Hemodynamic Evaluation of Embolic Trajectory in an Arterial Bifurcation
An In-Vitro Experimental Model

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Background and Purpose—Despite the importance of embolism as a major cause of brain infarction, little is known about the hemodynamic factors governing the path large emboli tend to follow. Our aim was to test in vitro, whether hemodynamic parameters other than flow ratios between bifurcation branches may affect the distribution of embolic particles in a Y-shaped bifurcation model, used as an analogue to an arterial bifurcation.

Methods—In vitro experiments were conducted using suspensions of sphere-shaped particles (0.6, 1.6, and 3.2 mm) in water-glycerin mixture, using steady and pulsatile laminar flow regimes in a Y-shaped bifurcation model (identical branching angles \( \theta_1 = \theta_2 = 45^\circ \) with one daughter branch diameter wider than the other \([D_1 = 6 \text{ mm}, D_2 = 4 \text{ mm}]; \) average Reynolds number 500).

Results—Experiments using naturally buoyant particles under steady flow conditions and four outlet-flow ratios revealed that small (0.6 mm) and mid-sized (1.6 mm) particles entered into either the narrower or wider bifurcation daughter branch nonpreferentially, proportionally to the flow ratios. Large particles (3.2 mm), however, preferentially entered the wider daughter branch. Moreover, as the flow ratio increases this phenomenon was augmented. Further experiments revealed that the preference of the wider daughter branch for high particle-to-branch diameter-ratios further increases under pulsatile flow and by the density ratio between particles and fluid.

Conclusion—Particles’ distribution in a bifurcation is affected, beyond its outlets-flow-ratios, by the particle-to-branch diameter-ratio. The tendency of large particles to preferentially enter the wider bifurcation branch, beyond the flow ratio, is augmented under pulsatile flow conditions and is affected by particle-to-fluid density-ratio. These findings may have important implications for understanding the hemodynamic mechanisms underlying the trajectory of large emboli.

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Key Words: embolism ■ experimental ■ hemodynamics

Materials and Methods

The Experimental System
A computer-controlled gear pump (Cole-Parmer) supplied flow of water-glycerin mixture through a circuit of tubing to a horizontally Y-shaped symmetrical bifurcation model (Figure 1A). The Y-model was made from a drilled block of polycarbonate, as an analogue to an arterial bifurcation. An external reservoir with temperature control was used in order to keep the fluid viscosity stable. A buffer for particles was located upstream \( \approx 75 \text{ diameters} \) from the Y-model. An ultrasonic flowmeter (Transonic, with flow probe) was used for measuring flow signal at the entrance to the model, and 2 turbine flow meters (Mckmillan) were placed downstream to the model branches and were used to measure the flow rate ratio between the 2 daughter branches. The flow rate signal was digitalized using a sampling card (PCI-MIO-16E; National Instrument, with “Labview” package) and was monitored online using a PC platform.

The Y-model had identical branching angles \( \theta_1 = \theta_2 = 45^\circ \) (Figure 1B).

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2696
narrower \( (D_2 = 2/3D_1 = 4 \text{ mm}) \). The flow rate ratios \( (Q_1/Q_2) \) were determined by 2 valves controlling the resistance for each daughter branch \( (D_1 \text{ and } D_2, \text{ respectively}) \). The diameters and flow ratios used were chosen to typically characterize the extracranial cerebral arteries.

Particles were individually released into the stream from the particles’ buffer. To determine their distribution between the 2 daughter branches, the particles were trapped at the distal end of each bifurcation side by filters. After the particles were dried, they were weighted and counted using an electronic balance \( (AX120 \text{ Electronic balance; Shimadzu Corporation}) \).

**Experimental Parameters**

**Particles**

Spherical particles \( (\text{Polysciences Inc}) \) of 3 different diameters \( (0.6, 1.6, \text{ and } 3.2 \text{ mm}; \text{ Figure 1C}) \) were used in order to simulate emboli of different sizes.

