complete disaggregation. Representation of the dimensionless backscattering coefficient against the shear stress show just one curve, independent of hematocrit, and directly indicated the same critical shear stress (0.45 N/m) for all the suspensions whatever hematocrit (Fig. 2).

***In vivo* spontaneous echo contrast study**

Table 1 presents the intra- and inter-observer variability between the two independent observers obtained after a dichotomic stratification of echogenicity of the left atrial found on echographic images between subjects with SPEC+ or SPEC. A perfect agreement between analyses by two observers corresponding to a kappa value +1, table I shows that detection of echogenicity is easier at 7 MHz than at 5 MHz, but the kappa test indicates a good agreement between both observers for the two ultrasonic frequencies.

Figure 3 presents a comparison of diameter, cross-sectional area and volume of the left atrial in the two groups, SPEC+ and SPEC-, after dichotomic stratification. For all these parameters, results found in the group SPEC+ were significantly higher than in the group SPEC– ($P < 10^{-3}$). Determinations of velocity at different locations in the left cavities (left atrial (LA), left atrial appendage (LAA), left pulmonary vein system (LPVS)) showed a significant decrease ($P < 10^{-2}$) of velocity in the group SPEC+ (Fig. 4).

Fibrinogen concentration and the BSC determined *in vitro* at rest, respectively on plasma and blood samples, for both groups of subjects are presented in Fig. 5. Levels of fibrinogen and the backscattering coefficient are significantly higher for subjects with SPEC+.

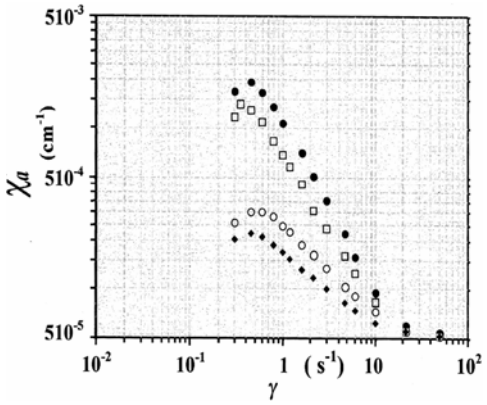

Fig. 1 — Ultrasonic backscattering coefficient χ_a *versus* shear rate γ for deformable red cells in 3g% dextran 70 - PBS. Particle volume fraction $\phi = 0.16$ (●), $\phi = 0.20$ (□), $\phi = 0.30$ (O) and $\phi = 0.35$ (♦).

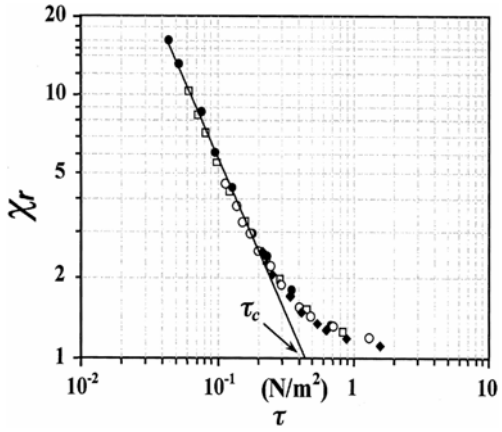

Fig. 2 — Dimensionless ultrasonic backscattering coefficient χ_a/χ *versus* shear stress τ for deformable red cells suspended in 3g% dextran 70 - PBS. Particle volume fraction $\phi = 0.16$ (●), $\phi = 0.20$ (□), $\phi = 0.30$ (O) and $\phi = 0.35$ (♦). τ_c is the critical shear stress. The slope of the straight line is 3/2.

Table 1 — Intra- and inter observer variability using the Kappa test after dichotomic stratification into two groups of patients, spontaneous echo contrast positive and spontaneous echo contrast negative, at two ultrasonic frequencies, 5 MHz and 7 MHz.

