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4pBA10. Probability distribution variation in high-frequency ultrasound blood echogenicity under in-vitro and in-vivo blood flow
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The dynamic phenomena of erythrocyte aggregation (EA) need to be analyzed statistically since EA varies spatially and temporally. In the present study, the cross-sectional B-mode images were acquired from a mock circulatory system with varying blood flow velocity (BFV) under steady flow, and the human radial artery using an ultrasound biomicroscopy system at 20 MHz. The kurtosis (K) and skewness (S) coefficients, and the Nakagami parameter (m) were computed for each image. For the in-vitro experiment, both K and S increased about 0.87±0.18 and 0.63±0.09 with increasing BFV from 12 to 44 cm/s, respectively; while m decreased about 0.90±0.20. In-vivo experimental results also showed that K, S, and m varied during a cycle. When BFV varied from 5 to 15 cm/s during a cardiac cycle, K and S increased about 0.36±0.14 and 0.13±0.06 for subject 1, and 0.60±0.24 and 0.15±0.07 for subject 2, respectively; while m was estimated as large as 4.73±0.14 and 2.74±0.09. The in-vivo results seemed to be consistent with the in-vitro results in the sense that K and S increased with BFV despite large m. This study suggests that the statistical analysis of blood echogenicity can be useful for in-vivo hemorheology and blood characterization.

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1. INTRODUCTION

Ultrasonic blood characterization (UBC) techniques are much related to understanding on red blood cell (RBC) aggregation. The phenomena of RBC aggregation may increase blood viscosity and flow resistance, so that UBC under flowing blood is very important in hemodynamics. Also, since RBC aggregation varies spatiotemporally in blood vessels, statistical studies on UBC have been emphasized.

Many studies on UBC have been performed up to now, in terms of ultrasonic\(^1,2\), hemodynamic\(^3,4\), and statistical properties\(^5-7\). However, statistical properties in blood imaging in-vivo have not been studied yet, since in-vivo condition is much more complex than in-vitro condition, resulting in complicated interpretation. Statistical UBC techniques depend on the model to describe the statistics of backscattered signal which have various types of probability density function\(^8\). In the present paper, hence, various types of probability distributions in ultrasound blood image were studied in order to characterize RBC aggregation in-vitro and in-vivo. The purpose of the present study was to statistical analysis of RBC aggregation computing the kurtosis (\(K\)) and skewness (\(S\)) coefficients and the Nakagami parameter (\(m\)) from ultrasound blood B-mode image. These parameters have been utilized in UBC methods under in-vitro conditions\(^5-7\). Thus, these parameters would be applicable methods even in-vivo condition.

2. MATERIALS AND METHODS

2.1. High-Frequency Ultrasound (HFUS) Imaging System

A HFUS imaging system was configured by an ultrasound imaging board (PCB v4.3, Capistrano Labs Inc., San Clemente, USA) and a probe (CLI 1600 Ti, Capistrano Labs Inc., San Clemente, USA) encapsulating a single-element 35 MHz broadband acoustic transducer (35 TiMHz, Capistrano Labs Inc., San Clemente, USA) as shown in Fig. 1. The system parameters were represented in Table 1. The transducer was sectorially reciprocated 30 times per second, and the B-mode image was acquired in one direction with 30 frames per second (fps). In order to save data in a PC, the maximal capacity, 256 frames were saved and the data length was 8.5 s approximately.

TABLE 1. System parameters of the UBM system and the transducer characteristics. ‘Amplitude’ defines the relative value of the power output of the high-voltage power supply, ‘Gain’ defines the gain to the servo, ‘Jitter control’ provides jitter compensation for 2-way scanning, ‘Scale factor’ provides correct linearity of the image, ‘Offset angle’ provides a means of adjusting probe centering in the display.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Value</th>
<th>Pulser</th>
<th>Value</th>
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<td>Frequency</td>
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<td>Frame Rate</td>
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<td>Servo</td>
<td>Value</td>
<td>Display</td>
<td>Value</td>
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<td>Look-Up-Table</td>
<td>Center</td>
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<tr>
<td>Scale Factor</td>
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<td>Time Gain Compensation</td>
<td>Min.</td>
</tr>
<tr>
<td>Offset Angle</td>
<td>128 (Center)</td>
<td></td>
<td>Max.</td>
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<table>
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<tr>
<th>Transducer Characteristics</th>
<th>Focal Length</th>
<th>BandWidth</th>
<th>F Number</th>
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<tr>
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<td>100 %</td>
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</table>

