Intramyocardial platelet aggregation in patients with unstable angina suffering sudden ischemic cardiac death

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ABSTRACT A specific search for intramyocardial platelet aggregates was made in 90 patients who died suddenly of ischemic heart disease. Platelet aggregates in small intramyocardial vessels were found in 27 (30%). There was a significant difference (p < .05) in the incidence of platelet aggregates in patients with chest pain of recent onset (unstable angina) before death (16/36, 44.4%) and that in those without it (11/54, 20.4%). Multifocal microscopic necrosis with involvement of the full thickness of the ventricular wall, including the subpericardial zone, was significantly more common (p = < .005) in the patients with platelet emboli (55.6% vs 12.7%). With one exception, aggregates were confined to the segment of myocardium immediately downstream of a major epicardial coronary artery containing an atheromatous plaque that had undergone fissuring and on which mural thrombus had developed. The results support the view that platelet aggregates in the myocardium represent an embolic phenomenon and are a potential cause of unstable angina. The association of myocardial necrosis with such emboli could precipitate sudden death from ventricular fibrillation.


WHEN PATIENTS with stable and unstable angina are compared the degree and distribution of stenosis is similar, but unstable angina is associated with stenotic lesions that have a characteristic angiographic morphology.1 These type II lesions have morphologic features, including an irregular outline and the presence of intraluminal filling defects outlined by contrast medium,2 that are observed on postmortem angiograms.3, 4 Under these circumstances histologic examination reveals atheromatous plaques that have undergone fissuring, rupture, or ulceration and over which nonocclusive (mural) thrombus has formed.

When thrombus projects into, but does not totally occlude, the lumen of an artery, there is the potential for emboli to pass distally into the segment of myocardium supplied by that artery. Such embolization has been postulated to be responsible for the crescendo form of unstable angina, particularly when it culminates in acute infarction, and for provoking ventricular fibrillation and sudden death.4, 6 Indirect evidence as to whether or not intramyocardial platelet emboli occur can be obtained, at present, only from autopsy studies since unlike the cerebrovascular circulation, for which the retina provides a window, the myocardial microcirculation cannot be visualized in life.

Previous studies of the myocardium at necropsy have revealed platelet aggregates in up to 54% of those suffering sudden ischemic death,7–9 but these findings have been regarded as indicative of hyperactive platelets that have undergone spontaneous intravascular agglutination in the microvascular bed. Falk,5 in a detailed study of 22 patients who died within 24 hr of the onset of acute ischemic myocardial damage, found platelet emboli in 11 (50%), but in his small series there was no significant difference in the incidence when he compared those patients with preceding unstable angina to those without it. Segments of myocardium that were not supplied by an artery containing mural thrombus were always devoid of platelet emboli. This finding was taken to confirm the embolic nature of the platelet masses since aggregation would occur more widely in the myocardium.
We report here the results of a specific search for platelet masses in the small vessels of the myocardium in patients who died suddenly within 6 hr of the onset of pain or other symptoms of the fatal attack and in whom there was no cause of death other than coronary stenosis due to atheroma so that death must be regarded as due to ischemic heart disease.

**Material and methods**

**Patients.** Ninety patients who died suddenly within 6 hr of the onset of symptoms of the last episode of chest pain and in whom there was no obvious cause of death other than over 75% coronary artery stenosis measured by cross-sectional area were studied. Only patients who had been leading their normal lifestyle and had not consulted a medical advisor within the last 2 weeks were included. Patients with a history of stable angina and/or previous myocardial infarction were included if they met the other criteria for entry into the study. Subsequent to death and before autopsy a coroner’s police officer obtained a clinical history from the next of kin who had been living with the patient. The specific question asked was whether the patient had complained, in the last 2 weeks, of any new symptoms. A complaint of intermittent chest or arm pain was regarded as significant; histories of weakness or tiredness were disregarded. A complaint of “indigestion” was taken as significant only if it was new and chest pain was mentioned specifically. On the basis of their previous medical histories, patients were subdivided into those who had been diagnosed as having chronic ischemic heart disease (stable angina and/or previous infarction) and those who were not known to have had ischemic heart disease. By chance the two groups, 45 in each, were exactly equal in size. The age range was from 34 to 69 years; 75 of the 90 were men. There was no significant difference in the distribution of men and women in the patient groups.

