Original Contributions

Differentiation Between Different Pathological Cerebral Embolic Materials Using Transcranial Doppler in an In Vitro Model

Hugh S. Markus, MA, MRCP, and Martin M. Brown, MD, MRCP

Abstract:

Background and Purpose: The detection of circulating particulate cerebral emboli using transcranial Doppler ultrasonography has been recently reported. It has been suggested that this method might allow discrimination between different embolic materials; this would be very useful for selecting specific pharmacological treatment in individual patients. This study was designed to identify those parameters of the Doppler signal that might prove useful in discriminating between different types and sizes of particulate cerebral emboli.

Methods: An extracorporeal circuit filled with a saline/Tween solution and driven by a peristaltic pump was used. The tubing was placed in a skull in the position of the middle cerebral artery. Using transcranial Doppler ultrasound, flow was insonated via the transtemporal window. The following embolic materials of measured sizes (range of maximum dimension, 0.5–5.0 mm) were introduced into the circuit: thrombus (n=20), platelet-rich aggregates (n=15), atheromatous material (n=20), and fat (n=20). The Doppler signal was recorded during the passage of each embolus. Off-line analysis was performed to measure the maximum amplitude and duration of the signal.

Results: For all embolic materials there was a highly significant relation between embolus size and maximum amplitude of the Doppler signal. The closest correlation was obtained when the logarithm of maximum amplitude was used (for thrombi, r=0.74; for platelet, r=0.87; for atheroma, r=0.46; and for fat, r=0.68). The slope of the regression line differed for the different embolic materials and was significantly steeper for platelets than for atheroma (p<0.01). Platelet emboli of maximum dimension ≤1.5 mm resulted in significantly lower maximum amplitude than similarly sized atheroma emboli (157 dB versus 206.7 dB, p<0.01). For larger emboli (>2 mm) there was little further increase in maximum amplitude with increases in embolus size. For all embolic materials there was a highly significant linear relation between embolus size and duration of the high-amplitude (>150 dB) signal (for thrombi, r=0.75; for platelet, r=0.90; for atheroma, r=0.77; and for fat, r=0.86).

Conclusions: Platelet emboli result in lower-amplitude signals and, therefore, analysis of maximum amplitude may provide information on the type of embolic material. However, it may be difficult to determine whether a given signal is associated with a large platelet embolus or a small atheroma embolus. Duration of the high-amplitude signal will allow accurate estimation of the size of emboli, particularly where the emboli are all of the same material. (Stroke 1993;24:1-5)

Key Words: cerebral vascular disorders, embolism, ultrasonics

Cerebral arterial embolism is believed to account for the majority of strokes. Although detection of potential sources of emboli is possible in some patients, the ability to detect circulating cerebral emboli would represent a major advance in determining the cause of stroke in individual patients and would also provide a useful tool for investigating the pathophysiology of stroke. It would be particularly useful if the technique also allowed differentiation between different embolic materials, therefore allowing appropriate treatment such as anticoagulants for thrombus emboli and antiplatelet agents for platelet emboli. The use of Doppler ultrasound to detect gas bubbles in the bloodstream was described as early as 1969 in divers with decompression sickness. The amplitude of the returned Doppler signal is dependent on the amount of sound that is reflected at the fluid–embolus interface. This depends on the acoustic impedance of the two media, which in turn is directly related to the density of each medium. One would therefore expect different embolic materials to return signals of different amplitudes. Gas emboli are easily detected due to their high reflectivity, and the detection of air emboli has proved useful in studying the pathogenesis of neuropsychological deficits associated with cardiopulmonary bypass. Recently the detection of particulate cerebral emboli has been described in patients undergoing carotid endarterectomy, and preliminary data suggest that such signals can be detected in patients with a variety of potential embolic...
sources including atrial fibrillation and cardiac valvular lesions. It has been suggested that the technique might prove useful in differentiating between different types of embolic material. In this study we have determined the characteristics of the Doppler signal associated with emboli of different sizes and materials in an in vitro pulsatile flow model, with the aim of determining those signal characteristics most useful in differentiating between different embolic materials.