2.2. In-Vitro Experiment

2.2.1. Blood Preparation

Fresh porcine blood, whose aggregation tendency was reported to be more similar to human blood compared with other livestock species\(^9\), was obtained from a local slaughterhouse in a 1 L bottle. The blood was prepared with a solution consisting of 3 g ethylenediamine tetraacetic acid dipotassium salt dissolved in 30 ml saline, for anticoagulation. Three different porcine blood samples were collected to confirm repeatability. A half amount of the
blood sample was used for whole blood (WB) experiments and the other half was prepared for RBC saline suspension (RBCS) experiments. For preparing the RBCS, the WB was centrifuged at 2,500 rpm for 15 min to separate the RBC from the plasma. The concentrated RBC was washed twice with 0.9 % normal saline solution buffered to pH 7.4. To prevent crenation of the red cells, 0.5% bovine albumin was added to the saline solution. The plasma and buffy coat layer, including white blood cells, platelets, and other minor cells, were removed through aforementioned procedures. Desired hematocrit was obtained at a later time by mixing the concentrated RBCS with 0.9 % saline solution for the RBCS experiments. Blood was circulated in the mock circulatory system for at least 1 h before any measurements were made to remove air bubbles inside the loop and to allow the blood to reach room temperature. The rest of the concentrated RBC was stored in a refrigerator at 4 °C.

2.2.2. Mock Circulatory System

A mock circulatory system was configured by a peristaltic pump (RP-1000, Eyela, Tokyo, Japan), a silicon tube (id4.76/od7.94, Eyela, Tokyo, Japan), a polyethylene terephthalate (PET) tube, a magnetic stirrer (HI 190M, Hanna Instruments, Seoul, Republic of Korea), and two triangle beakers (500 ml, Samduk-lab, Guri, Republic of Korea) for the steady state flow. The diameter of the silicon tube for the peristaltic pump should be minimally 7.94 mm outer diameter. However, the tube wall and the inner diameter were too thick to measure the backscattered signals using HFUS. Hence, the PET tube was used at the measurement site. The PET tube was fabricated by rolling a 100 -mm-thick PET membrane to a diameter of 2.5 mm with a length of 15 cm. The measuring site was chosen at one end of the PET tube, considering both the inlet length and the Reynolds number less than 2,000. Two triangular beakers were used as the blood reservoirs reducing velocity fluctuations. The experimental setup was represented in Fig. 1. The dynamic parameters for the mock circulatory system are represented in Table 2. Blood flow velocity was measured at the central axis of the mock vessel by an ultrasound imaging system (Voluson e, GE Healthcare Austria GmbH & Co OG, Zipf, Austria).

B-mode images were acquired at 20 MHz of ultrasonic frequency based on the Rayleigh scattering theory with varying Doppler peak velocities (12, 20, 28, 36, and 44 cm/s) while blood was flowing. The blood flow velocity was decided based on the velocity range at the radial artery. Three WB samples were used in order to verify repeatability. In the same way, three RBCS samples were used after the WB experiment.