**Procedures.** Postmortem coronary arteriography was carried out by perfusing the arteries with a warmed suspension of gelatin and barium sulphate at physiologic pressure. The epicardial coronary arteries were examined subsequently, by histology, at 3 mm intervals throughout their entirety. Arterial segments were quantified for stenosis by comparing the cross-sectional area of the lumen to the area within the internal elastic lamina on a digitizing pad. All segments were coded for the presence or absence of plaque fissuring and mural or occlusive intraluminal thrombus.

A 1 cm thick transverse slice of myocardium was taken through the short axis of the ventricles at mid septal level and examined histologically after division into 17 numbered segments. Examination of the myocardium was made without knowledge of the coronary artery pathology. The sections of myocardium were stained initially by the hematoxylin and eosin method and screened for occlusion of small vessels. Sections from any segment of myocardium in which vessels were identified as containing intravascular material that might be fibrin and/or platelets were stained by a modified trichrome method that differentiates fibrin as red, platelets as mauve, and red cells as yellow. Only intravascular masses in excess of 50 μm in diameter and made up of platelets or platelets and fibrin were considered as emboli. Smaller platelet masses intimately admixed with agglutinated red cells were ignored. The presence or absence of regional coagulative myocardial necrosis and of microscopic areas of focal necrosis contained individually with a low-power microscopic field were noted. An assessment was made of their distribution in the inner (subendocardial), and mid or outer (subepicardial) layers of the ventricular wall. In the presence of a large area of coagulative necrosis occupying more than a low-power field focal necrosis was not recorded as a separate entity.

**Results**

Platelet fissures were found in 86 of the 90 patients (90.5%). In 31 of 86 (36%) the artery was occluded by thrombus to a degree that distal filling did not occur and in the remainder the vessel distal to the fissure was filled by angiographic media. Intravascular masses of platelets were found in 27 of the 90 patients overall (30%) and were in segments of myocardium downstream from fissured atheromatous plaques (figure 1) with exposed mural thrombi in 26 of the 27 patients. In these 26 patients other segments of myocardium were free of emboli. In only one case were emboli found in a posterior segment of the left ventricle with thrombosis in the left anterior descending coronary artery.

The majority of platelet aggregates (figures 2 to 6) had a minimal or even totally absent fibrin component. A few larger intramyocardial vessels contained masses with a substantial amount of fibrin (figures 7 and 8). Platelet masses were found in vessels ranging in size from precapillary (figure 5) arterioles to arteries 1 mm in diameter (figure 7) and usually but not inevitably occluded the vessel. The incidence of platelet aggregates in the myocardium was eight of 31 patients in whom the vessel was occluded (25.9%) and 19 of 59 (32.2%) in whom the vessel subtending that segment of myocardium was patent. Two patients had evidence of embolization of cholesterol and atheromatous debris in addition to platelet aggregates.

Of the 90 patients, 36 had experienced episodic chest or arm pain in the 2 weeks preceding death and 54 had not. Three of the 54 patients without and 1 of the 36 patients with chest pain in the last 2 weeks had no plaque fissure and no acute vascular event to explain the timing of sudden death. The difference was not significant. Sixteen of the 36 (44.4%) and 11 of the 54 (20.4%) had intramyocardial platelet emboli. In comparing the incidence of platelet aggregates (table 1) between the two groups there was a significant difference ($p < .05$) by $\chi^2$ test ($\chi^2 = 4.87$ corrected for continuity). When cases with and without a known history of ischemic heart disease are compared there is no significant difference in the incidence of platelet aggregates (table 1).

