Identification Of Echocardiographic “Smoke” in a Bench Model With Transcranial Doppler Ultrasound

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Background and Purpose—Spontaneous echo contrast in cardiac chamber has been indicated as a source of cerebral embolism. The nature of the echocardiographic smokelike signal is still not fully understood. This study was designed to regenerate spontaneous echo contrast and verify its thromboembolic characters in an in vitro model.

Methods—Spontaneous echo contrast was reproduced in an expansion chamber under low flow conditions in a close circulation system. The spontaneous echo contrast was monitored and recorded with a 2-dimensional cardiosonography system and a transcranial Doppler device. Meanwhile, clinically commonly encountered embolic materials such as whole-blood clots, platelet aggregate–rich plasma, air bubbles, and 100-mL normal saline were injected into this monitored circuit. The differentiation of spontaneous echo contrast from emboli was performed by both visual observations of the echo images and offline Doppler signal intensity analysis. Average signal intensities produced by spontaneous contrast and injection of embolic materials and saline were compared. Furthermore, the effect of Doppler-detected flow velocity on generation of spontaneous contrast was also evaluated.

Results—Spontaneous echo contrast was reproduced at low flow settings (90 to 120 mL/min) in this model. There was no significant difference in average signal intensity between the flow with spontaneous echo contrast and that without the echo ($P=0.71$). However, injection of embolic materials or normal saline did not generate smokelike image but caused much higher average signal intensity than the flow with spontaneous contrast ($P<0.001$). Injection of normal saline also increased average signal intensity.

Conclusions—Our results suggest that smokelike echo is a special echo phenomenon occurring at low flow situations and does not itself produce material capable of embolizing into the systemic circulation. (Stroke. 2000;31:907-914.)

Key Words: cerebral embolism - spontaneous echo contrast - ultrasound, Doppler, transcranial

The echocardiographic appearance of spontaneous contrast, or echocardiographic “smoke,” is recognized by mobile increased intensity of the blood within left and right heart chambers, great vessels, and veins, which was first described by Feigenbaum, using M-mode echocardiography in patients with coronary artery disease. This phenomenon is occasionally noted in the state of regional stasis of blood, such as larger left atrial or ventricle diameter, and it may disappear when contractility of the heart is improved.

Previous clinical investigations have shown that the presence of “smoke” is associated with a greater incidence of left atrial thrombus and has been considered one of independent predictors for cardiac thrombus development after mitral valve replacement. To determine the incidence of smoke and its relation to the intracardiac thrombus, transesophageal and transthoracic echocardiography have been commonly used. It has been suggested that patients with left atrium and appendage smoke were at higher risk for intracardiac thrombus, and the latter play an important role as a cardiac source of cerebral embolism. Knowledge of the relationship between echocardiographic smoke and intracardiac thrombus and the nature of the smoke may help the management of patients with cerebral embolism when the need for anticoagulant or antiplatelet aggregatory agent is to be considered.

While the association between ischemic stroke and cardiac thrombi and spontaneous echo contrast has been under investigation, several methods have been used to investigate the pathogenesis of spontaneous contrast. In the previous studies, although blood stasis, platelet aggregation, red blood cell (RBC) coagulation, and flow-related interaction of RBCs and plasma have been implicated in such echogenicity, the mechanism responsible for the formation of echocardiographic smoke is still not fully understood.

To understand the putative cellular elements responsible for spontaneous echocardiographic contrast in the blood pool, Tarkkia et al recently examined, using a 7.5-MHz mechanical sector scanner, the roles of blood components, including packed RBCs, plasma, washed RBCs, and exogenous fibrinogen, and found that ultrasonic echo gray scale was an exponential function of fibrinogen and hematocrit (HCT) but was not...

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influenced by leukocyte count, platelet count, or serum protein level. In another experimental study, spontaneous contrast was imaged from the rabbit heart after administration of xylazine and ketamine while RBC aggregation was prevented by a photometric method. In addition to the factors of pathological cellular components, the shear rate of circulating blood has also been considered a possible contributor to the echocardiographic smoke. To evaluate this assumption, a variety of models have been designed to mimic the dilated left atrium and examine spontaneous contrast at different flow rate and shear rate conditions. In an experiment by Merino et al, ultrasound imaging was performed with a fixed 7-MHz linear probe in a tubular chamber with circulating heparinized whole blood. It was observed that characteristic swirling waves of spontaneous contrast were seen in the circulating blood at low flow rate and low shear rate.