	Intra	Inter
5 MHz 2D Echo	0.81	0.76
7 MHZ 2D Echo	0.90	0.86

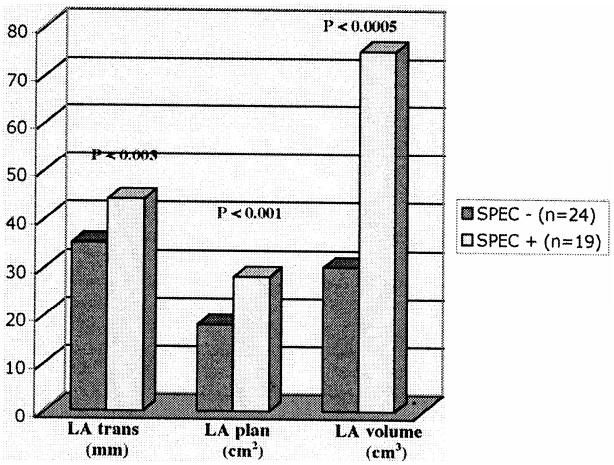

Fig. 3 — Comparison of the left atrial dimensions (diameter, cross-sectional area, volume) between the two groups of patients: spontaneous echo contrast positive (SPEC+) and spontaneous echo contrast negative (SPEC-).

Discussion

Ultrasonic pulse-echo systems can provide range-finding, time-position and real-time two-dimensional images of soft-tissue structures[25]. Therefore, mechanical behaviours of RBC suspensions can be analyzed by ultrasound either *in vitro* or *in vivo*. Moreover, the ultrasonic-wave Doppler effect can be used to study tissue motion and blood flow inside the vessels. Using appropriate ultrasonic instruments, characterization of tissues, blood flow volume rates, blood flow velocity profiles, pressure gradients, orifice area, volume determinations, and flow disturbance can be investigated[19,20]. Thus, such *in vivo* investigations have clinical applications in the exploration of cardiac, cerebral and peripheral blood flow[23].

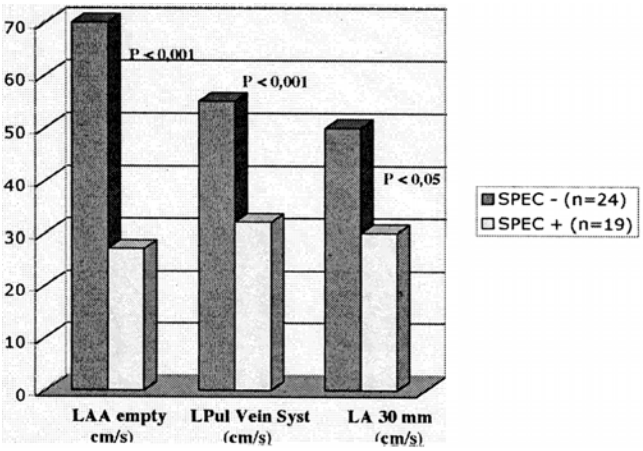

Fig. 4 — Comparison of the blood velocity in the left atrial appendage (LAA), the left atrial (LA), the left pulmonary vein system (LPVS) between the two groups of patients: spontaneous echo contrast positive (SPEC+) and spontaneous echo contrast negative (SPEC-).

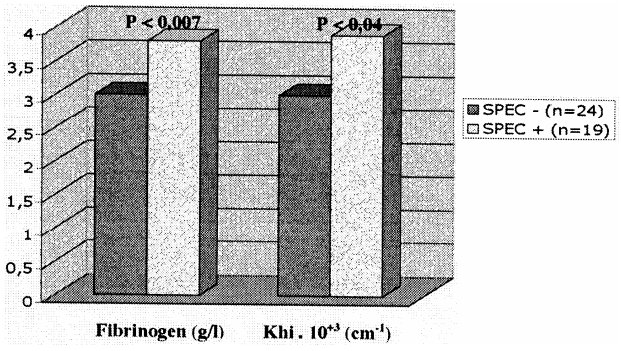

Fig. 5 — Comparison of the *in vitro* measured parameters (plasma fibrinogen concentration, ultrasonic backscattering coefficient « khi ») between the two groups of patients: spontaneous echo contrast positive (SPEC+) and spontaneous echo contrast negative (SPEC-).