![FIGURE 1](image.png)

2.3. In-Vivo Experiment

The in-vivo experiment was approved by the Institutional Review Board (IRB) of the Jeju National University Hospital (grant No. IRB 2009-37). Healthy two males (31.2±1.3 years old, the body mass index 25.7±1.7 kg/m²) were recruited and examined. The probe was placed on the right wrist near the RA. The angle between the probe and the wrist was carefully adjusted to maintain a rounded shape in the cross-sectional B-mode images of the vessel. The Doppler peak velocity of blood flow was measured at the central axis of the blood vessel by an ultrasound imaging system (Voluson e, GE Healthcare Austria GmbH & Co OG, Zipf, Austria). The experimental protocol in detail was described in the previous study of our group.
2.4. Data Processing

$K^9$-$12$, $S^8$, $9$, and $m^{11,12}$ of the probability distribution have been widely utilized in the field of ultrasound tissue characterization. $K$, $S$, and $m$ were defined by the following equations:

$$K = \frac{E[(X - E[X])^4]}{E[(X - E[X])^2]^2}, \quad S = \frac{E[(X - E[X])^4]}{E[(X - E[X])^2]^2}, \quad m = \frac{E[A^4]}{E[(A - E[A])^2]^2}$$

where $E[\cdot]$ represents the ensemble average, $X$ and $A$ are a random variable and the envelope of a time sequence signal, respectively. In the present study, $X$ is considered as $A$ which is supposed to be randomly distributed. The envelope $A$ is composed of the pixel values in the ROI since the B-mode image is laterally combined by A-mode signals which are the envelope of the backscattered echo. For $K$, if the random variables have the Gaussian distribution, then $K = 3$, however if the variables have a peaking distribution, then $K > 3$. For $S$, if the distribution is symmetric, then $S = 0$, while if the distribution is concentrated on the left, then $S < 0$, and if it is concentrated on the right, then $S > 0$. For $m$, if the random variables have the Rayleigh distribution, then $m = 1$, while if they have the pre-Rayleigh distribution, then $m < 1$, and if they have the post-Rayleigh distribution, then $m > 1$. These three parameters were computed in order to analyze the B-mode images acquired from both the mock vessel in the \textit{in-vitro} experiment and the RA in the \textit{in-vivo} experiment.

3. RESULTS AND DISCUSSION

3.1. \textit{In-Vitro} Results

The mean blood echogenicity for both the WB and RBCS were computed depending on the blood flow velocity at 20 MHz as shown in Fig. 2. The error bars were the standard deviations of three blood samples and 256 B-mode images per each sample ($n=768$). For the WB, the blood echogenicity decreased from $-8.9 \pm 1.5$ to $-11.8 \pm 0.5$ dB, while the blood echogenicity were not much changed from RBCS, depending on the velocity. These results mean that RBC can aggregate at low shear rate in the WB, which is an agreement with the literatures $^{13,14}$.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2}
\caption{Blood echogenicity depending on the blood flow velocity of both whole blood and RBCS. The error bars were the standard deviations of three blood samples and 256 B-mode images per each sample ($n=768$).}
\end{figure}

$K$, $S$, and $m$ for both the WB and RBCS were computed depending on the blood flow velocity as shown in Fig. 3. The error bars were the standard deviations of three blood samples and 256 B-mode images per each sample ($n=768$). $K$ increased about $0.87 \pm 0.18$ for WB, while it wasn’t much varied for RBCS, depending on the blood flow velocity (Fig. 5a). Increased $K$ for WB means that RBC disaggregated at higher shear rate, resulting in the narrowband distribution of the blood echogenicity in the cross-sectional B-mode image. On the other hand, RBC aggregation didn’t occur in RBCS at any shear rate, resulting in similar $K$ for all shear rates. $S$ increased about $0.63 \pm 0.09$ from $-0.36 \pm 0.07$ as a negative integer to $0.27 \pm 0.12$ as a positive integer for WB, while it wasn’t much varied as a positive integer for RBCS, depending on the blood flow velocity (Fig. 5b). Negative $S$ for WB means that the blood echogenicity distribution was concentrated on the right since RBC aggregated and the blood echogenicity increased. For RBCS, however, the blood echogenicity was not changed and RBC aggregation didn’t occur, so that the blood echogenicity distribution was concentrated on the left. In Fig. 5c, $m$ decreased about $0.90 \pm 0.20$ from $2.50 \pm 0.15$ to $1.59 \pm 0.14$ for WB, meaning that the blood echogenicity distribution varied from the post-Rayleigh ($m > 1$) to Rayleigh ($m = 1$) distribution. This result can be also interpreted with RBC aggregation depending shear rate. Since RBC aggregated at low shear rate for WB, the blood echogenicity increased, resulting in
the post-Rayleigh distribution of the blood echogenicity in the cross-sectional image. At higher shear rate, RBC disaggregated and the blood echogenicity decreased, resulting in the Rayleigh distribution of the blood echogenicity. Similarly with $K$ and $S$, $m$ was also not much changed ($1.14 \pm 0.10 \sim 1.02 \pm 0.08$) for RBCS since RBC aggregation didn’t occur in RBCS.

