Characteristics of Vulnerable Plaque: Structural Observations and Natural History Insights from the Core Pathology Laboratory

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Conflict of Interest Statement

Within the past 12 months, I or my spouse/partner have had a financial interest/arrangement or affiliation with the organization(s) listed below.

Physician Name: Renu Virmani, M.D.

Company/Relationship: Research Grants

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Employment 25%: Cardiovascular Research Foundation

Do not own any stock in any company.
Natural History of Atherosclerosis

- Systemic factors - hyperlipidemia, diabetes mellitus, smoking, hypertension, age and sex, hsCRP, Lp-PLA$_2$, etc.

- Local factors: at branch points, e.g., carotid bifurcation, abdominal aorta just above bifurcation coronary branch point, and arch vessels at take off, are the sites of atherosclerosis manifestation

- Thrombosis occurs in the coronary arteries at focal points and is most often seen in the proximal segments of the three main coronary arteries (systemic coagulation factors play a role), and occur at sites where there are underlying plaque characteristic that result in thrombosis
Branch points are the sites of atherosclerosis and occur in areas of low shear.

A Carotid Artery

B

Left Coronary artery

C Low shear

Blood flow

High shear

LM

PLAD

RI

PLCx
Lesions with Thrombi

- Plaque Rupture
- Plaque Erosion
- Calcified Nodule
Causes of Coronary Thrombosis

Rupture

Erosion

Calcified nodule

Gross and Light Microscopic Features of Plaque Rupture

60% of Thrombi in Sudden Coronary Death occur from Plaque Rupture
Plaque Erosion: 30-35% of thrombi in SCD

Plaque erosion in a 33 year-old female complaining of chest pain for two-weeks and discharged from the emergency room with a diagnoses of anxiety.
Clinical and Morphologic Difference in Plaques Associated with Luminal Thrombi

Plaque rupture

- Necrotic core
- Th
- Lumen

Plaque erosion

- Th
- Lumen

Calcified nodule

- Th

60% thrombi in SCD
M>F, Older, Ca++
Eccentric =
concentric
Greater % stenosis
Macs, T cells, HLADr

30-35% thrombi in SCD
M=F, younger
Usually eccentric
Lesser % stenosis
SMC rich, proteoglycans

2-7% thrombi in SCD, calcified plates
M>F, older, mid RCA
Usually eccentric
Stenosis variable
Nodules of bone
Acute Coronary Syndrome

- Acute Myocardial Infarction
  - >90% have coronary thrombi, usually occlusive
  - 65-75% from plaque rupture

- Unstable Angina Pectoris
  - Distribution weather rupture or erosion, unknown

- Sudden Coronary Death
  - 60% have thrombi- 60% rupture, 35% erosion, 2-5% calcified nodule)
  - 40% have stable plaques with >75% x-sectional narrowing (no thrombus)
  - 40% have HMI and 15% AMI (Virmani R, et al. ATVB 2000)
Development of Human Coronary Atherosclerosis

Intimal thickening

Intimal xanthoma

Pathologic intimal thickening

Fibrous cap atheroma

Thin-cap Fibroatheroma

Smooth muscle cells

Calcified plaque

Macrophage foam cells

Hemorrhage

Extracellular lipid

Thrombus

Cholesterol clefts

Healed thrombus

Necrotic core

Collagen

FC = fibrous cap
LP = lipid pool
NC = necrotic core
Thin-Cap Atheroma (Vulnerable Plaque) Components

- Necrotic core
- Thin fibrous cap (< 65 µm)
- Cap infiltrated by macrophages and lymphocytes
- Cap composition – type 1 collagen and few smooth muscle cells
Thin cap Fibroatheroma (Vulnerable Plaque)

A. Movat
B. nc
C. CD68
D. vWF
E. CD68
F. nc
### Morphologic Characteristics of Plaque Rupture and Thin-cap Fibroatheromas

