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Relationship of Thrombus Healing to Underlying Plaque Morphology in Sudden Coronary Death

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Objectives The aim of this study was to assess differences in thrombus healing between ruptured and eroded plaques, given the natural difference in lesion substrate and that thrombi might exist days to weeks before the presentation of sudden coronary death.

Background Although the ability to distinguish ruptures and erosions remains a major clinical challenge, in-hospital patients dying with acute myocardial infarction establish that erosions account for 25% of all deaths, where women experience a higher incidence compared with men.

Methods Coronary lesions with thrombi (ruptures, n = 65; erosions, n = 50) received in consultation from the Medical Examiner's Office from 111 sudden death victims were studied. Thrombus healing was classified as early (<1 day) or late stage characterized in phases of lytic (1 to 3 days), infiltrating (4 to 7 days), or healing (>7 days). Morphometric analysis included vessel dimensions, necrotic core size, and macrophage density.

Results Late-stage thrombi were identified in 79 of 115 (69%) culprit plaques. Women more frequently had erosion with a greater prevalence of late-stage thrombi (44 of 50, 88%) than ruptures (35 of 65, 54%, $p < 0.0001$). The internal elastic lamina area and percent stenosis were significantly smaller in erosions compared with ruptures ($p < 0.0001$ and $p = 0.02$), where plaque burden was greater ($p = 0.008$). Although macrophage infiltration in erosions was significantly less than ruptures ($p = 0.03$), there was no established relationship with thrombus organization. Other parameters of thrombus length and occlusive versus nonocclusive showed no association with healing.

Conclusions Approximately two-thirds of coronary thrombi in sudden coronary deaths are organizing, particularly in young individuals—especially women, who perhaps might require a different strategy of treatment. (J Am Coll Cardiol 2010;55:122–32) © 2010 by the American College of Cardiology Foundation

Acute myocardial infarction (AMI) and sudden death predominantly arise from coronary thrombosis caused by rupture of a thin fibrous cap or surface erosion in the absence of cap disruption (1–5). Although the morphology of the culprit plaque has been extensively studied, especially rupture, relatively little is known about the temporal relationship between the onset of acute coronary events and thrombus maturation. The occurrence of nonlethal ruptures recognized by accumulated fibrous tissue at healed repair sites suggests that healing thrombi represent an episodic cycle of lesion progression (6,7). Moreover, thrombi from fatal plaques are in various stages of healing, further suggesting that death might not necessarily coincide with the initial onset of thrombus formation.

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The presentation of acute ST-segment elevation myocardial infarction (STEMI), attributed to thrombi, is mainly conceptualized as a rapid onset of thrombotic occlusion of

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the infarct related artery, followed shortly by the onset of symptoms and/or death. The finding of healing in >50% of aspirated thrombi from patients presenting with STEMI during percutaneous coronary intervention (PCI), however, challenges this theory (8,9). These data indicate that sudden coronary occlusion is often preceded by a variable period of plaque instability and thrombus evolution before the onset of symptoms. Thus, acute coronary occlusion might represent the final phase in a series of nonocclusive atherothrombotic events transpiring in the foregoing days or even weeks (9). Furthermore, the age of the thrombus, in addition to other risk factors, seems to be an independent predictor of long-term mortality, particularly within the first 2 weeks after primary PCI (8).

Although the pathologic analysis of material retrieved by thrombectomy is informative, there is a limited understanding of the healing properties of coronary thrombi in relation to the underlying plaque morphology. Important issues regarding why a number of thrombi smolder with later presentation of AMI and whether the rate of thrombus maturation differs between events caused by plaque rupture or erosion are unclear. In addition, the mechanisms and potential triggers involved in the occurrence of a fresh occlusive thrombus superimposed on the healing thrombus are unknown. For example, occlusive thrombi in early stages of healing might be more prevalent in ruptures, because the initiating event involves a physical tear in the fibrous cap, with the exposure of a highly thrombogenic necrotic core. On the contrary, the precise trigger(s) for erosion remain less clear, where the nonruptured luminal surface might play an even greater role in thrombus organization and healing.

The current study focuses on the healing of coronary thrombi in sudden death victims. It is our contention that an established thrombus is present days or weeks before sudden death where the natural course of healing is vitally dependent on the underlying culprit plaque in relation to rupture or erosion.

Methods

Selection of cases. Case enrollment involved examination of all 345 sudden coronary death (SCD) cases received in consultation from the Maryland Medical Examiner's Office between years 2005 and 2008. Of these, 181 patients died of coronary artery thrombi (129 ruptures and 52 erosions), and the remaining died with severe coronary atherosclerosis in the absence of an acute thrombus. Included in the analysis are 111 cases of thrombi, on the basis of similar demographic data and the availability of sections suitable for staining and morphometric analysis. The examination included 65 ruptures and 50 erosions. The number of erosions as compared with ruptures was maximized while maintaining age and sex differences that occurred in subjects dying with plaque rupture during the aforementioned period.

Sudden death was defined as natural deaths without extracardiac causes occurring within 6 h after the onset of angina symptoms (witnessed cardiac arrest) or last seen alive within 24 h in a normal, healthy state (10). Registry files were also reviewed for the incidence of witnessed cardiac arrests with typical (chest pain, malaise, dyspnea, and nausea/vomiting) or atypical symptoms (paresthesia, back pain, and indigestion) associated with unstable angina and MI. Cardiovascular risks of smoking, diabetes, and hypertension were assessed as described previously (11).

Characterization of coronary lesions. Plaques with acute coronary thrombi were categorized as plaque rupture or erosion, as previously described (5). Ruptures showed areas of necrosis underlying a thin disrupted fibrous cap with a superimposed luminal thrombus, whereas eroded plaque showed surface thrombi in the absence of cap disruption. The necrotic core, when present in erosions, did not communicate with the lumen. Overall plaque burden was derived by adding the maximal percent cross-sectional area luminal narrowing in 4 arterial beds: left main, left anterior descending with diagonals, left circumflex with marginal branches, and right coronary with posterior descending artery (12).

