Mechanisms and Pathology of Calcification in Aortic Stenosis

British Heart Valve Society Annual Meeting
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Royal Brompton Campus, London

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BHF John Wheatley Chair of Cardiology
University of Edinburgh, UK
Anatomy of Aortic Stenosis

- Endothelium - Aorta
- Fibrosa - Fibroblasts
- Collagen rich
- Spongiosa - Mucopolysaccharides
- Mesenchymal Cells
- Ventricularis - Elastin Rich
- Endothelium - Ventricle

O’Brien et al. ATVB 1996;16:523-532
Aortic Valve Disease

Calcific Aortic Stenosis

Rigidity of the cusps rather than cusp fusion
Pathology of Aortic Stenosis

- Lipid Deposition
- Apolipoprotein B & (a)
- Macrophages
- Calcification
Pathology of Aortic Stenosis
Patients with Hypercholesterolaemia

Fibrosis

Cholesterol Deposition

A Randomized Trial of Intensive Lipid-Lowering Therapy in Calcific Aortic Stenosis

S. Joanna Cowell, B.M., David E. Newby, M.D., Robin J. Prescott, Ph.D., Peter Bloomfield, M.D., John Reid, M.B., Ch.B., David B. Northridge, M.D., and Nicholas A. Boon, M.D., for the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE) Investigators
SALTIRE Trial
Atorvastatin & LDL Cholesterol

**Graph 1:**
- **X-axis:** Months
- **Y-axis:** Serum LDL Cholesterol Concentration (mg/dl)
- **Legend:**
  - Placebo
  - Atorvastatin
- **Data Points:**
  - Placebo: 78, 70, 54, 31
  - Atorvastatin: 77, 65, 58, 35
- **Statistical Significance:** P<0.001

**Graph 2:**
- **X-axis:** Months
- **Y-axis:** Aortic-Jet Velocity (m/sec)
- **Legend:**
  - Placebo
  - Atorvastatin
- **Data Points:**
  - Placebo: 77, 69, 55, 30
  - Atorvastatin: 77, 65, 60, 34
The SEAS Trial

A  Serum LDL Cholesterol

- Placebo
- Simvastatin plus ezetimibe

\[ P < 0.001 \]

B  Peak Aortic-Jet Velocity

- Simvastatin plus ezetimibe
- Placebo

\[ P = 0.83 \]

The ASTRONOMER Trial
Rosuvastatin in 269 Patients with Aortic Stenosis

Newby et al. Heart 2006;92:729-734
Role of Lipid in Aortic Stenosis

Histology of Aortic Valve

<table>
<thead>
<tr>
<th></th>
<th>Calcific aortic valve disease</th>
<th>Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased prevalence with age</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Association with clinical factors</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Pathologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lipid infiltration</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Calcification</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>Genetic factors</td>
<td>?+</td>
<td>+++</td>
</tr>
<tr>
<td>Mechanism of clinical events</td>
<td></td>
<td>Leaflet stiffness</td>
</tr>
</tbody>
</table>


Where is the Fat?
Carotid Endarterectomy

Histology

Movat’s Pentachrome

Alkaline Phosphatase

Cleaved Caspase-3

Joshi et al. Lancet 2014;383:705-713
Calcification & Inflammation
Histology of Aortic Valve

# Calcific Aortic Stenosis

## Predictors of Incident Disease

**The Cardiovascular Health Study**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Valve (n = 3,917)</th>
<th>Aortic Sclerosis (n = 1,610)</th>
<th>Aortic Stenosis (n = 94)</th>
<th>p Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD, yrs</td>
<td>72 ± 5</td>
<td>74 ± 6</td>
<td>75 ± 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>1,547 (40)</td>
<td>792 (49)</td>
<td>47 (50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>African-American ethnicity, n (%)</td>
<td>371 (10)</td>
<td>361 (22)</td>
<td>9 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165 (9)</td>
<td>165 (10)</td>
<td>165 (10)</td>
<td>0.596</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>452 (12)</td>
<td>197 (12)</td>
<td>14 (15)</td>
<td>0.277</td>
</tr>
<tr>
<td>Hypertension history, n (%)*</td>
<td>2,179 (56)</td>
<td>1,035 (64)</td>
<td>63 (67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes history, n (%)†</td>
<td>279 (8)</td>
<td>130 (9)</td>
<td>9 (10)</td>
<td>0.113</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>678 (17)</td>
<td>380 (24)</td>
<td>22 (23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal insufficiency, n (%)‡</td>
<td>368 (9)</td>
<td>218 (14)</td>
<td>18 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>129 (36)</td>
<td>131 (36)</td>
<td>128 (37)</td>
<td>0.253</td>
</tr>
<tr>
<td>Lipoprotein (a), mg/dl§</td>
<td>40 (15, 72)</td>
<td>51 (21, 81)</td>
<td>39 (14, 74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>83 (2)</td>
<td>36 (2)</td>
<td>5 (5)</td>
<td>0.249</td>
</tr>
<tr>
<td>CRP, mg/day</td>
<td></td>
<td></td>
<td>1.90 (0.95, 3.36)</td>
<td>1.90 (1.01, 3.42)</td>
</tr>
</tbody>
</table>
# Calcific Aortic Stenosis