The ability of transcranial Doppler ultrasound (TCD) to detect embolic particles such as blood clots, platelet clots, and air bubbles has been recently confirmed both experimentally and clinically. An embolus causes a Doppler signal of increased intensity because its size and acoustic impedance are different from blood.

In this study, using an in vitro model to generate spontaneous contrast, we aimed to (1) identify whether smokelike signals result in production of embolus signals, (2) investigate whether whole-blood clots or platelet clots or microbubbles can produce smokelike signals, and (3) evaluate the effect of the smoke state on the formation of thrombi.

**Methods**

**Definition of Spontaneous Contrast**

Echogenic smoke was defined according to the previous description of (1) low-amplitude echogenic haze; (2) slow, repetitive movement in the cavity; and (3) dissipation and disappearance of the image when blood flow increases and reappearance as flow decreases.

**In Vitro Models for the Reproduction of Smokelike Echoes and TCD Evaluation**

The model used to generate spontaneous contrast was introduced by Merino et al. It was constructed with a 28-mm-diameter plastic cylindrical chamber containing a 4-mm-diameter tube. A 5-MHz linear transducer (LS18, Acuson) was fixed longitudinally to the chamber. In this study, we added a plastic tube of the same internal diameter that bypassed the expansion chamber to help us to identify the nature of smoke. A middle cerebral artery (MCA) model for TCD detection was formed with a plastic box filled with normal saline and inlaid with a piece of human temporal bone in one of the walls and a plastic tube with internal diameter of 4.5 mm. A 2-MHz TCD probe was placed on the temporal bone to record the Doppler signal produced by blood flow passing through the tube inside the MCA model. The models were connected together, and whole human blood (anticoagulated with 1:7 citrate phosphate dextrose adenine) was circulated by a constant pump, with the flow rate constantly monitored with a magnetic flowmeter (Figure 1).

**Ultrasonic Signal Recording and Offline Study**

Two-dimensional echography was performed with an ACUSON 128 Computed Sonography System (Acuson, Inc), and the images were recorded onto videotape for subsequent examination. Continuous Doppler monitoring was performed with a TC2000 TCD device (Nicolet). All parameters, including gain, power, and depth for both ultrasonic devices were maintained constant throughout. Echography and Doppler signals were synchronously recorded for 1 minute before and after each processing. TCD signal intensities were calculated with an intensity analysis software developed according to the validated and published method of Muller et al.

**TCD Detection During Appearance of Smokelike Echoes**

The TCD signal was continuously monitored for 30 minutes. Ten TCD frames were consecutively saved for later offline intensity analysis during the period when typical smokelike echoes were imaged in the expansion chamber. At the same time, the blood flow was also allowed to travel through the bypass tube, and the same TCD manipulation was repeated.

**Preparation and Injection of Embolic Materials**

**Whole-Blood Clots**

One milliliter of 0.2 mol/L CaCl₂ solution was added to 10 mL whole blood obtained from a donor and then gently shaken. This blood was incubated at 37°C for 24 hours to clot. To prepare clots with a volume of 1×1×1 mm³, 1 g of whole-blood clot was then sliced with a McIlwain tissue chopper (Mickle Laboratory Engineering) setting at a cutting distance of 1 mm. To determine the clot size and the number of clots, microscopy with a calibrated eyepiece was used to measure the clots dropped on the glass slide. The mean clot size was obtained by measuring 50 clots, and the concentration was determined by counting.
the number of clots in 0.1 mL blood clot-rich saline. Prepared clots were stored in 10 mL Dulbecco’s phosphate buffer.