The particles’ sizes were from the same order like in the in vivo situation. Each experimental point consisted of 4 iterations with \( \approx 300 \text{ particles of the 3.2 and } 1.6 \text{ mm particles and 1 gr (} \approx 10^4 \text{ particles) for the 0.6 mm particles.} \)

**Flow Conditions**

Two flow conditions were assessed: steady and pulsatile flow. The parameters that characterized the flow were the Reynolds number \( (Re) \) and the Womersley number \( (\alpha) \). Re is a dimensionless number, defined as the ratio of the inertia force to the viscous force in the blood flow, and is determined by flow rate, vessel diameter, and fluid viscosity. \( \alpha \) is the ratio of the transient or oscillatory inertia force to the shear force in the blood flow. In order to simulate flow conditions in the extracranial human cerebral circulation, Re for steady flow was set in the range present in the extracranial cerebrovascular system and maintained at 500. Pulsatile flow was used in the frequency of normal resting heart rate so that the sinusoidal flow waveform was maintained within an average \( Re = 500 \) and 75 pulses/min \( (\alpha = 5) \) at the inlet tube. Under physiological flow conditions, the flow through the wider daughter branch of an arterial bifurcation is always larger than the flow through the narrower branch. Therefore, we chose several flow rate ratios between the wider to the narrower bifurcation branches, all 1 or above.

**Density Ratio Between Particles and Fluid**

Emboli may differ by composition and density. The difference between the densities of a particle to that of the fluid dominate the body force acting on the particle. When the particle’s density is equal to the fluid’s density, the particles are in naturally buoyant condition, and no body force is acting on the particles. The main experimental design used naturally buoyant particles, and in another set of experiments floating particles were used by adjusting the water/glycerin ratio of the fluid.

**The Experimental Design**

To assess the parameters that govern the route and distribution of the particles in the model, we carried a set of experiments. The basic experiment was carried under steady flow with naturally buoyant particles, using 4 \( Q_1/Q_2 \) ratios and the 3 sizes of particles. Subsequently, the similar experiments were repeated but under pulsatile flow. Finally, the effect of buoyancy was assessed using both pulsatile and steady flow regimes.

**Statistical Methods**

Data were analyzed with SPSS software version 12.0. Two-way ANOVA was used to evaluate overall difference between the actual particles’ distribution to the hypothetical particles’ distribution according to flow, by particle diameter and the relative flow. One-way ANOVA with Tukey post-hoc test in each flow ratio was used to assess differences between the flow distribution by particle sizes.

**Results**

Validation of the Experimental Set Up

To validate the experimental setup, a basic calibration was first carried out using a comparable Y-model with symmetrical branching angles \( \theta_1 = \theta_2 = 45^\circ \) but identical inlet and outlet branches \( (D_0 = D_1 = D_2 = 6 \text{ mm}) \). Large Particles \( (3.2 \text{ mm}) \) were released using equal flow rates \( (Q_1 = Q_2) \) in the daughter branches under steady flow at \( Re = 500 \). Particles were found to be symmetrically distributed, showing that the tubing, adaptors, and filtering system do not effect particle distribution, and thus validating the experimental setup.

**Basic Experiment**

Small \( (0.6 \text{ mm}) \) and mid-sized \( (1.6 \text{ mm}) \) particles entered into either the narrow or wide bifurcation daughter branches non-preferentially, in proportion to the flow ratios \( (X = Y) \), whereas large particles \( (3.2 \text{ mm}) \) entered preferentially the wide daughter branch more than the flow rate ratio \( (Figure 2A) \). Moreover, as the flow rate ratio increased, this phenomenon was augmented.

A significant interaction effect \( (P < 0.001) \) was identified, using 2-way ANOVA, between the particles’ diameter and flow \( (ie, \text{ the particles’ tendency to enter a daughter bifurcation is a combination of the relative flow and the particles’ diameter}) \). By using 1-way ANOVA with Tukey post-hoc test in each flow ratio, significant difference was found between the flow distribution of the 3.2 mm particles versus the 1.6 and 0.6 mm particles \( (P < 0.05) \).

Figure 2B depicts the logarithm of the distribution ratios versus the logarithm of the flow rate ratios and reveals linear relations between them. This linear relation is independent of the particles’ diameter, but the slope of the linear trend line is different for each size of particles.

**Effect of Pulsatile Flow**

Next, we assessed the effect of pulsatile versus steady flow conditions at an average \( Re = 500 \) and \( \alpha = 5 \) for the mid-sized
and large (3.2 mm) particles. The distribution of particles under pulsatile flow did not differ from steady flow conditions for mid-sized particles, and particles were distributed proportionally to the flow ratios (Figure 3A). Pulsatile flow, however, in comparison with steady flow, has further augmented the tendency of the large particles to preferentially enter the wide daughter branch over the flow rate ratios (Figure 3B).