Information given by ultrasonic devices can be quantitative by measuring, from the echographic signal, dimensions, velocities, or the BSC, and semi-quantitative or qualitative by evaluating directly on echographic images shape, geometry or echogenicity of organs. Therefore, ultrasound is a good means to evaluate RBC aggregation in different conditions by quantification of the ultrasound backscattering coefficient considered as an index of RBC aggregation, blood flow by quantification of blood velocity, and blood echogenicity.

In vitro measurement of the backscattering coefficient from blood and red blood cell suspensions and analysis of their variations at rest or in controlled shear conditions give information on the medium structure. From a theoretical rheo-acoustical model of fractal RBC aggregates in concentrated suspensions [1], present results are explained. All the curves obtained for the shear rate dependence of the backscattering coefficient (Fig. 1) present a bell shaped curve indicating a restructuration and a compaction of RBC aggregates in the low shear regime. Higher shear rates induce RBC disaggregation and a decrease in the ultrasound backscattering coefficient. Particle crowding increases the suspension viscosity and the shear stress experienced on RBC clusters then reduces the cluster size. If we consider the variations of the dimensionless BSC $\chi_r = \chi_a / \chi$ versus the shear stress $\tau = \mu_a(\gamma)\gamma$ for red cells suspended in dextran 70 saline solution to take account for the microrheological conditions around the clusters whatever the particle volume fraction (Fig. 2) we can first define the experimental critical shear stress τ_c inducing a near complete dispersion, in term of extrapolated intercept [1,2]. Moreover, χ_r displays no significant dependence on particle volume fraction. The master curve $\chi_r(\tau)$ thus well establishes the validity of the effective medium approximation used in the microrheological models [26,27]. The experimental critical disaggregation shear stress for normal red blood cells $\tau_c \approx 0.45\,\mathrm{N/m^2}$ (Fig. 2) is representative of cell adhesiveness and reflects the mechanical force required to disrupt adhesive bonds between two particles. With a slope of the straight part of the curve $\chi_r(\tau)$ equal to 3/2, red cell clusters may be considered as soft clusters undergoing irreversible deformation under the action of external shear stresses, because of weak bonding energy between particles[1,2].

Quantification of tissue echogenicity is not obvious *in vivo* because of the attenuation of the echographic signal and ultrasound frequency shift with exploration depth. However, a semi-quantification of blood echogenicity associated with a velocity quantification of blood flow provide complementary diagnosis information useful for the physician. Simultaneously data of blood echogenicity and blood flow velocity measured in the left atrial of patients with SPEC+ (Table 1) are correlated, first, with an increase of the size of the left atrial, and secondly, with a decrease of blood velocity inside the left cavity. These physio-pathological conditions promote RBC aggregation inside the heart cavity, aggregation explaining spontaneous echo-contrast[28,29]. Moreover, a significant increase in both plasmatic fibrinogen concentration and *in vitro* ultrasonic backscattering level of blood in patients of group SPEC+ confirms hyperaggregation of blood.

Conclusion

Ultrasonic techniques, either echographic imaging or Doppler velocimetry, used in medical diagnosis provide useful information for the physician both on mechanical and flow properties of blood. From *in vitro* quantification of the ultrasonic backscattering coefficient, the structure of blood or RBC suspensions at rest or in shear flow can be approached. Determination of the experimental critical shear stress inducing a near complete disaggregated suspension leads to evaluation of the adhesion energy between cells. Moreover, from simultaneously *in vivo* acoustical and color Doppler imaging, spontaneous echocardiographic contrast found sometimes in patients and considered as an indicator of thromboembolic risk is qualitatively characterized. In future, it can be expected that *in vivo* quantification of blood echogenicity could be presented on commercially available ultrasonic imaging devices.

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