**Platelet Aggregate–Rich Plasma**

9 mL fresh human blood was used for preparation of platelet aggregate-rich plasma. Ten milliliters of whole blood from a donor was anticoagulated with 0.5 mL 0.1 mol/L citrate anticoagulant and centrifuged with a refrigerated centrifuge (Sorvall RT6000B; Refrigerated Centrifuge, Du Pont) at 1000g for 15 minutes to prepare platelet-rich plasma. One milliliter of platelet-rich plasma was added to 2 mL of 10 U/mL bovine thrombus (Sigma Chemical Co) and stored at 20°C for 30 minutes. Platelet-rich thrombus, containing >99% platelets, was then formed. After the platelet aggregate–rich plasma was formed, the same method as that used in evaluation of blood clots was applied to determine the mean size and concentration of platelet clots. We assumed that whole-blood and platelet clots used in this study were cubic.

**Air Bubbles**

Microbubbles were prepared in 15 mL of Ultravist 370, a nonionic contrast medium (Schering Health Care Ltd) by stirring with a polytron for 40 seconds with power set at 10 W. With this setting, microbubbles with a diameter of approximately 30 μm were generated. The size of prepared microbubbles was further controlled by transferring into a size control set. This set was constructed with a 20-μL plastic syringe, which was inlaid by 2 microfilter membranes, the first with a pore size of 40 μm (AF-10250 arterial blood filter, Baxter Healthcare) and the second with a pore size of 30 μm (AN 3H, Millipore). A side port was located between the 2 microfilter membranes. Once the agitated, bubble-rich medium was transferred into the bottom of the syringe, certain prompting pressure was given manually. This allowed microbubbles with a diameter of >40 μm to stay outside the first filter and those with a diameter of <30 μm to pass through the second filter. Thus, the filtrate containing microbubbles with diameters ranging from 30 μm to 40 μm stayed between the 2 microfilters and were obtained through a side port of this set. A blood cell count chamber was used to estimate the bubble concentration. The method to determine bubble size was the same as that for blood clots.

**Degassed Normal Saline**

A 100-mL infusion of normal saline was degassed in the sonicator bath for 15 minutes before injection; 2.5 mL emboli-suspended solution or degassed normal saline was then manually injected into the circuit through a 3-way tap within 2 seconds.

**Correlation of Smokelike Echo With Doppler Flow Velocity**

In addition to echographic detection of smokelike signals, a pulsed-wave Doppler device was combined to record the Doppler flow velocity from the central part of outflow jet, the area in the presence of smokelike echo, and the area near the border of the chamber. Mean velocity was calculated from 10 peaks of the flow velocities.

**Investigation of the Role of Smoke State in Thrombus Formation**

A calcium chloride solution was used to supply Ca\(^{2+}\) for whole blood to coagulate: 0.2 mol/L CaCl\(_2\) was added into blood at the ratio of 1:20 in volume. The time required for formation of blood thrombus was recorded. We investigated the coagulation time during the following 3 conditions: (1) 270 mL blood flow passing through the cylindrical expansion chamber after the addition of 13.5 mL 0.2 mol/L CaCl\(_2\), (2) the same volume of blood flow passing through the bypass tube after the addition of the same amount of CaCl\(_2\) solution, and (3) 9.5 mL blood in a 25-mL glass beaker after the addition of 0.5 mL 0.2 mol/L CaCl\(_2\) solution. The formation of solid thrombus was confirmed with 2-dimensional echographies detected from the chamber, bypass tube, and the beaker.

The RBC concentration and HCT of sampled circulating whole blood were examined with Sysmex NE 8000 (TOA Medical Electronic Co).

**Statistical Analysis**

Statistical analyses were performed with 1-way analysis of variance for more than 2 groups of animals, with subsequent individual comparisons by the Scheffe test. All the data in this study were expressed as mean ±1 SD. A value of P<0.05 (2-sided) was considered statistically significant.

**Results**

**RBC and HCT**

RBC concentrations and HCTs of the circulating blood used in this study ranged from 2.97 to 4.19×10\(^3\)/μL and from 0.27 to 0.42, respectively.