<table>
<thead>
<tr>
<th>Plaque type</th>
<th>Necrotic Core (%)</th>
<th>Fibrous cap Thickness (µm)</th>
<th>MΦs (%)</th>
<th>SMCs (%)</th>
<th>T-lymph</th>
<th>Calcification Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rupture</td>
<td>34±17</td>
<td>23±19</td>
<td>26±20</td>
<td>0.002±0.004</td>
<td>4.9±4.3</td>
<td>1.53±1.03</td>
</tr>
<tr>
<td>Thin-cap Fibroatheroma</td>
<td>23±17</td>
<td>&lt;65µm</td>
<td>14±10</td>
<td>6.6±10.4</td>
<td>6.6±10.4</td>
<td>0.97±1.1</td>
</tr>
<tr>
<td>P value</td>
<td>0.01</td>
<td></td>
<td>0.005</td>
<td>ns</td>
<td>ns</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Mean values are represented ± standard deviation. Abbreviations: MΦs= macrophages, SMCs= smooth muscle cells, T-lymph= T-lymphocytes

Relationship of Fibrous Cap Thickness to Macrophage Infiltration

Cell Mean for % Kp-1

- Less than 65 µm
- 66 - 200 µm
- 201 - 300 µm
- More than 300 µm

P = 0.03
P = 0.06
Remodeling in Varying Coronary Lesion Morphologies

IEL-Expected IEL (mm$^2$)

A.

IEL-Expected IEL (plaque area)

B.
Plaque rupture with mild non-occlusive thrombus: mechanism by which plaques progress.
Do TCFAs lead to plaque progression?

Movat pentachrome

Sirius red

Sirius red with polarized light

Mean % stenosis increases with number of prior rupture sites

Thin-cap Fibroatheroma
Recent Intraplaque Hemorrhage is seen at Multiple sites in Patients Dying SCD

Virmani R, et al. ATVB 2005
Consequence of Extravasated Erythrocytes Outside the Vasculature

- Free cholesterol content of erythrocyte membrane exceeds that of all other cells in the body, with lipid constituting 40% of the weight.
- Yeagle in 1985 showed that extravasated erythrocytes contain free cholesterol and Arbustini et al. in 2002 showed macrophage infiltration in intimal plaques in pulmonary trunk of patients with pulmonary hypertension at sites containing erythrocyte membranes.

We examined tissues from nonvascular location to determine the effect of hemorrhage.

- Pericardial hemorrhage
- Intratumor hemorrhage (atrial hemangiomas, papillary carcinoma of kidney etc)

Phase Separation of Erythrocyte-Derived Cholesterol in Coronary and Non-Coronary Diseases

Thin Fibrous Cap Atheroma

Hemorrhagic Pericarditis

Hemorrhage Periphery

Hemorrhage Core

A

B

C

D

E

F

G

H

RBCs

MΦ

GpA
Plaque Types Studied

A. Pathologic Intima Thickening

B. Fibroatheroma ‘Early’ Core

C. Fibroatheroma ‘Late’ Core

D. Thin Cap Fibroatheroma
Morphometric Analysis of Hemorrhagic Events in Human Coronary Plaques from Sudden Death Victims

<table>
<thead>
<tr>
<th>Plaque Type</th>
<th>GpA Score (mm²)</th>
<th>Iron (mm²)</th>
<th>Necrotic Core (mm²)</th>
<th>MΦ (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIT no core (n=129)</td>
<td>0.09±0.04</td>
<td>0.07±0.05</td>
<td>0.0</td>
<td>0.002±0.001</td>
</tr>
<tr>
<td>FA early core (n=79)</td>
<td>0.23±0.07</td>
<td>0.17±0.08</td>
<td>0.06±0.02</td>
<td>0.018±0.004</td>
</tr>
<tr>
<td>FA late core (n=105)</td>
<td>*0.94±0.11</td>
<td>*0.41±0.09</td>
<td>*0.84±0.08</td>
<td>*0.059±0.007</td>
</tr>
<tr>
<td>TCFA (n=52)</td>
<td>*1.60±0.20</td>
<td>*1.24±0.24</td>
<td>*1.95±0.30</td>
<td>*0.142±0.016</td>
</tr>
</tbody>
</table>