### Materials and Methods

Flow was studied in an extracorporeal circuit. Pulsatile flow was produced by a peristaltic pump (HR Flow Inducer Type 2000, Watson Marlow, Cornwall, UK) set to obtain a flow rate of 50 ml/min through a circuit of plastic tubing. A 5-mm i.d. tube entered the skull via the carotid canal and followed the approximate course of the middle cerebral artery. The intracranial cavity was filled with acoustic coupling gel.

A multifrequency transcranial pulsed Doppler ultrasound machine (TC2000, EME, Uberlingen, FRG) with a 2-MHz probe was used. Signal analysis employs a 128-point fast Fourier transform. Power was set at 58%, and a sample volume of 9 mm and a sweep speed of 10 seconds were used. Flow was insonated via the trans-temporal window through the skull at a depth of 50 mm. The angle between the transmitted ultrasound beam and forward flow was approximately 20°.

The circuit was filled with saline mixed with a 0.001% solution of Tween 80 in saline. Emboli were introduced via a plastic 1-ml syringe and a side arm. The embolic materials used were: 1) thrombus prepared by adding 1 ml (containing 10 National Institutes of Health units) human thrombin (Sigma Chemical Co., Poole, UK) to 10 ml fresh human blood; 2) platelet-rich aggregates prepared by adding 1 ml aged (48 hours) human thrombin (Sigma) to 5 ml platelet-rich plasma; 3) atheromatous material obtained from fresh (<24 hours) postmortem human aorta; and 4) fat obtained from the mesenteric fat from fresh (<24 hours) postmortem human material.

Embolic material was cut under a dissecting microscope into cuboid pieces of determined dimensions. From these measurements the maximum length of the longest edge (maximum dimension) and the maximum cross-sectional area were recorded for each embolus. The embolic material was then resuspended in the saline/Tween liquid and injected into the extracorporeal circuit. A three-way tap downstream of the ultrasound probe allowed fluid containing the embolic material to be removed from the circuit to prevent recirculation of the embolic material.

The Doppler signal was recorded onto an IBM-compatible microcomputer and analyzed off-line. Using specifically designed software (EME), individual time frames of the Fourier transform were analyzed, and the maximal amplitude (in decibels) in each successive time frame was recorded. Correlations were examined between embolus maximum dimension or maximum cross-sectional area and amplitude or duration of the Doppler signal. Duration of the embolic signal was measured by recording the number of time frames in which the signal occurred. Each 10-second sweep period contained 510 time frames, which therefore represented 19.6 msec each. The total duration of the embolic signal (all frames where amplitude was >50 dB) and the duration of the high-amplitude (>150 dB) signal were recorded for subsequent analysis.

For each embolic material the relation between embolus size and characteristics of the Doppler signal were analyzed by calculating Pearson correlation coefficients. Significance was declared at a p<0.05 level. Regression analysis was performed, and linear and quadratic terms were fitted. Significance was assessed using F tests.

### Results

In the absence of emboli in the system, mean±SD maximum amplitude of the Doppler signal was 13.1±2.0 dB. The following emboli were introduced into the flow model: 1) 20 thrombus emboli; mean (range) maximum dimension, 2.6 (1.0–5.0) mm and mean (range) maximum cross-sectional area, 7.2 (0.7–25.0) mm²; 2) 15

#### TABLE 1. Correlation Coefficients Between Size of Embolus and Doppler Signal Characteristics for Different Embolic Materials

<table>
<thead>
<tr>
<th>Signal characteristic</th>
<th>Maximum dimension</th>
<th>Logarithm of maximum dimension</th>
<th>Maximum cross-sectional area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Thrombus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum amplitude</td>
<td>0.65</td>
<td>0.0018</td>
<td>0.74</td>
</tr>
<tr>
<td>Duration of high-amplitude signal</td>
<td>0.75</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum amplitude</td>
<td>0.79</td>
<td>0.0008</td>
<td>0.87</td>
</tr>
<tr>
<td>Duration of high-amplitude signal</td>
<td>0.90</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Atheroma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum amplitude</td>
<td>0.46</td>
<td>0.04</td>
<td>0.46</td>
</tr>
<tr>
<td>Duration of high-amplitude signal</td>
<td>0.77</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum amplitude</td>
<td>0.66</td>
<td>0.0015</td>
<td>0.68</td>
</tr>
<tr>
<td>Duration of high-amplitude signal</td>
<td>0.86</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

