Measured Turbulence and Its Effect on Thrombus Formation

By Paul D. Stein and Hani N. Sabbah

ABSTRACT
Turbulence is one of the hydraulic disturbances implicated in thrombus formation, even though absolute proof of its contributory effect is lacking. Because of the importance of a possible effect of turbulence on thrombus formation, the relation was studied in eight dogs. In each dog, two arteriovenous shunts were established, one from each femoral artery to the contralateral femoral vein. Only one shunt contained a turbulence-producing device; otherwise, the shunts were identical in shape, size, and material. The intensity of turbulence distal to the turbulence generator was quantified in vitro by measuring the relative magnitude of the randomly fluctuating velocities. In each of the eight dogs, more thrombi, by weight, accumulated in the turbulent shunt than in the laminar shunt (P < 0.001). Thrombi from the turbulent shunt weighed 180 ± 30 (SE) mg, whereas those from the laminar shunt weighed 0.9 ± 0.6 mg. The weight of thrombi that accumulated within the turbulent system appeared to be related to the intensity of turbulence. A linear relation was observed between the Reynolds number in the region of the turbulence-producing orifice and the weight of the thrombi within the turbulent shunt (r = 0.90). The relative intensity and the absolute intensity of turbulence distal to the turbulence generator were also linearly related to the Reynolds number (r = 0.97 and 0.99, respectively). The results of this study therefore indicate that turbulence is a characteristic of blood flow that can contribute to the formation of thrombi.

KEY WORDS
hemodynamics  blood coagulation  hemorheology
blood flow  fluid dynamics  disturbed flow  mongrel dogs

Attempts to detect turbulence in arterial blood flow prior to the introduction of hot-film anemometry tended to indicate that turbulence occurs (1, 2). Subsequent point velocity measurements within arteries by constant-temperature hot-film anemometry strengthened the earlier observations by demonstrating velocity fluctuations characteristic of disturbed flow (3-7). Hydraulic factors (8), including turbulence (9), probably contribute to atherosclerosis. Disturbances of flow have been shown to produce intimal damage in blood vessel walls (10), and turbulent blood flow appears to augment the sickling process of sickle homoglobin-containing erythrocytes (11). Of particular interest to us is the possibility that turbulent flow contributes to thrombus formation.

A variety of local alterations in blood flow have been thought to contribute to thrombus formation (12). Slowing of the blood and its effect on the distribution of platelets in the axial stream were considered by Eberth and Schimmelbusch in 1888 (13). Vortexes, which occur at sites where there are rapid changes in the rate or the direction of flow, were implicated by Von Recklinghausen in 1893 as possible contributing factors to thrombus formation (14). In 1927, Shionoya observed that thrombi in extracorporeal shunts are consistently deposited at sites where eddies and stagnation seem to occur (15). Geissinger et al. (16) in 1962 and Packham et al. (17) in 1967 showed that platelet deposits occur at bifurcations and the orifices of vessels. Disturbed flow appears to contribute to the deposition of platelets in vivo (18) and in shunts (8). The hydraulic disturbances that occur at bifurcations could involve boundary layer separation, local turbulence, vortex formation, cavitation, or a combination of these phenomena (19). Turbulence, supplemented by boundary layer separation and cavitation, has been labeled as the primary mechanical cause of thrombosis (19), although no firm evidence has specifically implicated this hydrodynamic factor or defined the relative importance of its role. A previous study from this laboratory has shown in dogs that platelet thrombi accumulate within...
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shunts in which turbulence is induced by flow through an orifice (20). Turbulence was identified in this study, but it was not quantified. In the present investigation, an effort was made to more firmly establish the premise that turbulence is a hydrodynamic factor that contributes to the formation of thrombi.

Methods

Studies were performed in eight mongrel dogs (19.5–28.5 kg) anesthetized with sodium pentobarbital (25–28 mg/kg body weight, iv). In all dogs, two arteriovenous shunts were established, one from each femoral artery to the contralateral femoral vein. In one shunt, a device for creating turbulent flow was incorporated. The turbulence generator consisted of a smooth tube with a tapered central constriction 1.6 mm in diameter (Fig. 1). In the laminar flow shunt, a similar length of tubing with no constriction was inserted so that flow would be undisturbed. The shunts were otherwise identical in size and material. They were fabricated from Teflon and Silastic tubing with an internal diameter of 4.8 mm. The tubes were tapered to 3 mm at the sites of the venous and arterial insertions. A particular effort was made to be certain that identical lengths of each type of tubing were inserted in both the laminar and the turbulent shunts. The nonconstricted section of tubing that was inserted in the laminar flow system served as a control to substitute for the foreign surface incorporated in the turbulence generator. Cannulating Biotronex electromagnetic flow transducers 3 mm in diameter were incorporated within the shunts to be certain that flow was comparable in both types of shunt. The transducers were coated with Siliclad to diminish the activation of the clotting mechanism. It was recognized, however, that some platelet adherence to the silicone-coated surfaces would occur. No anticoagulants were administered at any time during the study.