## Predictors of Clinical Outcome

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P )</td>
<td>Risk ratio</td>
</tr>
<tr>
<td>Age ( \geq 50 ) years</td>
<td>0.0001</td>
<td>2.6 (1.7–4.8)</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.28</td>
<td>0.9 (0.7–1.1)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>0.0002</td>
<td>1.7 (1.3–2.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.18</td>
<td>1.2 (0.9–1.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.52</td>
<td>1.1 (0.8–1.5)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0.75</td>
<td>1.0 (0.8–1.4)</td>
</tr>
<tr>
<td>Aortic valve peak velocity ( \geq 3 ) m/s</td>
<td>0.0079</td>
<td>1.5 (1.1–2.0)</td>
</tr>
<tr>
<td>Aortic valve calcification (score 3 or 4)</td>
<td>0.0001</td>
<td>2.1 (1.5–3.0)</td>
</tr>
</tbody>
</table>

## No. of Patients with Variable (%)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P ) value</td>
<td>Risk ratio (95% CI)</td>
</tr>
<tr>
<td>Age ( \geq 50 ) yr</td>
<td>&lt;0.001</td>
<td>2.7 (1.5–5.2)</td>
</tr>
<tr>
<td>Female sex</td>
<td>NS</td>
<td>0.9 (0.7–1.2)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>&lt;0.05</td>
<td>1.7 (1.0–2.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>NS</td>
<td>0.9 (0.5–1.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>&lt;0.05</td>
<td>1.9 (1.0–3.3)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>NS</td>
<td>1.2 (0.7–2.0)</td>
</tr>
<tr>
<td>Aortic-jet velocity ( \geq 4.5 ) m/sec</td>
<td>NS</td>
<td>1.3 (0.8–2.1)</td>
</tr>
<tr>
<td>Aortic-valve calcification score 3 or 4†</td>
<td>&lt;0.001</td>
<td>5.2 (2.4–13.5)</td>
</tr>
</tbody>
</table>
Patients with Aortic Stenosis

Calcification and Stenosis

Peak Post-Aortic Valve Velocity (m/s)

Aortic Valve Calcium Score (HU)

Patients with Aortic Stenosis
Calcification and Stenosis

CT with highlighted calcium
Aortic valve calcification

Spline Curve Analysis
Different thresholds for excess mortality in women and men

Overall survival with medical treatment, all patients (N = 794)

<table>
<thead>
<tr>
<th></th>
<th>Severe Absolute AVC*</th>
<th>Severe AVCdensity†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (95% CI)</strong></td>
<td><strong>HR (95% CI)</strong></td>
<td><strong>p Value</strong></td>
</tr>
<tr>
<td>Univariate</td>
<td>5.63 (3.82-8.46)</td>
<td>7.06 (4.66-11.02)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariate‡</td>
<td>1.75 (1.04-2.92)</td>
<td>2.44 (1.37-4.37)</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Overall survival with medical/surgical treatment

<table>
<thead>
<tr>
<th></th>
<th>Severe Absolute AVC*</th>
<th>Severe AVCdensity†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (95% CI)</strong></td>
<td><strong>HR (95% CI)</strong></td>
<td><strong>p Value</strong></td>
</tr>
<tr>
<td>All patients (N = 794)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td>3.04 (2.20-4.19)</td>
<td>3.76 (2.58-5.22)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariate§</td>
<td>1.71 (1.12-2.62)</td>
<td>2.22 (1.40-3.52)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Subgroup with coronary calcium scoring (n = 635), multivariate§§</td>
<td>1.58 (1.02-2.47)</td>
<td>1.89 (1.14-3.09)</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