Coronary artery sectioning and histology. Hearts were sectioned before or after fixation in 10% neutral buffered formalin. The major epicardial coronary arteries were serially sectioned at 3- to 4-mm intervals intact on the heart where segments of interest were removed and decalcified if necessary, before paraffin processing. The right coronary artery (RCA), left anterior descending coronary artery (LAD), and left circumflex coronary artery (LCx) were divided into proximal and mid-to-distal regions. The proximal regions consisted of the first 3 cm for the RCA, before the first diagonal branch for the LAD, and before the obtuse marginal for the LCx. Middle segments were between the first and second diagonals for the LAD, between left obtuse marginal 1 and left obtuse marginal 2 for the LCx, and beyond 3 cm of the right up to the level of the right marginal branch for the RCA (13). The majority of coronary arteries with thrombi were embedded serially in paraffin and divided into segments maintaining proximal-to-distal orientation. Histologic sections were prepared at 6 μm at 3 different equally spaced levels (1 to 2 mm apart) to best identify rupture sites. Cut sections were mounted on charged slides and stained with hematoxylin and eosin and Movat pentachrome. Unstained slides were held in reserve for immunohistochemistry. The thrombus length was estimated both on gross and histologic sections where it was available in 89 lesions.

Abbreviations and Acronyms

AMI = acute myocardial infarction
H&E = hematoxylin and eosin (staining)
IEL = internal elastic lamina
LAD = left anterior descending coronary artery
LCx = left circumflex coronary artery
MI = myocardial infarction
PCI = percutaneous coronary intervention
RCA = right coronary artery
SCD = sudden coronary death
SMC = smooth muscle cell
STEMI = ST-segment elevation myocardial infarction

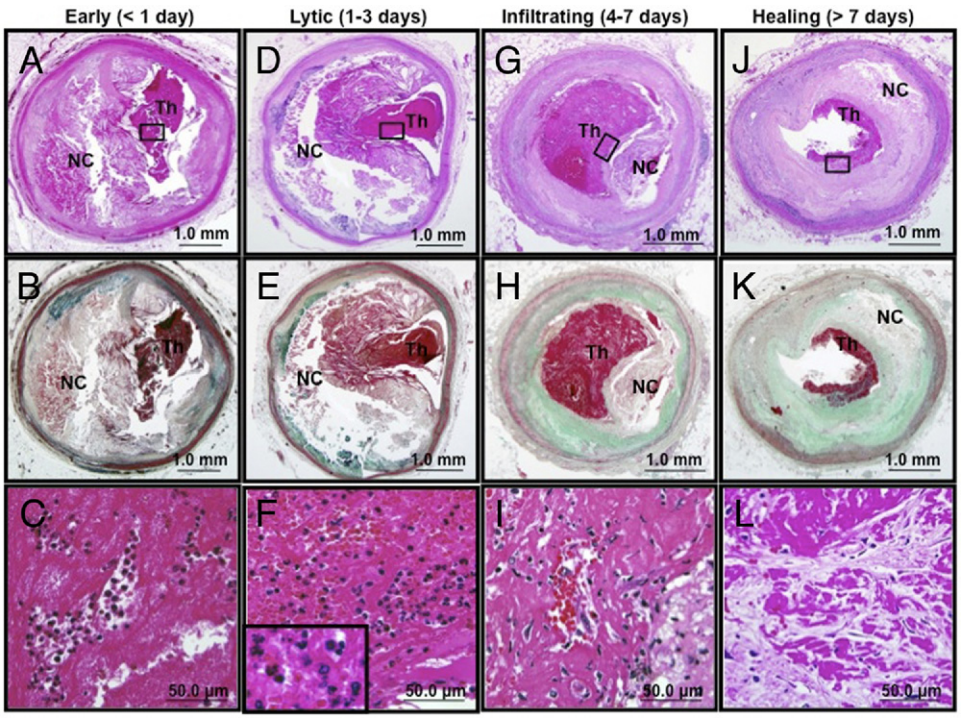

Figure 1 **Morphology of Coronary Thrombi With Early, Late (Lytic), Infiltrating, and Healing Maturation in Plaque Rupture**

(A and B) Corresponding low-power views of a human ruptured coronary lesion showing a relatively large necrotic core with an acute superimposed thrombus (<1 day in age). **(C)** Higher magnification showing platelets and fibrin, and focal collections of neutrophils without inflammatory cells lysis. **(D and E)** Rupture with a superimposed lytic thrombus (1 to 3 days in age). **(F)** Higher-power views of the thrombus with degrading inflammatory cells (see **inset** ×1,000 magnification). **(G and H)** Rupture with an occlusive infiltrative thrombus (4 to 7 days in age). **(I)** Corresponding higher-power view of an infiltrative thrombus demonstrating invading mesenchymal cells resembling smooth muscle cells (SMCs) and endothelial cells. **(J and K)** Rupture with a healing thrombus (>7 days in age). **(L)** Higher-power view of a healing thrombus characterized by organized layers of SMCs and proteoglycan-collagen matrix. **A**, **D**, **G**, **J**: ×20 magnification, hematoxylin and eosin (H&E) staining; **B**, **E**, **J**, **K**: ×20 magnification, Movat Pentachrome staining; and **C**, **F**, **I**, **L**: ×400 magnification; image fields represent the areas within the **black boxes of A**, **D**, **G**, **J**, respectively. H&E staining. NC = necrotic core; Th = thrombus.

Pathologic staging of coronary thrombi. Coronary thrombi were classified into 4 stages of healing and organization modified from previously reported criteria (8,9) as shown by specific examples illustrated in Figures 1 to 3. Stage 1 (early thrombus, 0 to 1 day) is composed of alternating layers of platelets mixed with fibrin and intact neutrophils. Stage 2 (lytic thrombus, 1 to 3 days) represents an acute thrombus with degraded acute inflammatory cells without evidence of cellular organization. Stage 3 (infiltrating thrombus, 4 to 7 days) shows a basal in-growth of smooth muscle cells (SMCs) and/or endothelial cells without accumulated proteoglycan matrix. The final stage, Stage 4 (healing thrombus, >7 days), is composed of layers of SMCs with proteoglycan deposition admixed and endothelial infiltration. Notably, all thrombi had acute fibrin and/or platelets on the most luminal edge, where the stage of organization was based on the most advanced healing characteristics of the thrombus.