**Reproduction of Smokelike Echoes**

At a flow rate of 0 mL/min, static blood in the chamber displayed the echographic image of grainy haze with equal intensity at all areas within the chamber (Figure 2A). At a flow rate of 30 to 60 mL/min, blood entering the chamber showed an echolucent jet stream line (Figure 2B). The typical swirling smokelike images of flow lines appeared between the outflow jet stream and the static blood near the border of chamber when flow rates were 90 to 120 mL/min (Figures 2C and 2D). This spontaneous contrast also characterized as a higher echodensity than the surroundings but without definite margins. At flow rate of <60 or >180 mL/min (Figure 2E), smokelike echoes disappeared but reappeared when the flow rate was reduced to 90 mL/min or 120 mL/min.

**TCD Examination of Smokelike Echoes**

During the presence of echogenic smoke, the blood passing through the chamber caused no significant difference in average TCD signal intensity (49.9±6.53 U) compared with that produced by the blood passing through the bypass tube (49.0±4.4, P=0.71; Figure 3). Additionally, no visible embolic signals were found from TCD recordings that corresponded to the appearance of smokelike echoes or the flow bypassing the chamber (Figures 4A and 4B).

**Injection of Embolic Particles and Normal Saline**

Injection of whole-blood clots (mean size 1.3±0.5 mm, concentration 67 clots/mL), platelet clots (0.9±0.5 mm, 47 clots/mL), microbubbles (30.2±9.8 μm, 1.4×10\(^3\) bubbles/mL) and degassed normal saline (2.5 mL) all produced flame-shaped echoes with a high echodensity when they were initially pumped into chamber (Figure 5). Figure 5A showed that along with this high-density echo of the inflow jet flow from in the middle of the chamber, injection of saline into the chamber at the given volume and rate also generated a transient smokelike echo for several seconds inside the expansion chamber (Figure 5A). The emboli in the chamber produced a general increase in average echodensity as well as starlike, high-echodensity points with clear margins (Figures 5B, 5C, and 5D). They floated inside the chamber and finally were exempted from chamber with the blood flow. The echographic appearance of emboli was quite distinct from the smokelike echoes. However, compared with the flow with presence of smokelike echoes (49.9±6.53 U), injection of whole-blood clots, platelet clots, and microbubbles caused a significant increase in average Doppler signal intensity (whole-blood clots 80.6±23.6 U, P<0.05; platelet clots...
72.4±14.4 U, \( P = 0.05 \); and microbubbles 2453.7±885.4 U, \( P = 0.001 \). In contrast, injection of an equivalent volume of normal saline decreased the average signal intensity (14.8±0.68 U, \( P = 0.05 \); Figure 3).

Moreover, injection of embolic materials but not normal saline (Figure 4C) produced high average signal intensity and visible embolic signals in the TCD velocity waveforms (Figures 4D, 4E, and 4F).

Correlation of Smokelike Echo With Doppler Velocity

When smokelike echoes occurred, mean Doppler flow velocity recorded in the middle of the outflow in the expansion chamber was higher (148.7±17.9 cm/s)(Figure 6a) than that obtained in the smokelike echo area (32.8±0.18 cm/s)(Figure 6b) \( P < 0.01 \). Both showed bi-directional wave patterns. However, the Doppler flow velocity was near to zero in the area near the side of this chamber (Figure 6c).

Smokelike Echoes and Thrombus Formation

To examine whether coagulation of the blood could increase under smoke state, CaCl\(_2\) was added into the circuit, and the time taken for thrombus formation was observed at different settings. At a flow rate of 90 mL/min, the time taken for thrombus formation was 4 minutes for undisturbed blood, 4.5 minutes for the blood passing through the expansion chamber.
with the presence of smokelike echoes, and 6 minutes for blood passing through bypass tube.

**Discussion**

Two-dimensional echocardiography is a well-established technique in the assessment of left atrial spontaneous echocardiographic contrast or smokelike echo and thrombi. Using Yorkshire albino pig blood, Merino et al. mimicked spontaneous echocardiographic contrast in an expansion chamber by pumping whole blood at a low flow velocity. This in vitro flow model was thought to emulate pulmonary vein flow into the left atrium or mitral flow into the left ventricle. With a similar model and flow velocity, we used human whole blood to generate a similar pattern of echoes, but produced pulsed flow because this is closer to the clinical situation.