High-amplitude signal, >150 dB.
platelet emboli; mean (range) maximum dimension, 1.7 (0.5–3.5) mm and mean (range) maximum cross-sectional area, 3.3 (0.2–10.5) mm²; 3) 20 atheroma emboli; mean (range) maximum dimension, 1.8 (0.5–3.5) mm and mean (range) cross-sectional area, 2.8 (0.2–6.0) mm²; and 4) 20 fat emboli; mean (range) maximum dimension, 2.5 (1.0–5.0) mm and mean (range) cross-sectional area, 7.7 (1.0–25.0) mm². The passage of an embolus through the sample volume was accompanied by a high-amplitude Doppler signal in all cases except for one platelet embolus, of dimension 0.5×0.5×0.5 mm, which resulted in no detectable Doppler signal.

For all embolic materials there was a highly significant positive relation between embolus size (both maximum dimension and maximum cross-sectional area) and maximum amplitude of the Doppler signal. Linear correlation coefficients are shown in Table 1. The correlations were stronger when maximum dimension was used as the parameter of embolus size, except in the case of atheroma, when maximum cross-sectional area resulted in the closer correlation. Results for the different embolic materials are shown graphically in Figure 1. This demonstrates that for larger emboli with a maximum dimension of approximately ≥2 mm there was no further rise in maximum amplitude of the Doppler signal with increases in embolus size, except in the case of platelet emboli. Large (>2 mm maximum dimension) emboli of all materials resulted in maximum-amplitude signals of a similar order (about 240 dB). However, for smaller emboli (<1.5 mm maximum dimension) platelet emboli resulted in a lower maximum amplitude than did the other materials.

A comparison was made between different embolic materials considering only emboli with a maximum dimension of <1.5 mm. In this group there were 10 thrombus emboli (mean size, 1.2 mm), six platelet emboli (mean size, 0.9 mm), nine atheroma emboli (mean size, 1.0 mm), and six fat emboli (mean size, 1.2 mm). There was no significant difference between the sizes of embolii of the different materials (Student's t, p>0.05). The mean ±SD maximum amplitude of the Doppler signal was lower for platelet emboli (157.0±46.6 dB) than for fat emboli (188.0±36.5 dB), thrombus emboli (201.3±16.7 dB), and atheroma emboli (206.7±13.6 dB), but the difference was significant only between platelet and atheroma emboli (Student's t, p=0.009).

In view of the apparently nonlinear relation between embolus size and maximum amplitude it was investigated whether the fit was improved by using the logarithm of embolus size and/or maximum amplitude in the regression analysis. The closest relation was found when the logarithm of maximum dimension was used. Regression lines using the logarithm of maximum dimension are shown in Figure 1, and correlation coefficients between this parameter and maximum amplitude are shown in Table 1. Fitting of quadratic terms in the regression analysis resulted in no improvement in the significance of the fit, except in the case of thrombus emboli, for which the inclusion of a squared term significantly improved the fit, with the r value increasing from 0.74 to 0.83. Regression coefficients for the relation between the logarithm of maximum dimension and maximum amplitude are shown in Table 2, demonstrating the different slopes of the best-fit line for different materials, with the greatest slope for platelet aggre-

**Table 2. Regression Coefficients for Relation Between Logarithm of Embolus Size and Maximum Amplitude of Doppler Signal and Between Embolus Size and Duration of High-Amplitude Signal**

<table>
<thead>
<tr>
<th>Embolic material</th>
<th>Maximum amplitude</th>
<th>Duration high-amplitude signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombus</td>
<td>45.0</td>
<td>1.61</td>
</tr>
<tr>
<td>Platelet</td>
<td>133.9</td>
<td>2.59</td>
</tr>
<tr>
<td>Atheroma</td>
<td>24.5</td>
<td>2.01</td>
</tr>
<tr>
<td>Fat</td>
<td>97.7</td>
<td>4.39</td>
</tr>
</tbody>
</table>

Significant difference between slopes for maximum amplitude of platelet and atheroma emboli (χ², p<0.01).
gates. The slope of the regression line was significantly greater for platelet aggregates than for atheroma emboli \( (\chi^2, p<0.01) \), but differences between the slopes of the other regression lines were not significant.