Immunohistochemistry. Paraffin sections were incubated with primary antibodies against human smooth muscle α-actin (1:400, Dako, Carpenteria, California) and the endothelial recognition marker CD34 (1:4,000, Monosan, Uden, the Netherlands) for further confirmation of the thrombus age in all cases or the macrophage marker CD68 (1:300, Dako). The labeling of primary antibodies was achieved by using a biotinylated link antibody, and positive staining was visualized by a 3,3′-diaminobenzidine substrate chromogen system with nickel tinting; the sections were counterstained with Gill's hematoxylin.

Evaluation of coronary lesions. Morphometric measurements of coronary sections were performed as described previously (14). Briefly, quantitative planimetry included area analysis of the internal elastic lamina (IEL), lumen, and necrotic core. The percent stenosis was derived from the formula: (1 − lumen area/IEL area) × 100, where the thrombus was excluded. Computer-assisted color image analysis segmentation with background correction was used to quantify immunohistochemical staining of CD68-positve macrophages, which were expressed as percentages of total plaque or thrombus area.

Myocardial examination. Paraffin sections of the left and right ventricle were examined for evidence of ischemia or infarction. Full thickness areas involving the left anterior, lateral free wall, posterior left and right ventricle, and interventricular septum were sampled. Acute infarction was

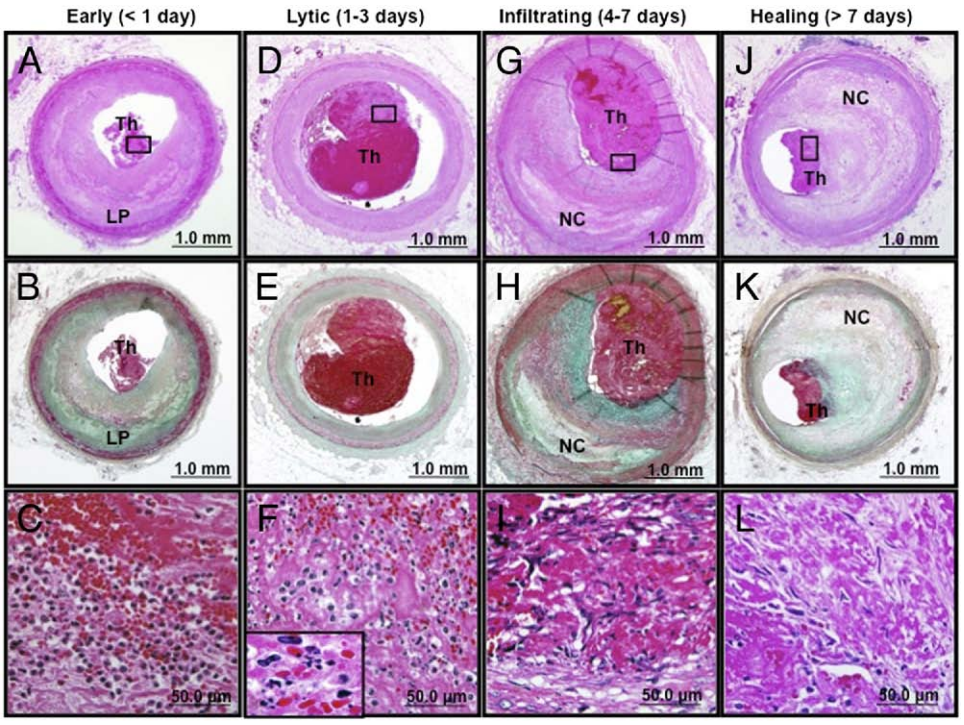

Figure 2 **Morphology of Coronary Thrombi With Early, Late (Lytic), Infiltrating, and Healing Maturation in Plaque Erosion**

(A and B) Low-power view of human coronary plaque erosion with a superimposed nonocclusive early thrombus (<1 day of age); the underlying plaque shows a "lipid pool" (LP). **(C)** Corresponding higher-power view shows platelets, fibrin, and clusters of intact neutrophils. **(D and E)** Human coronary plaque erosions with a superimposed lytic thrombus (1 to 3 days in age). **(F)** Higher-power view of the thrombus with degrading inflammatory cells (see **inset** ×1,000 magnification). **(G and H)** Low-power view of a fibroatheroma with a superimposed infiltrative thrombus consistent with plaque erosion (4 to 7 days in age). **(I)** Corresponding higher-power view of the infiltrative thrombus with invading mesenchymal cells resembling SMCs and endothelial cells. **(J and K)** Macrophage-rich early fibroatheroma with coronary plaque erosion and superimposed healing thrombus (>7 days of age). **(L)** Higher-power view of the thrombus composed of layers of SMCs and proteoglycan-collagen matrix. **A**, **D**, **G**, **J**: ×20 magnification, H&E staining; **B**, **E**, **J**, **K**: ×20 magnification, Movat Pentachrome staining; and **C**, **F**, **I**, **L**: ×400 magnification, H&E staining; imaging fields represent the areas within **black boxes of A**, **D**, **G**, **J**, respectively. Abbreviations as in Figure 1.

defined as myocyte necrosis with or without acute inflammatory cells and/or focal granulation tissue measuring at least 1 cm^2. A healing infarction was identified by granulation tissue accompanied by chronic inflammation with or without acute necrosis, whereas healed MI consisted predominantly of scar tissue with or without lymphocytic infiltrates. Cases that showed no evidence of infarction were referred to as "no infarct" group. The acute and healing infarct were assessed together and assigned to a group entitled "acute ± healing infarction," whereas hearts with acute ± healing infarction in the presence of previous healed infarct are referred to as "acute ± healing infarction + healed infarction."

Statistical analysis. Continuous and categorical variables are expressed as mean ± SD and frequency values and proportions, respectively. The means of normally distributed data were compared with Student *t* test. For analysis of lesion morphology and morphometry, groups were compared with the Wilcoxon/Kruskal-Wallis (rank sums) test, whereas dichotomous variables were compared with the chi-square test. A probability value <0.05 was considered statistically significant. All analyses were performed with JMP software (SAS Institute, Cary, North Carolina).