**Effect of Buoyancy**

To test the effect of the density difference between particles and fluid, the distribution of floating particles (particles’ density of 1.05 gr/mL versus fluid density’s of 1.1 gr/mL) were compared with that of naturally buoyant particles using 3.2 mm particles under steady flow conditions (Re=500).

Figure 2. A, The basic steady flow experiment using naturally buoyant particles at Re=500. The dots describe the various experimental particle distributions for different flow rate ratios and for different particle sizes. The x axis (Q1/Q2) depicts the flow ratio between the wide to the narrow outlet branches, whereas the y axis (N1/N2) depicts the ratio between the number of particles that entered into the wide bifurcation branch (D1) to the number that entered the narrow bifurcation branch (D2). The dash line depicts a theoretical particle distribution, which is proportional to the flow ratio between the branches (X=Y). Each of the data points presented in the graphs consists of the mean of the results of 4 iterations with standard error. B, Logarithmic presentation of the basic experiment’s results. The trend lines exhibit linear relations between Log(Q1/Q2) to Log(N1/N2) independent of the particle diameter (for the 3.2 mm, Log(N1/N2)=1.64×Log(Q1/Q2)−0.01, R²=0.99; 1.6 mm, Log(N1/N2)=0.96×Log(Q1/Q2)+0.12, R²=0.98; 0.6 mm, Log(N1/N2)=1.04×Log(Q1/Q2)−0.05, R²=0.98).

Because pulsatile flow and density difference both exhibited further increase of the tendency of larger particles...
enter the wider daughter branch, we tested their combined effect. Floating particles (3.2 mm) under pulsatile flow conditions were compared with the identically sized naturally buoyant and floated particles under steady flow conditions at Re=500. Floating particles under pulsatile flow preferentially entered the wider daughter branch over the particles under steady flow (both floating and naturally buoyant particles).

Discussion

Although the prevailing opinion is that the distribution of emboli in a bifurcation is determined solely by flow ratios, we found that the distribution of particles in a Y-bifurcation is affected by additional parameters. The particle-to-branch diameter ratio also affected the distribution of particles in that the tendency of particles to preferentially enter the wider daughter branch more than expected according to the flow rate ratio increased for high particle-to-branch diameter ratios. Moreover, this tendency was augmented under pulsatile flow conditions and was affected by the density ratio between particles and fluid. These findings may have important implications for understanding the hemodynamic mechanisms underlying the trajectory of large emboli.

As expected according to fluid dynamics, small particles entered into the narrow or the wide daughter branches nonpreferentially, similarly to the flow ratios. An illustrative example to this effect is the flow of red blood cells in large arteries that distribute in the vascular tree proportional to the flow rate ratio. Large particles (3.2 mm; 80% particle-to-narrow daughter branch diameter ratio, d/D2), however, preferentially entered the wider daughter branch, more than expected by the flow rate ratio. Generally, for large particles, sized almost as the narrow tube diameter (d/D2→1) it is intuitively expected that most of the particles will enter into the larger branch. The exact d/D2 for which this effect occurs is not yet clear, but it was observed in the current experiments only for large particles with d/D2=80%. A support for the unique behavior of the large particles may be found also in the theoretical analysis by Yen and Fung,7 in which an unique behavior of the large particles may be found also in association with more intense anticoagulation in atrial fibrillation, the best studied etiology for cardio-embolism.4,5,14 The bigger the arteries of the circle of Willis (ie, middle cerebral artery) is 2 mm, and the main tributaries 1 to 2 mm,15 in the same order size of the particles used in these experiments. Based on our findings, one might hypothesize that large emboli would

The tendency to preferentially enter the wider daughter branch in high particle-to-branch diameter-ratios is augmented under pulsatile flow. Under pulsatile flow, the fluid dynamic forces (oscillatory forces) cause the particle to vibrate, the particle is less stabilized, and its chance to enter the narrower branch decreases. This phenomenon is pronounced for large particle and negligible for very small ones.

As the density ratio between particles and fluid increases, the body force acting on it increases and causes the particle to move toward the tube wall. Because the narrow branch’s orifice is in the middle of the bifurcation’s cross section, deviation of the particle from the tube’s centerline decreases its chance to enter this branch. Because the body force is proportional to the particle’s volume (and to the third power of particles’ diameter) this phenomena is pronounced for large particles. In these experiments the bifurcation is horizontally oriented so that the branches are at the same plane in 90° with the body force direction, thus ensuring that no component of force is directed into a specific branch.

