HOW TO FIND THE VULNERABLE PLAQUE?

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Morphology and histology of silent and symptom-causing atherosclerotic carotid plaques – Rationale and design of the Helsinki Carotid Endarterectomy Study 2 (the HeCES2)

CONFLICTS OF INTEREST
- NONE
HECES 1 1997-2000, SYMPT >70% N=92

- histology
- gene expression
- iron/heme metabolism
- intraplaque hemorrhage
- gene profile symptom giving vs silent
- radiological findings, other emboli...
ALL CASES RE-ANALYSED BY 2 STROKE NEUROLOGISTS

Plaque collection 2012-15

HECES2 STUDY PATIENTS AND PLAQUES – ORIGIN OF SYMPTOMS

STUDY PATIENTS N=500

ASYMPTOMATIC PATIENTS N=67

PATIENTS WITH UNSPECIFIED SYMPTOM N=20

13%

PATIENTS WITH CEREBROVASCULAR SYMPTOM N=413

SYMPTOM-CAUSING PLAQUES N=324

ETIOLOGY OF SYMPTOM NOT DETERMINED N=54

SILENT CAROTID PLAQUES N=35

INFARCTION N=161

HEMISPHERIC N=137

RETINAL N=24

TRANSIENT ISCHEMIA N=163

HEMISPHERIC N=79

AMAUROSIS FUGAX N=84
IMAGING

- CTA
- (MRA)
- No contrast US nor gray-scale

E = Bulky calcification
F = Soft plaque
G = Ulcer pouch
H = Calcified nodules
**EXPERT PANEL BLINDED TO CLINICAL DATA GRADED THE PICTURES**

We practiced with 100 px, which were reanalysed later

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>EVALUATION CRITERIA</th>
<th>REPRESENTATIVE IMAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Hot spot&quot;</td>
<td>A part of the specimen deemed to be the most vulnerable, thrombogenic or most liable to having induced the recent symptom.</td>
<td></td>
</tr>
<tr>
<td>Luminal thrombus</td>
<td>0 = no thrombus; 1 = thin clot layer through which the shape of specimen surface and size of ulceration can be monitored; can be small separate clot areas; 2 = large thrombus concealing the shape of specimen surface, potential underlying ulceration cannot be detected.</td>
<td></td>
</tr>
<tr>
<td>Ulceration</td>
<td>Injury of the specimen surface where continuous surface is absent. Artefactual mechanical injury was excluded. Size of ulceration was recorded in millimeters (mm). In case of multiple ulcerations (A), the longest was measured. In addition, ulcerative pouches (B) with ≥ 2 mm depth were recorded.</td>
<td></td>
</tr>
<tr>
<td>Smoothness</td>
<td>Specimen was considered smooth or mostly smooth if the surface was predominantly regular and intact. Small ulcerations may still exist.</td>
<td></td>
</tr>
<tr>
<td>Intraplaque hemorrhage</td>
<td>Reddish, black, greenish or brownish substance under the surface of specimen, consistent with evolution of blood pigment degradation under the endothelium.</td>
<td></td>
</tr>
<tr>
<td>Atheromatous gruel</td>
<td>Soft yellowish mass under the surface of specimen. Colour can be different due to haemorrhage. The consistence of the atheromatous mass was recorded as either firm or fluid. Recorded only as dominant characteristic.</td>
<td></td>
</tr>
</tbody>
</table>
...AND NATURE OF CALCIFICATION

<table>
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<tr>
<th>Intramural calcification</th>
<th>Evaluated when divided into 4-6 slices. 0 = soft specimen, no calcification; 1 = can be dissected with lancet, or smaller calcified areas; 2 = strongly calcified, needs to be cut with scissors.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral-type calcification</td>
<td>Hard coral-type projection into the lumen that breaks the surface of specimen.</td>
</tr>
</tbody>
</table>


Warfarin associates with intramural calcification as do female gender, increased age and ex smoking.
Macroscopic characteristics of plaques (%)

- Hot spot
- Luminal thrombus
- Ulceration
- Ulceration pouch
- Smoothness
- Intraplaque hemorrhage
- Atheromatous gruel
- Intramural calcification
- Coral-type calcification

0 = not present, 1 = present OR
0 = not present, 1 = moderately present, 2 = abundantly present
The "ROI" method

EXAMPLE: GLUTAMINE SYNTHETASE EXPRESSION

1. Place the ROI grid on the photo of each section.
2. Calculate the distance of the center of each ROI from either endothelium or intima-media border.
3. Each ROI is classified according to the histology it mainly represents and graded semiquantitatively for GS immunoreactivity, cholesterol clefts, red blood cell shadows, and calcification.

4. ROI classification:
   - a: "thickened intima" ROI
   - b: "core" ROI with cholesterol clefts
   - c: "cap" ROI with GS+ cells
   - d: "edge of the core" ROI with GS+ cells
   - e: an "inflammation" ROI with GS+ cells
   - f: an "edge of core" with calcification

5. The same regions of adjacent sections stained for CD68, Alpha-Actin, Prussian Blue, Heme Oxigenase 1, and CD163 (thickness 4μm) are graded semiquantitatively as well, if the morphology is sufficiently analogous.

6. Liver and skin were used as positive and negative control samples. Liver shows the typical staining pattern, where GS is expressed in hepatocytes around the terminal venules.

BY PIA ISOVIITA
This is the first study to show GS immunoreactivity in advanced atherosclerosis.

In the schematic illustration, black spots are macrophages and red spots are GS-expressing macrophages. The plaque drawing is luminal side up.

- A thrombus might also develop on an area with erosion of the endothelium. GS expression is increased in subendothelial areas with overlying luminal thrombotic mass (p = 0.035).
- Not all CD68-expressing cells with macrophage foam cell morphology express GS. Here GS is seen only in a subgroup of foam cells under the fibrous cap of a large, complex plaque with an intraplaque bleeding.
- Increased local GS expression in the ROI is associated with a decreased fibrous cap thickness in the same area. In symptom-causing plaques the amount of cholesterol clefts in ROI associates with increased GS expression in fibrous cap ROIs (p=0.004).

The fibrous cap is thinner in ROIs where the amount of GS is increased.

**Subgroups of macrophages express GS. The amount of GS varies greatly.**

- GS is expressed in a subgroup of macrophages surrounding area where cholesterol crystals seem to form from erythrocytes.
- GS expression is increased in ROIs with red blood cell shadows or ferric iron. The picture below shows an CD68/GS-positive macrophage, which might represent an active erythrophagocyte at work.

**Increased GS expression in ROIs with thrombotic tissue on luminal side.**

**Cholesterol clefts**

**GS associates with intraplaque blood components.**

**Crystallizing cholesterol and GS expression in ROIs.**

**GS is not expressed in thickened intima or in the media.**

**The deeper, anoxic areas of the necrotic core are totally devoid of GS.**

In this sample, the only GS expression is seen in the corner of a large atheroma. In this region also sign of intraplaque hemorrhage are found: ferric iron and red blood cell shadows. In some lesions with intraplaque hemorrhage, the amount of GS is significantly higher. This lesion gives the impression, that “something is going on” in the corner.
GS IS PREVIOUSLY UNKNOWN TO Atherosclerosis – Loop to Clinic

- A longer delay between symptom to surgery was associated with lower plaque GLUL mRNA ($Rs=-0.423$, $p=0.050$), indicating a role in acute plaque events, potentially through modulating macrophage activity.
CONCLUSION

• bench to bedside co-operation is stimulating