Results

Patient demographic data and cardiovascular risks. Coronary thrombi were identified in 111 SCD victims in a total of 115 culprit plaques, 1 heart showed 2 rupture sites in different vessels, 2 showed 2 erosion sites in different vessels, and 1 showed a rupture and an erosion in a single vessel. A total of 74 witnessed deaths were identified, where 51 subjects exhibited typical cardiac symptoms indicative of unstable angina or MI (chest pain = 33, malaise = 13, dyspnea = 4, and nausea/vomiting = 1), with 8 cases showing atypical cardiac symptoms (indigestion = 5, paresthesia = 1, and back pain = 2); and 15 cases were without evidence of symptoms. Sudden death secondary to rupture of the fibrous cap occurred in 65 plaques, whereas 50 of the lesions showed plaque erosion (Table 1). Sudden death victims with ruptures were generally older (mean age: ruptures = 52 ± 10 years vs. erosions = 43 ± 9 years, $p < 0.0001$). The proportion of women was significantly

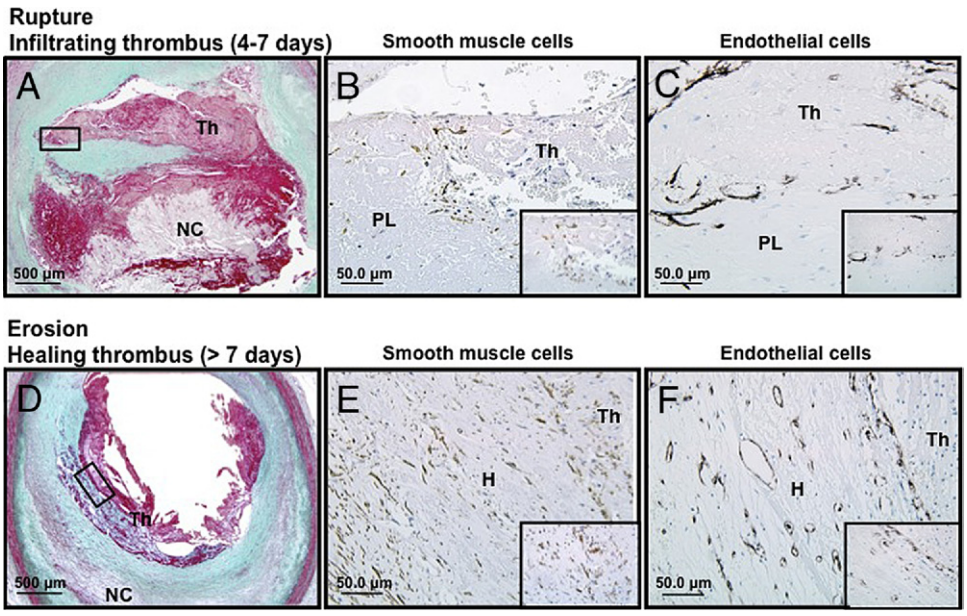

Figure 3 **Immunohistochemical Identification of Invading SMCs (Anti-α-SMC Actin) and Endothelium (Microvessels, Anti-CD34) in Late-Stage Thrombi in Plaque Ruptures (Infiltrating, 4 to 7 Days of Age) and Erosions (Healing, >7 Days of Age)**

(A) Higher-power view of a late-stage infiltrating thrombus in plaque rupture (Movat pentachrome, ×400). **(B)** Area within the **inset in A** shows infiltrating SMCs at the plaque/thrombus interface (α-SMC actin, **brownish-black** reaction product, ×400 magnification). **(C)** Similar region as **B**, showing numerous $CD34^+$ microvessels within the thrombus (anti-CD34, ×400 magnification). **(D)** Healing thrombus (>7 days in age) in plaque erosion (Movat pentachrome, ×400 magnification). Note the presence of proteoglycan matrix within the deeper regions of the plaque/thrombus interface **highlighted by the inset**. **(E)** α-SMC actin immunostaining showing organized layers of SMCs at the base of the thrombus (×400 magnification). **(F)** Similar area as in **E**, showing an abundance of endothelial cells with microvessels formation (anti-CD34, ×400 magnification). PL = plaque substrate; other abbreviations as in Figure 1.

higher in erosions than ruptures (rupture = 7 of 65 [11%] vs. erosion = 13 of 50 [26%], p = 0.03). The incidence of cardiovascular risk for smoking, diabetic status, and hypertension, however, was not statistically significant between lesion types. There was a trend, however, toward a greater incidence of subjects with hypertension in plaque ruptures than erosion, where the lack of statistical significance is possibly dependent on the limited number of cases. Notably, however, the same trends were observed in a previously study from our laboratory, where plaque ruptures were more frequently associated with hypertension (15).

Histomorphometric analysis of culprit plaques. A summary of histomorphometric measurements including IEL, plaque area, stenosis, necrotic core area (%), plaque burden, and overall macrophage content is shown is Table 2. Coronary artery area measurements of IEL were significantly greater in ruptures than erosion (ruptures = 13.66 ± 6.04 vs. erosion = 9.53 ± 5.18, p < 0.0001). Similarly, plaque area, necrotic core size, and plaque burden were also significantly greater in ruptures than erosion together with the percentage of intimal macrophages (ruptures = 3.44 ± 2.77% vs. erosion = 2.53 ± 2.65%, p = 0.03). Although the frequencies of occlusive thrombi were slightly greater in erosions, significant differences with ruptures were not achieved (p = 0.53).

Thrombus healing. The coronary plaque phenotype and thrombus characterized by the age of healing are summarized in Table 3. The majority of early thrombi (<1 day) were observed in plaque rupture (n = 30 [46%]) as compared with erosions (n = 6 [12%], p < 0.0001); similarly, more lytic thrombi (1 to 3 days) were also observed in ruptures. In contrast, the majority of thrombi in erosions were infiltrating (4 to 7 days) or healing (>7 days) (healing erosions = 23 [46%] vs. ruptures = 6 [9%], p < 0.001). The reciprocal-healing trends with greater maturation of

Table 1 **Patient Data, by Culprit Lesion**

Culprit Lesion (n = 115)	Patient Age (yrs)	Male	Diabetic	Hypertensive	Smokers
Rupture (n = 65)	52 ± 10	58 (89)	7 (11)	15 (23)	11 (17)
Erosion (n = 50)	43 ± 9	37 (74)	6 (12)	6 (12)	10 (20)
p value	<0.0001	0.03	0.84	0.13	0.67

Values are n (%), and continuous variables are expressed as mean ± SD.