Values are reported as the means±SE, *p<0.001 versus early core. The number in parenthesis represent the number of lesions examined; the total number= 365. MΦ = macrophages

Plaque Vasa Vasorum

- Plaque capillaries are observed in atherosclerotic plaques with plaque thickness > 0.5 mm, suggesting that wall ischemia may be a determinant of neovascularization.
- Heistead and Armstrong reported a 5 fold increase in intimal/medial blood flow from proliferating micro vessels in monkeys fed a high cholesterol diet for 17 months. (Arteriosclerosis 1986)
- Plaque Vv may be a potential source of inflammation within the plaque [expression of VCAM-1, ICAM-1 and E-selectin has been shown in plaque Vv (O’Brian, et al. AJP 1994).
- Inflammation and matrix composition of atherosclerotic plaques may also influence angiogenesis.

Low vasa vasorum density in internal thoracic artery may be responsible for the low incidence of atherosclerosis

Vasa vasorum at various stages of plaque development

Adaptive Intimal thickening
Vv in adventitia

Fibroatherma Abnormal Vv

Fibroatherma with leaky Vv
Intraplaque Vasa Vasorum in Coronary Plaques with a Necrotic Core

150 μm thick sections stained with Ulex

**Vasa Vasorum by Plaque Type**

### Thin-Cap Fibroatheroma

- **A**: NC
- **B**: I
- **C**: I

### Plaque Rupture

- **D**: NC
- **E**: Th
- **F**: I

### Stable Plaque

- **G**: NC
- **H**: I
- **I**: vWF
- **J**: vWF

**K** Mean No. of Vasa Vasorum by Plaque Type

<table>
<thead>
<tr>
<th>TCFA</th>
<th>Rupture</th>
<th>Stable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P = 0.07</td>
<td>P = 0.01</td>
</tr>
</tbody>
</table>

Source: JACC In Press
Adventitial Vasa Vasorum In Varying Plaque Morphologies (Ulex Europaeus)

Intimal-Medial Border Vasa Vasorum in Varying Plaque Morphologies (CD31/CD34)
Intimal-Medial Border T Cell Densities and % Macrophage Infiltration at Vaso Vasorum Hotspots in Varying Plaque Morphologies

T Cell Densities (UCHL-1)

PIT

Late FA

Rupture

Macrophage Infiltration (CD68)
**Pericore**

- PIT: 0.02
- Early Core: *P=0.02*
- Late Core: *
- TCFA: *
- Rupture: *

**I-M Border**

- Normal: 0.0007
- PIT: *
- Early Core: *
- Late Core: *
- TCFA: *
- Rupture: *

**Neointima**

- Normal: *
- PIT: *
- Early Core: *
- Late Core: *
- TCFA: *
- Rupture: *

**Adventitia**

- Normal: P=0.20
- PIT: *
- Early Core: *
- Late Core: *
- TCFA: *
- Rupture: *

* = significant VS. normal
Conclusions

- Plaques occur focally at branch points in the presence of systemic risk factors.
- The morphologic characteristics most predictive for the presence of unstable vs. stable plaque is necrotic core size, plaque area and to a lesser extent macrophage infiltration.
- Intra plaque hemorrhages from leaky vv are responsible for enlargement of necrotic core, macrophage infiltration and progressive luminal narrowing.
- Non invasive detection of vulnerable plaques is the only mechanism through which morbidity and mortality for CAD can be reduced or eliminated.
Progression of stenosis causing Stable Angina Pectoris

Asymptomatic progression of stenosis

Acute Coronary Syndrome
- Unstable Angina
- Myocardial Infarction
- Sudden Coronary Death

Thrombosed Plaque

Asymptomatic progression of stenosis

Terminology for high risk and Vulnerable Coronary Artery Plaque, Aug 29, 2003, Santorini, Greece