![Graph showing kurtosis, skewness, and Nakagami parameter](image)

**FIGURE 3.** Kurtosis (a) and skewness (b) coefficients and Nakagami parameter (c) depending on the blood flow velocity of both whole blood and RBCS. The error bars were the standard deviations of three blood samples and 256 B-mode images per each sample (n=768).

### 3.2. *In-Vivo* Results

The cyclic variations of blood echogenicity were observed for all subjects as shown in Fig. 4. The error bars were the standard deviations of nine cycles per subject (n = 9). The magnitudes of variation were 0.6 (Fig. 4a) and 0.7 (Fig. 4b) dB, respectively. This observation was in good agreement with the *in-vitro* results that the blood echogenicity increased as the blood flow velocity decreased. The maximal blood echogenicity were seen at 0.8 and 0.9 of the normalized time, respectively, which was estimated as an end diastole. The minimal blood echogenicity were seen at 0 or 1 of the normalized time, which was estimated as a peak systole.

![Graph showing echogenicity variation](image)

**FIGURE 4.** Ensemble averaged blood echogenicity during a cardiac cycle from two subjects. The cyclic variations of blood echogenicity were shown in all subjects. The magnitudes of variation were (a) 0.6 and (b) 0.7 dB, respectively. The error bars were the standard deviations of nine cycles per subject (n=9).
$K$, $S$, and $m$ for all subjects were ensemble-averaged and overlapped with the blood echogenicity as shown in Fig. 5. The error bars for $K$, $S$, and $m$ were the standard deviations of nine cycles per subject ($n = 9$). The error bars for the blood echogenicity are not shown for clarity. The magnitudes of the variations were $0.36 \pm 0.14$ and $0.13 \pm 0.06$ for $K$ and $S$ of subject 1, respectively (Fig. 5a). Similarly, the magnitudes of the variations were $0.60 \pm 0.24$ and $0.15 \pm 0.07$ for $K$ and $S$ of subject 2, respectively (Fig. 5b). The temporal variations of both $K$ and $S$ were almost out-of-phase with those of the blood echogenicity, which was in agreement with the in-vitro results. However, $m$ was very large as $4.73 \pm 0.14$ and $2.74 \pm 0.09$, which means the post-Rayleigh distribution, respectively, so that it couldn’t be easily interpreted with respect to the cyclic variation of blood echogenicity.

**FIGURE 5.** Kurtosis, skewness, and Nakagami parameter of blood echogenicity during a cardiac cycle from two subjects. The error bars for the echogenicity are not shown for clarity.

4. CONCLUSION

The present paper shows that the probability distribution of high-frequency ultrasound blood echogenicity varied with the blood flow velocity under in-vitro and in-vivo blood flow. The kurtosis and skewness coefficients of the blood echogenicity from the cross-sectional ultrasound B-mode image increased with blood flow velocity in both in-vitro and in-vivo experiments. The Nakagami parameter, however, decreased with that in in-vitro experiment, and was very large during a cardiac cycle. This preliminary study suggests that the probability distribution such as the kurtosis and skewness coefficients and the Nakagami parameter can characterize ultrasound blood image in-vivo. Accordingly, the ultrasound blood characterization should be studied with understanding on the probability density function from the ultrasound image, in addition to the hemorheology.

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REFERENCES