Table 2 Plaque and Thrombi Characteristics, by Culprit Lesion

Culprit Lesion (n = 115)	IEL (mm^2)	Plaque Area (mm^2)	Stenosis (%)	Necrotic Core Area (%)	Plaque Burden	Occlusive Thrombus (%)	Macrophage Intima (%)
Rupture (n = 65)	13.66 ± 6.04	12.83 ± 15.2	77.1 ± 13.8	38.3 ± 23.4	231 ± 67	30 (46)	3.44 ± 2.77
Erosion (n = 50)	9.53 ± 5.18	6.82 ± 4.3	71.3 ± 14.9	18.3 ± 24.4	190 ± 72	26 (52)	2.53 ± 2.65
p value	<0.0001	<0.0001	0.02	<0.0001	0.008	0.53	0.03

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequency values and proportion.
IEL = internal elastic lamina.

thrombi associated with erosions relative to ruptures were also highly significant ($p < 0.0001$).

Lesion findings and thrombus healing. The effect of luminal stenosis, IEL area, thrombus length, and macrophage content of the thrombus by age of healing was also compared (Table 3). Erosions showed smaller IEL areas and less-severe narrowing than ruptures at all phases of healing. Although overall IEL area decreased with thrombus organization ($p = 0.01$), this trend was no longer significant for individual plaque phenotypes. The mean thrombus length ranged from 8.7 ± 4.9 mm to 10.8 ± 4.5 mm for ruptures and 8.2 ± 4.1 mm to 10.2 ± 7.2 mm for erosions, respectively, with no differences found between lesion types. Finally, macrophage content of the thrombus area was increased for lytic and infiltrating thrombi regardless of lesion type, with the least found in healing thrombi, although none of these differences were significant.

Coronary distribution of culprit plaques and association with healing thrombi. The coronary distribution of ruptures and erosions and thrombus age are summarized in Figure 4. The majority of culprit plaques were found in the proximal coronary arteries, specifically in the LAD, followed by the RCA, with markedly fewer (<10%) in the LCx and left main. Greater frequencies of erosions were found in the LAD (n = 33 [66%]), with fewer lesions in the RCA (n = 11 [22%]), whereas ruptures were more equally distributed in both vessels (LAD = 26 [40%]; RCA = 23 [35%]).

Plaque location seemed to have minimal effect on the maturation of thrombi for both ruptures and erosions. The percentage of ruptures with early thrombi (<1 day) were slightly lower in the LAD (11 of 26 [42%]) compared with the RCA (12 of 23 [52%]), whereas healed thrombi were slightly higher in the LAD (15 of 26 [58%]) compared with the RCA (11 of 23 [48%]); the differences, however, were not statistically significant ($p = 0.49$). In contrast, few early thrombi were noted in erosions in both the LAD and RCA (6 of 44 [14% of arteries]), where the percentage of healed thrombi in the LAD and RCA were similar (LAD = 29 of 33 [88%]; RCA = 9 of 11 [82%], $p = 0.61$).

The influence of noncritical and critical stenosis on thrombus healing. CULPRIT LESIONS WITH NONCRITICAL STENOSIS. A total of 53 lesions (ruptures = 23 [43%], and erosions = 30 [57%]) were identified with <75% cross-sectional luminal narrowing (Table 4). In this data subset,

Table 3 Characteristics of Culprit Lesions and Thrombus, by Thrombus Age

Culprit Lesion/ Thrombus Characteristics (n = 115)	Early Thrombi (<1 Day)	Late Thrombi (≥1 Day)	Late Thrombi			p Value*	p Value†
			Lytic (1–3 Days)	Infiltrating (4–7 Days)	Healing (>7 Days)		
All lesions	36 (31)	79 (69)	24 (30)	26 (33)	29 (37)		
Rupture	30 (46)	35 (54)	17 (26)	12 (19)	6 (9)	<0.0001	<0.0001
Erosion	6 (12)	44 (88)	7 (14)	14 (28)	23 (46)		
Stenosis (%)	77 ± 12	71 ± 15	73 ± 15	73 ± 17	75 ± 15‡	0.40	0.82
Rupture	77 ± 13	76 ± 15	78 ± 11	75 ± 20	79 ± 15	0.84	0.97
Erosion	74 ± 11	71 ± 15	61 ± 18	71 ± 15	75 ± 11	0.65	0.35
IEL area (mm^2)	13.9 ± 6.6	10.9 ± 5.5	12.2 ± 5.4	10.8 ± 5.1	10.0 ± 6.0‡	0.01	0.01
Rupture	14.7 ± 6.7	12.8 ± 5.3	13.7 ± 5.5	11.2 ± 3.6	13.2 ± 7.5	0.36	0.57
Erosion	10.1 ± 4.5	9.5 ± 5.2	8.5 ± 2.7	10.5 ± 6.2	9.1 ± 5.4	0.65	0.55
Thrombus length (mm)§	8.7 ± 4.6	9.5 ± 5.0	8.6 ± 4.6	9.6 ± 4.4	10.2 ± 6.3	0.41	0.83
Rupture	8.7 ± 4.9	9.7 ± 4.4	8.7 ± 4.5	10.8 ± 4.5	9.0 ± 2.4	0.28	0.61
Erosion	9.0 ± 2.4	9.3 ± 4.6	9.5 ± 4.8	8.2 ± 4.1	10.2 ± 7.2	0.98	0.95
Macrophages thrombus (%)‖	3.7 ± 3.7	4.2 ± 5.0	5.5 ± 6.69	4.9 ± 4.8	2.4 ± 2.3	0.78	0.11
Rupture	4.0 ± 3.9	6.81 ± 11.1	4.6 ± 5.3	6.5 ± 6.0	3.0 ± 3.0	0.44	0.70
Erosion	2.3 ± 1.6	3.4 ± 4.5	7.8 ± 10.4	3.8 ± 3.5	2.3 ± 2.2	0.70	0.22

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequency values and proportion. Total numbers of ruptures and erosions as culprit lesion were 65 and 50, respectively. *The p values of comparisons between early thrombi (<1 day) and late thrombi (lytic, infiltrating, or healing [≥1 day]). †The p values of comparisons among early (<1 day), lytic (1–3 days), infiltrating (4–7 days), and healing (>7 days) thrombi. ‡Significant difference between early thrombi (<1 day) and healing thrombi (>7 days). §Thrombus length was available in 89 culprit lesions with thrombi, 58 ruptures and 48 erosions, respectively. ‖Percentage of macrophages in thrombus substrate was available in 106 culprit lesions, 58 ruptures and 48 erosions, respectively.
IEL = internal elastic lamina.