For all embolic materials there was a highly significant relation between embolus size (both maximum dimension and maximum cross-sectional area) and both total duration of the signal and duration of the high-amplitude signal. The relation with duration of the high-amplitude signal was the stronger of the two, and this relation was stronger than that with maximum amplitude of the Doppler signal for all embolic materials (Table 1). For all materials this correlation was best fitted by a linear relation (Figure 2), and no improvement in fit was found when quadratic terms were inserted or logarithmic transformations performed.

**Discussion**

Our results demonstrate a highly significant positive relation between size of an embolus and amplitude of the Doppler signal for each embolic material for all materials studied. Russell and coworkers\(^6\) have also reported a significant relation between size of an embolus and intensity of the signal. They used a similar transcranial Doppler machine to record the signal from rabbit aorta while blood clot, platelet, and atheroma emboli introduced proximally into the renal artery passed through the sample volume.\(^6\) Our results also demonstrate that as embolus size increases above a maximum dimension of 2 mm, further increases in embolus size result in much smaller increases in signal amplitude. This implies that for larger emboli maximum amplitude may not be so useful in determining the size of the embolus, whereas for smaller emboli it may be possible to derive information about their composition from the maximum amplitude. Monitoring, with detection of a number of emboli rather than a single embolus, may also allow additional information to be gained.

For all embolic materials there was a highly significant positive linear correlation between embolus size and duration of the high-amplitude signal. This implies that in the clinical situation in which the embolic material remains constant, such as thromboembolism from clot on a vascular wall, it will be possible to determine the size of any emboli detected in vivo using this method. The duration of the high-amplitude signal probably represents the most useful parameter as being most equivalent to the abnormal “embolic” signals reported in clinical practice, which represent a short duration of signal amplitude above that of the blood, usually by a relative level of 15–30 dB. If blood is run in our in vitro circuit, then mean maximum amplitude of the Doppler signal is 190 dB during systole and 100 dB during diastole. The relation between embolus size and duration of the high-amplitude signal holds for both small and large emboli.

We used saline as the medium in which to study emboli detection for two reasons. First, it allowed direct visualization of the embolic material passing the Doppler probe and correlation of the Doppler signal with the individual embolus and exact determination of the duration of the signal associated with each embolus. Second, it allowed visualization of air accidently introduced with the embolus. This was a particular problem initially with the introduction of fat emboli. Accidentally introduced air can lead to an overestimation of the amplitude of the returned signal associated with a particular embolic material and cannot be detected readily if blood is used as the medium. Tegeler and coworkers\(^7\) examined the signals associated with microspheres in an in vitro model using simultaneous Doppler and B-mode ultrasound and demonstrated that 6.4% of the signals were in fact due to air accidentally introduced rather than to the microspheres. This may explain why the relative amplitude of the Doppler signal associated with fat emboli was lower in our study than in the study of Russell et al.\(^6\) For macroscopic emboli the results obtained from our model using saline are likely

![Figure 2. Scatterplots of relation between embolus size (maximum dimension) and duration of high-amplitude (>150 dB) Doppler signal for four different types of embolic material. Linear regression lines are shown.](http://stroke.ahajournals.org/)
to approximate well those obtained using blood, and the relative differences in amplitude of the Doppler signal associated with different embolic materials will apply. For microscopic emboli with a size less than or of the order of the wavelength of the ultrasound (0.77 mm for 2 MHz), Rayleigh scattering will play a part in determination of the Doppler signal and therefore our results may not apply. Determination of the Doppler signal caused by such small emboli is currently hampered by the difficulty in preparing and introducing such small emboli into a model.

In summary, our results demonstrate that analysis of the maximum amplitude of the Doppler signal may help in discriminating between different embolic materials, although it may be difficult to determine whether a given signal is associated with, for example, a large platelet embolus or a small atheroma embolus. Duration of the high-amplitude signal will be particularly useful in estimating embolus size, particularly in those cases in which emboli are all of the same material, and this parameter is likely to be of use over the full range of embolus size, from microscopic to large macroscopic emboli.

Acknowledgments

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References

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