Patients without platelet aggregates had a higher incidence of a normal myocardium (table 2). The null hypothesis that the presence or absence of such platelet masses does not affect the occurrence of ischemic changes in the myocardium may be rejected ($\chi^2 =$}
FIGURE 1. Thrombus (TH) projecting into the lumen (L) of a major pericardial coronary artery through a fissure (arrows) in the cap of an atheromatous plaque (P). Thrombus is also present within the plaque in the intima. The lumen contains contrast media used in angiography (C). (Original magnification × 20.)

FIGURE 2. An intramyocardial artery containing a large mass of platelets occupying most of the lumen. The contrast media used in angiography (arrow) is easily distinguished from platelets by a smaller granule size and different color (Original magnification × 250.)
FIGURE 3. An artery within the myocardium completely occluded by a mass of platelets appearing as small dark dots. In addition there are a few small masses of fibrin (arrow). No injection media is present (Original magnification × 450.)

FIGURE 4. Two intramyocardial arteries completely occluded by masses of platelets recognized by their punctate nature. In one artery (arrow) the mass of platelets also contains polymorphonuclear leucocytes. No injection media is present. (Original magnification × 250.)
19.61, p < .005, with continuity correction). Multifocal microscopic myocardial necrosis (figures 9 and 10) was found in 15 of 27 patients with platelet fibrin aggregates, but in only eight of the 63 patients in whom they were not observed. When the two groups are compared this difference was significant ($\chi^2 = 16.85$, $p < .005$, with continuity correction). The foci of microscopic necrosis were present throughout the left ventricular wall and in all cases the subpericardial zone was involved. There was no significant difference in the proportion of patients with larger areas of regional coagulative infarction (six of 27 [22.2%] and 16 of 63 [25.4%]) in the two groups. Of the 22 patients with regional infarction, 17 (77.3%) had no filling of the related artery distal to a plaque fissure and thrombus. Of the remaining 68 patients, who had either normal myocardium or multifocal microscopic necrosis, 14 (30.6%) had no distal flow in the related artery.

**Discussion**

This study and that of Falk establish that a proportion of patients who die suddenly from ischemic heart disease, with or without acute myocardial infarction, can be shown at autopsy to have platelet aggregates in the myocardium. Both studies agree that platelet masses are found only in segments of myocardium downstream of an atheromatous plaque over which thrombus has developed. These results could have a number of explanations. Nonspecific agonal aggregation of platelets is excluded by the selectivity to segments of myocardium downstream of mural thrombi. However, this selectivity could itself be the result of postmortem angiography. There are a number of factors pointing to the platelet aggregates being true emboli that represent a phenomenon in vivo, although it is impossible to prove absolutely that their incidence is not increased by the technique of postmortem coronary injection.

Platelet emboli were found predominantly in small vessels (figures 4 to 7) to which injection media had not penetrated due to its viscosity. If the injection media itself displaced platelet aggregates the two would be expected to be intimately mixed. The incidence of platelet aggregates downstream of an occlud-
FIGURE 6. A small artery sectioned in the longitudinal axis. There is an aggregate of platelets (arrows) on either side of which are red cells. The artery is lined by an intact endothelium. No injection media is present. (Original magnification × 350.)

FIGURE 7. A large intramyocardial artery completely occluded by a mass that comprises a network of dark-staining fibrin between which platelets are packed. (Original magnification × 200.)
ing thrombus is not different from that related to a mural thrombus. Platelet aggregates can be found in hearts in which postmortem angiography has not been carried out (figure 8), although we do not have figures as to its exact incidence from our own experience since our standard practice is to carry out postmortem angiography. Previous studies reporting platelet microthrombi have been carried out on uninjected hearts; in one such study 22 out of 47 patients (53%) who had suffered sudden death had platelet aggregates in the myocardium. Only a minority of plaques with fissures are associated with distal microthrombi, but artifactual induction might be expected to produce a higher incidence. Finally, if platelet aggregates were solely artifactual it is unlikely that there would have been a difference in incidence between the two patient groups.