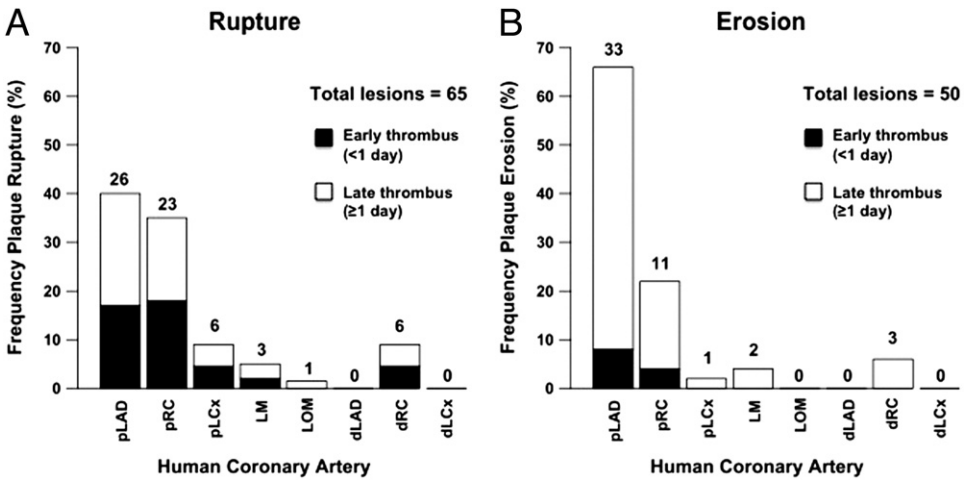

Figure 4 **Bar Graphs Illustrating the Frequency and Distribution of Ruptures and Erosions in the Major Coronary Arteries Relative to the Degree of Thrombus Maturation**

Frequency and distribution of **(A)** ruptures and **(B)** erosions in the major coronary arteries relative to the degree of thrombus maturation. The proximal regions consisted of the first 3 cm for the right coronary artery (pRC), before the first diagonal branch for the left anterior descending artery (pLAD), and the obtuse marginal for the left circumflex artery (pLCx). The mid-segments were between the first and second diagonals for the LAD, between left obtuse marginal (LOM) 1 and LOM 2 for the LCx, and beyond 3 cm of the right coronary artery (RCA) to the right marginal branch. The **solid bars** represent culprit plaques with early stage thrombi (<1 day of age), whereas the unfilled **(open bars)** represent culprit lesions with late-stage thrombi characterized as lytic (1 to 3 days), infiltrating (4 to 7 days), or healing (>7 days of age). Note the majority of ruptures and erosions were localized to the proximal LAD or RCA where there were far greater numbers of plaque erosions with late thrombi. dLAD = distal left anterior descending coronary artery; dLCx = distal left circumflex coronary artery; dRC = distal right coronary artery; LM = left main.

the proportion of men was significantly greater in ruptures (n = 22 [96%]) than erosion (n = 22 [73%], p = 0.02). Furthermore, the mean age at death was also significantly greater in coronary ruptures when compared with erosion (p = 0.02). Early thrombi (<1 day) were present in 12 of 23 (52%) ruptures, whereas only 3 of 30 (10%) thrombi in erosions were considered early. In contrast, 27 of 30 (90%) erosions showed late thrombi (≥1 day) compared with only 11 of 23 (48%) ruptures (p = 0.001). Artery size determined by IEL area was also significantly smaller in erosions than ruptures (p = 0.001), consistent with negative remodeling. Overall plaque burden was greater in ruptures, despite further maturation of coronary thrombi attributed to erosion, although differences were of borderline significance (p = 0.08). Lesions with necrotic cores were apparent in approximately one-half (47%) of erosions where the percentage of necrotic core area was significantly less than ruptures (erosion = 15.5 ± 23.2% vs. ruptures = 33.6 ± 23.5%, p < 0.0001). Furthermore, the percentages of lesional macrophages were significantly greater in ruptures

Table 4 **Characteristics of Thrombi, by Underlying Cross-Sectional Area Luminal Narrowing**

	Critical and Noncritical Stenosis in Erosions and Ruptures With Thrombi					
	<75% Stenosis (n = 53)			>75% Stenosis (n = 62)		
Patient/Plaque Characteristics	Rupture (n = 23)	Erosion (n = 30)	p Value	Rupture (n = 42)	Erosion (n = 20)	p Value
Patient age (yrs)	52 ± 12	43 ± 9	0.02	52 ± 9	44 ± 8	0.003
Male	22 (96)	22 (73)	0.02	36 (86)	15 (75)	0.3
Thrombus age						
Early*	12 (52)	3 (10)	0.001	18 (43)	3 (15)	0.03
Late†	11 (48)	27 (90)		24 (57)	17 (85)	
IEL area (mm^2)	13.6 ± 5.2	9.2 ± 3.9	0.001	13.7 ± 6.5	10.0 ± 6.7	0.005
Plaque burden	217 ± 72	179 ± 69	0.08	237 ± 65	207 ± 73	0.18
Necrotic core	23 (100)	14 (47)	<0.0001	42 (100)	11 (55)	<0.0001
Necrotic core area (mm^2)	2.99 ± 2.74	0.63 ± 1.19	<0.0001	6.30 ± 10.42	1.16 ± 1.46	<0.0001
Necrotic core area (%)	33.6 ± 23.5	15.5 ± 23.3	<0.0001	40.0 ± 23.9	22.4 ± 26.0	0.009
Macrophage area (intima) (%)	4.3 ± 2.7	2.2 ± 2.2	0.003	3.0 ± 2.7	3.1 ± 3.2	0.86

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequency values and proportion. *Early thrombus indicates stage 1 thrombus (<1 day). †Late thrombus indicates stage 2 (lytic, 1 to 3 days), 3 (infiltrating, 4 to 7 days), and 4 (healing, >7 days) thrombus.
IEL = internal elastic lamina.