Before insertion of the shunts into the dogs, the flow transducers were connected to a two-channel Biotronex BL 610 electromagnetic blood flowmeter, and the transducers were made equisensitive. In all but one dog, flow was kept at comparable levels in both shunts by adjusting a screw clamp on the distal portion of each shunt. The flow transducers were calibrated using the dog’s own blood at the conclusion of each study.

Both extracorporeal systems were filled with saline and clamped to prevent blood from entering the tubing while the shunts were being inserted into the vessels. After the shunts had been inserted, all clamps were simultaneously removed, allowing blood to flow through both systems at the same time. Both shunts were clamped after 7 minutes of flow. The shunts were then promptly removed, and thrombi were carefully taken from them and immediately weighed. It has previously been shown that the deposits that occur in this system have the characteristics of platelet thrombi (20).

During each study, the Reynolds number (Re) calculated as Re = ρνD/μ, where ρ = density of fluid (g/ml), ν = velocity of fluid (cm/sec), D = diameter of orifice (cm), and μ = viscosity of fluid (poise), was measured in both the turbulent and the laminar shunt. The diameter of the orifice, 1.6 mm, was used to calculate the Reynolds number within the turbulent shunt, and the uniform diameter of 4.8 mm was used to calculate the Reynolds number within the laminar shunt. The density of blood was measured with a hydrometer, and its viscosity was measured with a Wells-Brookfield microviscometer equipped with a 1.565-degree cone spindle and a sample cup. The viscometer was calibrated using calibrated oil. A circulating type of constant-temperature (37°C) bath was used with the sample for temperature control. Velocity, for purposes of calculating the Reynolds number, was determined as the ratio of mean flow to cross-sectional area. Flow was measured with an electromagnetic flowmeter.

Twenty-one separate studies of the effects of turbulent flow on thrombus formation were performed in these eight dogs. Various levels of flow and various Reynolds numbers were produced within the shunts by partially constricting them near the inflow. Following each study, the shunts were removed and thoroughly rinsed with cold water. Prior to reinsertion within the dog, any thrombi that had developed at the site of the ligatures around the arteriotomy or venotomy were removed with an embolectomy catheter. Control measurements of the prothrombin time, the partial thromboplastin time, the platelet count, and the hematocrit were obtained before each study and remeasured after the entire series of studies on each dog (Table 1).

The range of Reynolds numbers required to produce a turbulent region just distal to the turbulence generator but laminar flow elsewhere was determined experimentally in vitro by inserting a hot-film sensor within the shunts. Instantaneous velocities distal to the turbulence-producing orifice of the turbulent shunt and within the laminar shunt were measured with the hot-film sensor. The existence of turbulence was documented by measur-
Hematological Measurements

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Post refers to values remeasured after the entire series of studies on each dog.

Flow and velocity recorded in turbulent and laminar shunts during an in vitro correlation of intensity of turbulence with Reynolds number. Flow in both shunts, measured with an electromagnetic flow meter, was equal. Velocity distal to the turbulence-producing orifice, recorded with the hot-film anemometer, showed prominent fluctuations. The relative intensity of turbulence, 0.19, was calculated as the ratio of $u'$ to $U$. Velocity within the laminar shunt (right) showed no disturbances; pulsatile velocity along the axis of flow reflected the fact that flow was pulsatile.
The intensity of turbulence could not be satisfactorily measured during in vivo studies in the dogs, because fibrinous material was deposited on the sensing surface of the hot-film probes and interfered with the measurements. The utilization of heparinized blood in vitro prevented this problem. A pulsatile pump (Harvard Instrument Co.) was used to move the heparinized blood at pump rates of 110 and 150 beats/min. The stroke output ranged between 0.5 and 2.3 ml/beat. Blood with a viscosity of 0.05 poise (measured at room temperature) and a density of 1.035 g/ml was used. These values of rate, stroke volume viscosity, and density are comparable to those measured in vivo and used to calculate the Reynolds numbers. Turbulence was observed distal to the orifice of the turbulence generator at Reynolds numbers of 180 and above. A linear relation between the Reynolds number and the intensity of the turbulence produced within the turbulent shunt was shown (Fig. 3).

Within the laminar shunt, laminar flow occurred at flows identical to those that produced turbulence within the turbulent shunt (Fig. 2). The Reynolds number, when expressed in terms of flow, is inversely related to the diameter of the tube; therefore, the Reynolds numbers at comparable flows were lower within the laminar shunt, because it contained no narrowing in the form of a turbulence-producing orifice. Of greater importance is the fact that the critical Reynolds number within the turbulent shunt was lower than that within the laminar shunt. As previously indicated, turbulent flow was observed distal to the turbulence-producing orifice at Reynolds numbers as low as 180. No disturbances of flow were observed within the laminar system, even at flows identical to those that produced marked turbulence within the turbulent shunt. This phenomenon was expected, since the Reynolds number within the laminar shunt did not exceed 310 during any of the studies. The critical Reynolds number within the smooth, uniform tubing of the laminar shunt should be greater than 2000, since the critical Reynolds number for pulsating flow is the same or higher than that for steady Poiseuille flow (22).