If it can be accepted that the platelet aggregates are emboli and are found in a certain proportion of patients suffering sudden ischemic death and acute infarction, it must still be questioned whether or not they are responsible for ill effects. Pathologic studies cannot give an absolute answer, but the present findings and those of Falk suggest that platelet emboli are significant because both studies show that multifocal microscopic necrosis is closely associated with the presence of platelet aggregates.

### TABLE 1
Presence of intramyocardial platelet/fibrin aggregation in relation to clinical history of chest pain in the preceding 2 weeks

<table>
<thead>
<tr>
<th></th>
<th>With platelet aggregates</th>
<th>Without platelet aggregates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstable angina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Known IHD</td>
<td>8/15</td>
<td>16/36 (44.4%)^A</td>
</tr>
<tr>
<td>No Known IHD</td>
<td>8/21</td>
<td>27/90 (30%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No unstable angina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Known IHD</td>
<td>4/30</td>
<td>11/54 (20.4%)^A</td>
</tr>
<tr>
<td>No Known IHD</td>
<td>7/24</td>
<td></td>
</tr>
</tbody>
</table>

IHD = ischemic heart disease.

^Ap < .05.

### TABLE 2
Presence of myocardial necrosis in patients with and without platelet/fibrin aggregates

<table>
<thead>
<tr>
<th></th>
<th>With platelet aggregates</th>
<th>Without platelet aggregates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated multifocal microscopic necrosis</td>
<td>15 (55.6%)^A</td>
<td>8 (12.7%)^A</td>
</tr>
<tr>
<td>Regional coagulative necrosis</td>
<td>6 (22.2%)</td>
<td>16 (25.4%)</td>
</tr>
<tr>
<td>No acute necrosis</td>
<td>6 (22.2%)</td>
<td>39 (61.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>63</td>
</tr>
</tbody>
</table>

^Ap < .005.
of platelet emboli. Any condition that causes even microscopic myocardial necrosis is, a priori, a potential precipitant of ventricular fibrillation and hence of sudden death.

Two forms of necrosis were encountered in the study: coagulative necrosis involving large areas, particularly in the subendocardial zone, and multifocal microscopic foci distributed throughout the thickness of the ventricular wall. A small proportion of the larger areas of necrosis had reached the colliquative stage in which microvascular damage had induced fibrin thrombi. Such fibrin thrombi in vessels surrounded by dead tissue were not counted in the present study.

All of the larger areas of infarction were associated at their periphery with additional small focal areas of necrosis, but these were predominantly subendocardial with sparing of the subpericardial layer and were in contrast to the transmural distribution of multifocal microscopic necrosis. Both forms of necrosis might be the result of occlusive epicardial arterial thrombus, but in the dog induction of infarction by ligation of epicardial arteries has been shown to induce necrosis that spreads from the endocardial zone outward. This fact suggests that microscopic foci throughout the wall, particularly in the subpericardial zone, represent a different mechanism. Platelet emboli would be one putative mechanism suggested by our data. In eight of the 25 examples of multifocal microscopic necrosis, however, no platelet emboli were demonstrated. This may mean that such emboli induce microscopic foci of necrosis but are readily cleared from the microcirculation and vanish or that there is another mechanism for the induction of microscopic multifocal necrosis.