Table 5 MI Status, by Culprit Lesion or Thrombus Age

Patients (n = 109)	MI by Culprit Lesion				
	No MI	Acute ± Healing MI	Healed MI	Acute ± Healing MI + Healed MI	p Value*
All lesions	36 (33)	38 (35)	23 (21)	12 (11)	
Rupture	17 (27)	22 (36)	15 (24)	8 (13)	0.47
Erosion	19 (40)	16 (34)	8 (17)	4 (9)	

	MI by Thrombus Age		
	Early Thrombus (<1 Day)†	Late Thrombus (≥1 Day)‡	p Value§
No MI (n = 36)			
All lesions	15 (42)	21 (58)	
Rupture	11 (65)	6 (35)	0.008
Erosion	4 (21)	15 (79)	
Healed MI (n = 23)			
All lesions	8 (35)	15 (65)	
Rupture	8 (53)	7 (47)	0.01
Erosion	0 (0)	8 (100)	
Acute ± healing MI (n = 38)			
All lesions	8 (21)	30 (79)	
Rupture	6 (27)	16 (67)	0.27
Erosion	2 (13)	14 (87)	
Acute ± healing MI + healed MI (n = 12)			
All lesions	4 (33)	8 (67)	
Rupture	4 (50)	4 (50)	0.08
Erosion	0 (0)	4 (100)	
(−) Acute or healing MI‖	23 (39)	36 (61)	0.1
(+) Acute or healing MI¶	12 (24)	38 (76)	

Values correspond to frequencies and proportions. Information regarding myocardial infarction (MI) was available in 109 of 111 patients, with plaque rupture as culprit lesion in 64 hearts and plaque erosion in 45 hearts, respectively. *The p value corresponds to the comparison between culprit lesion (rupture or erosion) and presence (and type) of MI. †Early thrombus indicates stage 1 thrombus. ‡Late thrombus indicates stage 2 (lytic), 3 (infiltrating), or 4 (healing thrombus). §The p values correspond to the comparison between culprit lesion (rupture or erosion) and thrombus age (early or late). ‖Patients with no MI or healed MI. ¶Patients with acute ± healing MI + healed MI.

when compared with erosion (ruptures = 4.3 ± 2.7% vs. erosion = 2.2 ± 2.2%, p = 0.003).

CULPRIT LESIONS WITH CRITICAL STENOSIS. A total of 62 culprit lesions with >75% cross-sectional luminal narrowing (erosions = 20, and ruptures = 42) was identified (Table 4). There was a greater percentage of men with critical stenosis presenting with ruptures (36 of 42 [86%]) relative to erosions (15 of 20 [75%]), although differences were not statistically significant (p = 0.3). The relationship of early and healing thrombi was similar to lesions with noncritical stenosis, with the majority of erosions (17 of 20 [85%]) showing late phases of healing compared with ruptures (24 of 42 [57%], p = 0.03). Artery size by IEL area was similarly less in erosions relative to ruptures, as in lesions with noncritical stenosis (p = 0.005), whereas calculated plaque burden was similar between phenotypes. Again, areas of necrosis were present in just over one-half the number of erosions where the percentage of area occupied by the necrotic core was significantly smaller as compared with ruptures (erosion = 22.4 ± 26.0%; rupture = 40.0 ± 23.0%, p = 0.009). Unlike lesions with noncritical stenosis, however, the percentage of lesional macrophages was similar in both phenotypes (p = 0.86).

Relationship of thrombus age and MI. Data regarding the incidence of MI was available in 109 hearts, where summaries of relationship to plaque phenotype are shown in Table 5. Overall, near equal percentages of SCD cases were found without MIs (33%) or with acute ± healing MI (35%), whereas fewer cases were observed for healed (21%) or acute ± healing with previous healed MI (11%). Significant differences in the incidence of MI relative to culprit plaque, however, were not observed (p = 0.47).

Further analysis of early and late thrombi in ruptures and erosions relative to infarction status is summarized in Table 5. No infarcts were observed in 33% of all thrombi cases, irrespective of the stage of thrombus healing. Regardless of infarct status, lesions with late thrombi were found in approximately two-thirds of hearts. Significant differences in the incidence of early and late thrombi between ruptures and erosion were noted for all infarct categories, except culprit plaques with acute ± healing MI. Consideration of infarct status revealed a positive trend toward increased frequencies of acute or evolving MI in coronary plaques with late thrombi (without MI = 36 [61%] vs. with MI = 38 [76%]) relative to early thrombi (without MI = 23 [39%] vs. with MI = 12 [24%]), although differences were not

statistically significant (p = 0.10). Nevertheless, culprit plaques with late thrombi are more likely to represent an increased risk for MI in at least two-thirds of cases.

Discussion

Coronary thrombi at autopsy exhibit diverse phases of healing, depending on the etiology of the underlying culprit plaque—either rupture or erosion. In ruptures, nearly one-half of thrombi showed a lack of healing, characterized by platelets/fibrin with entrapped polymorphonuclear leukocytes without evidence of lysis, whereas the remaining (50%) demonstrated various phases of healing. In contrast, >85% of thrombi in erosions exhibited late stages of healing characterized by inflammatory cell lysis, invasion by SMCs and/or endothelial cells, or organized layers of SMCs and proteoglycans with varying degrees of platelet/fibrin layering.

In the context of previous studies, erosions are generally more prevalent in younger men and women (<40 and <50 years of age, respectively) (16). Moreover, erosions generally exhibit smaller IEL areas than ruptures, together with absent or smaller necrotic cores. In the present study, noncritical stenosis was apparent in at least 40% of lesions where >60% were erosions with most (90%) showing greater maturation of thrombi; in contrast, ruptures equally showed early and late phases of healing thrombi. These data further support the finding that thrombus initiation, in a substantial number of cases, occurs before the onset of symptomatic coronary events. Moreover, it is now clear that thrombus maturation in fatal lesions is highly dependent on the underlying lesion morphology, where healing is considerably more advanced in erosions.

The present data confirm earlier reports, where examination of thrombus material collected during adjunctive thrombectomy during PCI from patients presenting with STEMI showed evidence of healing in at least 50% of cases (8,9). Moreover, older thrombi together with other cardiovascular risks (prior history of coronary bypass surgery, presence of cardiogenic shock, diabetes, female sex, and post-procedural Thrombolysis In Myocardial Infarction flow grades of 0 to 1) were considered independent predictors of long-term mortality (8). Pathologic evidence supporting the important role of erosion in AMI are reported by Arbustini et al. (17), where erosions accounted for 25% of 291 in-hospital deaths from AMI that were autopsied, with the majority occurring in women.