Results

More thrombi, by weight, occurred within the turbulence-producing shunt than within the laminar flow shunt. Thrombi invariably occurred in the region of the turbulence generator, and most of the thrombi were located just distal to the orifice. The weight of thrombi that accumulated in the turbulent system (considering only the first observation in each dog) was 180 ± 30 (SE) mg, whereas the weight of thrombi that accumulated in the laminar system was 0.9 ± 0.6 mg \( (P < 0.001) \). Flows, velocities, Reynolds numbers, and weights of thrombi during each observation are shown in Table 2.

A linear relation was observed between the Reynolds number in the region of the turbulence-producing orifice and the weight of the thrombi within the turbulent shunt \( (r = 0.90) \) (Fig. 4). The relative intensity of turbulence distal to the turbulence generator was also linearly related to the Reynolds number \( (r = 0.97) \) (Fig. 3). Similarly, the absolute intensity of turbulence (the root-mean-square value of the fluctuating components) was linearly related to the Reynolds number \( (r = 0.90) \). The weight of thrombi that accumulated within the turbulent system, therefore, appears to be related to the intensity of turbulence.

At no time did turbulent flow occur within the laminar flow system. The Reynolds number within this system never exceeded 310.

![Figure 3](image_url)

Turbulence intensity within the turbulent shunt as a function of Reynolds number. Pulsatile flow from the pump at simulated heart rates (HR) of 110 beats/min (triangles) and 150 beats/min (circles) was studied. The correlation coefficient (R) and the regression equation are shown.

_Circulation Research, Vol. 35, October 1974_
TABLE 2
Flow Characteristics of Blood and Weight of Thrombi

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Trial no.</th>
<th>Viscosity (poise)</th>
<th>Density (g/ml)</th>
<th>Mean flow (ml/sec)</th>
<th>Mean velocity (cm/sec)</th>
<th>Reynolds number (dimensionless)</th>
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Lam = laminar flow system, and Turb = turbulent flow system.

Discussion

The orifice within the turbulent shunt produced turbulent jets with well-described boundaries, the structure of which has been extensively studied by others (21). Care was taken to ensure that all factors except turbulence were the same in both flow systems. Since the same amount and type of foreign surface was present in each shunt, the presence of the foreign surfaces does not appear to be the cause of the observed differences in thrombus formation. Of course, truly intravascular thrombosis was not demonstrated, since both shunts were extracorporeal systems.

The mechanism of thrombus formation induced
by turbulence is presumably related to the effects of turbulence on formed elements in the blood. Such effects could have been caused by (1) shear, (2) collision with the walls of the tubing, or (3) prolonged contact with the foreign surface. Both high shear and agitated random flow are intrinsic characteristics of turbulence (23). The possibility that high rates of shear contribute to the activation of platelets has been previously considered (24). Collision of formed elements with the walls of the tubing does occur, since turbulence causes eddies of flow perpendicular to the channel. Injury of the blood cells due to such collisions could have contributed to the deposition of thrombi. Collision of the formed elements with the turbulence generator cannot be excluded. This phenomenon could also have contributed to the deposition of thrombi, although the generator was tapered to diminish this effect.

Agitated random motion of the blood within the turbulent region could have caused the blood cells to be in contact with the walls of the tubing (a foreign surface) for a longer period of time in the turbulent system compared with the laminar flow system. This situation could have contributed to the observed increase in thrombus formation in the turbulent system. Such a mechanism, if valid, could apply to the mechanism of thrombus formation in some clinical circumstances such as thrombosis in the vicinity of intravascular prostheses.

The possible contribution of thrombus formation of a recirculating region or a stationary vortex in the region of the turbulence generator merits consideration. Stagnation, such as that which can occur in vortexes (24), has been shown by others to be associated with platelet deposition (15). Previous observations during dye injections made in a transparent, geometrically similar apparatus during both laminar and turbulent flow in vitro have shown that there is rapid flow into and out of any recirculating region. The rapid clearing of dye from these regions indicates that stagnation does not occur. The behavior of the formed elements within the liquid is assumed to be similar to that of the flowing system, although migration of particles is known to occur (24).

In conclusion, turbulence appears to contribute to the formation of thrombi. In each of the eight dogs in which turbulent flow was present, more thrombi were recovered from the turbulent system than from the nonturbulent system, and the amount of thrombus formation (weight of thrombi) seemed to be related to the relative intensity of turbulence.

References


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