Falk in his small series of 22 patients found that the frequency with which platelet emboli were found in patients with unstable angina was above that in patients without unstable angina but the difference did not reach statistical significance. On the other hand, the present rather larger series has demonstrated a significant increase in the incidence of intramyocardial platelet emboli in patients with unstable angina who die suddenly. Platelet emboli in the myocardium may cause symptoms by release of pharmacologically active substances, including thromboxane A2 and sero-

FIGURE 9. Microscopic focus of acute myocardial necrosis with dark, deeply eosinophilic hypercontracted muscle cells surrounded by normal myocardial cells. (Original magnification × 350.)
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FIGURE 10. Foci of acute myocardial necrosis (N) at the stage by which necrotic muscle cells have been resorbed, leaving a lattice of fine connective tissue stroma. Such foci collapse and lead ultimately to small collagenous scars. The pericardium is close by (P). A small artery (arrow) adjacent to the foci of necrosis shows an intraluminal lattice of fibrin, but platelets are absent at this stage (Original magnification × 5.)

tonin, which increase vasomotor tone to a degree similar to that induced by the physical plugging of small vessels.6 Less direct evidence for the role of platelets in unstable angina comes from the improvement in prognosis in such patients after aspirin therapy.12, 13

Inevitably, an autopsy study is biased because it contains only those patients who have died and it cannot therefore be taken to establish that all or even the majority of patients with unstable angina have plaque fissuring with overlying mural thrombus and platelet emboli. Pathologic studies, while they can suggest that platelet embolization exists, are not ideally suited for establishing its exact incidence. The techniques for demonstrating platelets in tissue sections are difficult and time consuming to carry out. In histologic sections stained by routine hematoxylin and eosin methods sludged or agglutinated red cells appear as a shade of pink virtually identical to that of platelets. The recognition of thrombi is usually dependent on the presence of bright red–staining fibrin; since platelet emboli do not frequently contain fibrin they often escape notice.

There are good differential staining methods for platelets,10, 14 but they are arduous technically and cannot be used to screen large numbers of sections. Unless pathologists take special care the true incidence of platelet emboli will be underestimated. Because platelet emboli are localized to the area of myocardium supplied by the artery in which a thrombus has developed, examination of random sections of the myocardium will also underestimate their frequency. These factors explain the infrequent recognition of platelet emboli in the past. At the other extreme it is possible to set criteria that are too sensitive; after death, red cells that have agglutinated within small intramyocardial blood vessels will contain small clumps of platelets. One study that reported platelet emboli in 15% of control hearts highlights this source of error.8 If the criteria are adopted of accepting as emboli only masses of platelets over 50 μm in diameter that are not admixed with agglutinated red cells and that plug vessels, this source of errors is avoided.

Patient selection will influence the proportion of patients considered positive for platelet emboli in any particular study. If platelet emboli are associated with an unstable phase of angina and with acute myocardial necrosis and if these conditions are used as criteria for excluding patients from a particular study, then the incidence of platelet emboli will fall dramatically; in the present series it would have resulted in only two positive findings out of 54 patients (3.7%). The ab-
sence of platelet emboli in other studies of sudden ischemic death\textsuperscript{15} may illustrate the effect of such exclusions.

Establishing cause-effect relationships in postmortem studies is very complex and can never provide absolute proof, yet despite this reservation the present data suggest that the analogy that has been drawn between transient cerebral ischemic attacks due to platelet emboli from carotid atheromatous plaques and unstable angina of the crescendo type is a valid one.\textsuperscript{5, 16} The results of many pathologic studies of fatal acute ischemic attacks, including cases of sudden death, acute infarction,\textsuperscript{4, 17–19} and crescendo angina,\textsuperscript{5} now stress the role that plaque fissuring plays in invoking both mural and occlusive coronary thrombosis. Our study presents evidence supporting the additional role of platelet aggregation in the acute ischemic syndromes. Such studies do not discount that other mechanisms may also cause unstable angina; plaque fissures may precipitate arterial spasm or, conversely, spasm may provoke fissures. High-grade stenosis may predispose to the development of platelet thrombi on an intact endothelial surface and the consequent release of vasoactive substances could cause vasospasm. Finally, spasm may occur in relation to an eccentrically placed plaque that leaves a segment of vessel wall with a normal media capable of undergoing contraction.\textsuperscript{20, 21}

References
11. Reimer KA, Jennings RB: The wave front phenomenon of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 40: 633, 1979
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