Acute MI in the present study was observed in nearly one-half of subjects dying suddenly (n = 50 of 111), with a slightly greater incidence in ruptures (ruptures = approximately 60% vs. erosions = approximately 40%). On the contrary, late thrombi were more frequent in erosions than ruptures in hearts without evidence of acute or healed MI. Consistent with these observations, autopsy examination of SCD hearts in another study from our laboratory showed intramyocardial microemboli in approximately one-half (54% of cases)—more common to eroded plaques (70% of cases) compared with ruptures (40% of cases), further indicating that thrombi in erosions are evolving for longer periods before presentation of the final event (18). These findings of myocardial emboli were considered significant, because microvascular obstruction was more frequently associated with focal myocardial necrosis, which might contribute to an arrhythmogenic focus in the absence of an overt infarction.

Along similar lines, our analysis fails to demonstrate a relationship of cardiac symptoms to acute and/or healing MI in subjects dying suddenly. A total of 51 subjects in our study exhibited typical cardiac symptoms indicative of MI or unstable angina (chest pain = 33, malaise = 13, dyspnea = 4, and nausea/vomiting = 1). No significant differences, however, were found in the incidence of chest pain with or without presentation of acute and/or healing MI (n = 17 and n = 14, respectively), although cases with typical cardiac symptoms were more prevalent in erosions than ruptures (erosions = 28 of 51 [55%] vs. ruptures = 23 of 51 [45%]).

Critical and noncritical stenosis and thrombus healing. Thrombus healing was examined in ruptured and eroded lesions with noncritical and critical stenosis. Although thrombus maturation is greater in erosions, this relationship was independent of the underlying degree of stenosis. Likewise, the frequency of plaque ruptures with early thrombi was unaffected by stenosis. Although IEL area progressively decreased with greater maturation of the thrombus, this relationship was not as stringent when plaques with significant stenosis were examined. These data are in concordance with an earlier study from our laboratory where marked expansion of the IEL was noted for plaque rupture and erosions demonstrated negative remodeling (13).

The present study highlights disparities in clinical presentation contingent on the etiology of the thrombus. For example, coronary lesions with ruptures from patients dying suddenly are generally more severely narrowed, thus providing a greater likelihood of early presentation with AMI and/or sudden death. On the contrary, the prevalence of erosions with severe luminal narrowing is markedly less, and thus these are less likely to limit flow. Accordingly, thrombi in coronary erosions might have more time to evolve and often show greater healing either at the time of AMI or sudden death, as exemplified in the current study. It is also important to emphasize that greater thrombus organization in erosion occurs in the deeper regions of the thrombus where it mixes with plaque despite a persistence of platelet aggregates near the luminal surface, where this dynamic process might constitute a persistent nidus for distal embolization.

Thrombus propagation, total occlusion, and healing. The thrombus length might represent another important determinant of overall healing, because maturation occurs more rapidly at the ends through formation of granulation tissue, whereas mid-segments remain enriched in platelets/fibrin; therefore, intuitively, shorter thrombi might heal faster. In the current study, thrombus maturation seemed to be unaffected by length, which was nearly identical in

erosions and ruptures (9.3 and 9.2 mm, respectively) despite the diverse plaque morphologies. Similarly, the occlusive or nonocclusive nature of the thrombus displayed little or no effect on healing, because both ruptures and erosions remarkably showed equal frequencies of total occlusions among all stages of thrombus healing.

The influence of lesion substrate and early thrombus composition on healing. There are few published studies of healing thrombi in animal models or human disease. In a seminal study by Geary et al. (19), healing thrombi were studied after angioplasty fracture of iliac artery atheroma in nonhuman primates. A thin mural thrombus was present at sites of plaque fracture by 2 to 7 days, which was invaded by leukocytes (days 2 to 4) and α-actin–positive SMCs at 4 to 7 days. The thrombus was replaced by SMCs expressing hyaluronan and the associated versican protein (day 14).

It is evident that differences in thrombus healing are highly contingent on the underlying plaque substrate and, together with inflammation, orchestrate healing responses, which ultimately require SMC infiltration for maturation to occur. A potential mechanism of delayed thrombus healing has been described for mural thrombi of aneurysms whereby matrix-degrading enzymes—namely, leukocyte elastase and MMP-8 and -9—trapped within fibrin might degrade the scaffolding required for cellular infiltration of the thrombus (20). This, together with the disappearance of mural SMCs capable of providing further thrombus stabilization, leads to incomplete healing. An analogous situation is plaque rupture where highly inflamed plaques relatively devoid of SMCs would expect to incur a delayed cellular reaction, thereby impairing maturation of the thrombus, albeit not to the extent with aneurysms.

Study limitations. The principal limitation of the current study is the inherent bias of examining subjects that come to autopsy. Unlike the hospital-based autopsy experience, the majority of Medical Examiner's autopsies are performed on younger individuals <50 years of age. Moreover, pertinent clinical information regarding cardiovascular risk, family history, medication (e.g., aspirin), or whether a physician was involved is generally gathered from investigator's statements and histories sought from family members rather than patients themselves and are thus less reliable. Artery size (remodeling index) was not normalized to reference vessels, unlike in a previous study (13), considering not all hearts were perfusion-fixed at physiologic pressure. Furthermore, additional techniques for recognition of early cell death and MI were not employed. Nonetheless, we contend that the underlying processes associated with thrombosis and healing attributed to rupture or erosion observed at autopsy also occur in living patients, as exemplified in the study by Kramer et al. (8), whereby 50% of aspirated thrombi in STEMI patients showed thrombus organization.

Clinical Perspective

There is considerable evidence to indicate that the etiology and pathogenesis of initiating event(s) causing rupture or erosion are distinct regarding inflammation, remodeling, and growth rates of the underlying plaque. The current data widens this view, where coronary thrombi in fatal erosions are in later stages of maturation as compared with ruptures. Considering that STEMI patients with healing thrombi of >1 day have poorer prognosis, the present findings that erosions are the main cause of healing thrombi—which occur predominantly in women and younger men—together with the increased risk for distal intramyocardial embolization would further indicate that women and younger men might require different strategies of treatment.

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