HR: 3.76(2.58-5.22); p<0.0001
Adjusted HR: 2.22 (1.40-3.52); p=0.001*

Follow-up, (years)

Clavel et al. J Am Coll Cardiol 2014;64:1202-1213
$^{18}$F-Fluorodeoxyglucose

‘The ring of fire’
Calcification versus Inflammation

$^{18}$F-Fluoride and $^{18}$F-FDG

**18F-FDG Versus 18F-NaF**

**Aorta versus Aortic Valve**

Dweck et al. Eur Heart J 2013;34:1567-1574
European Synchotron Radiation Facility
European Synchotron Radiation Facility
Spectral Patterns of Tissue Calcification

Atherosclerosis

Aortic Stenosis
What is the Mechanism?
Aortic Stenosis and Osteoporosis
Animal Models

Aortic Stenosis & Osteoprotegerin

**OPG Deficient Mice**

**OPG (+/+)**

**OPG (+/-)**

**OPG (-/-)**

H&E

Azan (Elastin)

von Kossa (Calcium)

Aortic Stenosis & Osteoprotegerin
Animal Models and Clinical Data

Weiss et al. PLOS ONE 2013;8:e65201
Kaden et al. J Mol Cell Cardiol 2004;36:57-66
Aortic Calcification & Denosumab
Murine Model of Osteoporosis and Aortic Calcification

Figure 2. Analysis of aortic calcium and phosphate content. Wild-type (WT) and human RANKL knock-in (huRANKL-KI) mice were treated with prednisolone (Pred), denosumab (Dmab), or both. A: Aortic calcium content was measured photometrically at 570 nm (n = 6). B: Aortic phosphate content was measured using a photometric assay at 340 nm (n = 6). Mineral content of aortas was evaluated histologically (C) and semiquantitatively (D) using alizarin red S (n = 6). Bars represent mean ± SEM. Insets show results of two-way analysis of variance.

Aortic Calcification and Warfarin
Animal Models and Clinical Data

2 Weeks

3 Weeks

4 Weeks

5 Weeks

TABLE III
FACTORS INDEPENDENTLY ASSOCIATED WITH ODDS OF HIGHER CATEGORY OF AV CALCIFICATION (ORDINAL REGRESSION)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;18 months warfarin*</td>
<td>3.77 (0.97-14.7)</td>
<td>0.055</td>
</tr>
<tr>
<td>Age (10-year increase)</td>
<td>1.69 (1.12-2.65)</td>
<td>0.012</td>
</tr>
<tr>
<td>Dialysis vintage (1-year increase)</td>
<td>1.13 (0.98-1.30)</td>
<td>0.089</td>
</tr>
<tr>
<td>Calcium (500-mg increments)</td>
<td>1.41 (1.03-1.91)</td>
<td>0.027</td>
</tr>
<tr>
<td>Calcitriol (0.25-μg increments)</td>
<td>1.73 (0.88-3.41)</td>
<td>0.113</td>
</tr>
</tbody>
</table>

The model accounted for 23.3% of the variance (Cox and Snell r²).

*Reference group was <18 months of warfarin exposure.
Calcific Aortic Stenosis

Yutzek et al. ATVB 2014;34: in press
Mechanisms and Pathology of Calcification

- Calcification is an important and active pathophysiological processes in aortic stenosis
- Lipid appears to play a minor (possibly initiatory) role
- Calcification in the valve appears to be amorphous
- Animal models suggest several putative mechanisms of valvular calcification
- Clinical experimental medicine models are needed to elucidate the pathways of valvular calcification and identify novel therapeutic targets
Acknowledgements

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William Wallace
Christophe Lucatelli

University of Cambridge
James Rudd
Anthony Davenport
Agnese Irkle

Cedars-Sinai
Dan Berman
Piotr Slomka

British Heart Foundation

MRC | Medical Research Council
Wellcome Trust
Chief Scientist Office
Bisphosphonate Use and Prevalence of Valvular and Vascular Calcification in Women

MESA (The Multi-Ethnic Study of Atherosclerosis)

Elmariah et al. J Am Coll Cardiol 2010;56:1752-1759
Role of Inflammation
Interleukin-1 Receptor Antagonism