We also noted that the mean Doppler peak velocity obtained in the smokelike echoes area in the chamber is close to that recorded in the human left atrium with the presence of spontaneous echo contrast and/or thrombi. This suggests that the model is adequate to mimic the clinical situation.

In this study, we used Doppler ultrasound, which has been proved to be very sensitive in detection of embolic materials in blood flow, to investigate whether smokelike echoes resulted in the production of embolic signals. Embolic particles produce a visibly high amplitude signal on the Doppler waveform as well as a remarkable increase in signal intensity. However, we found that the components that caused intracavitary smokelike echoes failed to produce any embolic Doppler signals and did not increase Doppler signal intensity compared with the signal intensity produced by blood passing through the bypass tube. This result is in accord with those of a previous experimental report that the components of spontaneous echo contrast are not solid but are liquid materials.
Although it has been reported that spontaneous echo contrast was acoustically distinct from endocardiac mural thrombus attached to the ventricular apex, no previous study has attempted to differentiate echographic smoke signals from the emboli. Furthermore, we have identified that the blood flow with smoke-like signals dose not produce embolic signals detectable with TCD. We also tested the reverse situation, whether emboli caused smokelike signals, because microthrombi, red blood and platelet aggregates, and air bubbles have been implicated in the development of smoke-like echo. We found that, as expected, these emboli produced TCD signals after they circulated within the echo chamber. However, there was no evidence that they caused smoke echoes.

The cardiac cavity is normally echo free on the echocardiogram because the ultrasound reflection from intracardiac blood is not dense enough to appear on the screen. This was confirmed as a flow-dependent phenomenon in the present study. The mechanism for the echogenicity of static blood as well as spontaneous echo contrast has been investigated by Sigel et al. Whole blood suspended in plasma produces visible echoes in vitro. However, plasma serum or erythrocytes do not produce echoes. The echogenicity of blood components may also depend on factors related to blood flow, including rheological and biochemical properties, and changes in the physical alignment of potentially echogenic blood components. Our findings that smokelike echoes usually occurred in the area between the inflow jet with higher flow velocity and static blood located in the side of the chamber without Doppler-detectable flow velocity may support this hypothesis. Thrombi were detected more frequently in patients with reduced left ventricular global systolic function. It has been suggested that shear stress, defined as the product of the velocity gradients between parallel flow lines located in the center and the periphery of blood vessels times the blood viscosity, contributed to the echogenicity of spontaneous contrast by exerting a mechanical force to change the physical layering.

It has been reported that direct shear-induced platelet aggregation may develop under certain pathologic flowing conditions, especially at a high shear stress condition in which stenosis of vessel usually existed, and such shear-induced platelet aggregates could be inhibited by administration of GPIIb/IIIa receptor antagonist. From a view of biofluid mechanics and blood biorheology, the formation of microparticles by platelet aggregation could influence the reflected Doppler ultrasound waveform by increasing the scattering intensity, since it increased the reflector size. Considering the scattering factor, in this study we analyzed the average intensity of total Doppler signal reflected (instead
of individual signal intensity at any interested points) at different testing protocols and found that the blood flow associated with smokelike echo did not increase the ultrasound backscattered power. This may further indicate that platelet aggregates are formed in the chamber when the spontaneous echo contrast is presented at the current settings. This may be due to the fact that the smokelike echo and shear-induced platelet aggregates are generated in different levels of flow shear.

Interestingly, injection of normal saline produced short-lived spontaneous contrast. This phenomena has also been seen in an animal model. This provides further evidence that smokelike echo is generated by fluid components. This may result from a change in physical alignment of blood components by temporally altered regional shear force or acoustic impedance between blood and normal saline from which more echoes are expected to be reflected from the blood-saline interfaces than those from the scattering effect of RBCs. Therefore, this lasts only for a short time before complete admixture of normal saline and blood.

Figure 6. Doppler velocity related to smokelike echo. Mean peak velocity in the center of outflow stream was higher (148.7±17.9 cm/s) than that in the smokelike signal area (32.8±0.18 cm/s; P<0.01). Both waveforms were in a biphasic wave pattern. The velocity in the area near the side of chamber was too low to record.
References


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