The study of the distribution of emboli in an arterial bifurcation is not new. Prior studies, however, assessed very different conditions in that small (micro) emboli were tested in in vivo and ex vivo experiments.8–10 Svensson et al11 investigated whether the anatomic origin of microemboli influences their intracranial distribution. The results of this study in baboon supports the hypothesis that microaggregates of cardiac origin distribute equally throughout the brain. Also, in a Transcranial Doppler study with agitated saline contrast injection, microemboli were distributed in both the anterior and posterior circulation, supporting our findings that small particles distribute proportional to flow.12 Macdonald et al13 assessed the distribution of microemboli in circumferential versus penetrating arteries in a monkey model and demonstrated that small emboli can enter penetrating arteries but that the majority enter circumferential arteries. Relatively larger emboli (92 μ) were more likely to enter the circumferential arteries.

The severity of embolic stroke depends on the size of the embolus and the location of the obstruction. Epidemiological data demonstrate poorer outcome and higher case-fatality rates from stroke associated with atrial fibrillation, the best studied etiology for cardio-embolism.4,5,14 The bigger the embolus and the larger the vessel obstruction, the larger the territory of brain at risk. A lessened severity of stroke was observed in association with more intense anticoagulation in patients with atrial fibrillation and may reflect smaller emboli or a reduced proportion of strokes from a cardiac source under more intense anticoagulation.15

The tendency of large particles to enter large branches more than the flow ratio might explain the distribution of large emboli attributable to atrial fibrillation in the human cerebrovascular system. The high rate of severe stroke and mortality that characterizes patients with embolic stroke attributable to atrial fibrillation supports the dislodgement of relatively large emboli.4,14 The diameter of the main cerebral arteries of the circle of Willis (ie, middle cerebral artery) is 2 to 4 mm, and their main tributaries 1 to 2 mm,16 in the same order size of the particles used in these experiment. Based on our findings, one might hypothesize that large emboli would
tend to preferentially enter the wider vessel more than expected by the flow rate ratio, and thus to preferentially enter the common carotid artery and then the internal carotid artery and middle cerebral artery rather than to enter narrower bifurcation branches, such as the vertebral arteries. Such a preferential distribution is in agreement with the common clinical observation of severe embolic strokes in the distribution of the anterior circulation.3,4

Strength and Limitations

We chose an in vitro experimental model to test our research question because an in vitro experimental model has many inherent advantages for hemodynamic assessment in a fully controlled experimental environment and may be a particularly useful experimental model for assessing the distribution of emboli in a bifurcation. The model enables selection and comparison of individual parameters studied one-by-one in a stable monitored system, not feasible in an in vivo model. Experiments in the current study were performed in a simple Y-bifurcation. The findings may be extrapolated to a more complex set of bifurcations, such as the cerebrovascular arterial tree, in that the path of an embolus from its source is considered as a superposition of the junctions that compose its path, as shown in a mathematical model by Pollanen.17

What may seem removed from in vivo conditions is therefore simplification and isolation of the most relevant physical parameters. The experimental model was designed as an analogue to an arterial bifurcation, as detailed in the Methods section. A limitation of the current study is that the effect of different bifurcation angles that may likely affect the embolic trajectory was not assessed. We used a symmetrical Y-model where the branching angle was not one of the tested parameters. Torvik and Skulphard18 proposed a mechanism to explain the preferential distribution of emboli in the cerebral circulation. They emphasized the sharp angulations of branching vessels, suggesting that emboli are unlikely to enter such branches. Bugliarello found, in a study from the mid-sixties,19 a very small effect of the angle of the bifurcation on the distribution of the particles, but a relative low particle to tube diameter ratio was used in these experiments.

There are infinite possibilities to characterize emboli’s shape, and we chose symmetrical spheres because spheres can be geometrically characterized by only one parameter (diameter), thus reducing the number of the tested parameters. Additional parameters that were not tested in the current experiments may likely affect the particles’ route (eg, vessels’ curvature, heart rate, etc) and the effects of such parameters on the distribution of emboli warrant further investigation. In vitro models should be supplemented by in vivo experiments designed to study the hemodynamic factors governing the path large emboli tend to follow.

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