TGF-β/ENG RATIO IN BICUSPID AORTOPATHY

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Disclosure of Interest

Speaker name: Alessandro Della Corte

- I do not have any potential conflict of interest
Surgical treatment of bicuspid aortic valve disease: Knowledge gaps and research perspectives

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Surgical treatment of bicuspid aortic valve-associated conditions is not a particular technical challenge. However, the scientific basis for management criteria is still rudimentary and persistent gaps in knowledge determine inconsistencies in surgical practice and lack of patient-tailored approaches. This review focuses on controversial or unexplored aspects of bicuspid disease.

A possible model for systematic risk stratification

Phenotype-based stratification

- Root Phenotype
- Ao Regurgitation

- Ascending/Normal Phenotype
- Ao Stenosis/Normal function

Higher risk of fast progression and/or aortic dissection

Further stratification

- Functional Imaging
- Wall Biomechanics
- Biomarkers
The ideal prognostic biomarker of BAV aortopathy

- Related to aberrant aortic wall tissue remodeling
- Serum/plasma concentration measurable
- Significantly different levels in controls
- Unique of BAV (?)
- Loosely/not correlated with the aortic diameter
TGF-β in BAV aortopathy pathogenesis

Early cell changes and TGFβ pathway alterations in the aortopathy associated with bicuspid aortic valve stenosis

Amalia FORTE*, Alessandro DELLA CORTE†, Mario GROSSI*, Ciro BANCONE†, Raffaella PROVENZANO†, Mauro FINICELLI*, Marisa DE FEO†, Luca S. DE SANTO†, Gianantonio NAPPI†, Maurizio COTRUFO†, Umberto GALDERISI* and Marilena CIPOLLARO*

Valve-Related Hemodynamics Mediate Human Bicuspid Aortopathy

Insights From Wall Shear Stress Mapping

David G. Guzzardi, BSc,† Alex J. Barker, PhD,‡ Pim van Ooij, PhD,‖ S. Chris Malaisrie, MD,‖ Jyothy J. Pathumana, MD,‖ Darrell D. Belke, PhD,‖ Holly E.M. Mewhort, MD,‖ Daniyil A. Svystonyuk, BSc,‡ Subodh Verma, MD, PhDr, Jeremy Collins, MD,‖ James Carr, MD,‖ Robert O. Bonow, MD,‖ Michael Markl, PhDr,‡ James D. Thomas, MD,‖ Patrick M. McCarthy, MD,‖ Paul W.M. Fedak, MD, PhDr‡

Original article

Hypothesis-free secretome analysis of thoracic aortic aneurysm reinforces the central role of TGF-β cascade in patients with bicuspid aortic valve

Silvia Rocchiccioli (MSc, PhD)‡, Antonella Cecchettini (MSc, PhD)‡, Paola Panesi (MSc)‡, Pier Andrea Farneti (MD)‡, Massimiliano Mariani (MD)‡, Nadia Ucciferri (MSc, PhD)‡, Lorenzo Citti (MSc, PhD)‡, Maria Grazia Andreassi (MSc, PhD)‡, Illenia Foffa (MSc, PhD)‡, Star
Serum TGF-β in BAV patients

Total Serum Transforming Growth Factor-β1 Is Elevated in the Entire Spectrum of Genetic Aortic Syndromes

Mathias Hillebrand, MD; Nathalie Millot, MD; Sara Sheikhzadeh, MD; Meike Rybczynski, MD; Sabine Gerth, MTA; Tilo Kölbl, MD; Britta Keyser, PhD; Kerstin Kutsche, PhD; Peter N. Robinson, MD, MSc; Jürgen Berger, PhD; Thomas S. Mir, MD, PhD; Tanja Zeller, PhD; Stefan Blankenberg, MD; Yskert von Kodolitsch, MD, MBA; Britta Goldmann, MD

Interactive CardioVascular and Thoracic Surgery (2017) 1-4
doi:10.1093/icvts/ivx153


Increased blood levels of transforming growth factor β in patients with aortic dilatation

Carmen Rueda-Martínez a, Oscar Lamas a, Fernando Carrasco-Chinchilla a, Juan Robledo-Carmona a, Carlos Porras a, Gemma Sánchez-Espín a, Manuel Jiménez Navarro a,b,* and Borja Fernández c

ICVTS 2017
A Possible Early Biomarker for Bicuspid Aortopathy: Circulating Transforming Growth Factor β-1 to Soluble Endoglin Ratio
Amalia Forte, Ciro Bancone, Gilda Cobellis, Marianna Buonocore, Giuseppe Santarpino, Theodor J.M. Fischlein, Marilena Cipollaro, Marisa De Feo and Alessandro Della Corte

_Circ Res._ 2017;120:1800-1811; originally published online April 18, 2017; doi: 10.1161/CIRCRESAHA.117.310833
**Study population**

n=167 patients undergoing surgical AVR for pure aortic stenosis

<table>
<thead>
<tr>
<th>Study Population</th>
<th>Aorta Donors</th>
<th>Serum Donors- Control Group I</th>
<th>Serum Donors- Control Group II</th>
<th>TAV</th>
<th>BAV\textsubscript{non-dil}</th>
<th>BAV\textsubscript{dil}</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, %</td>
<td>54.5</td>
<td>63.6</td>
<td>55.0</td>
<td>52.0</td>
<td>61.5</td>
<td>64.5</td>
<td>0.72</td>
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<tr>
<td>Age, y</td>
<td>39.7±13</td>
<td>59.7±4.8\textdagger</td>
<td>59.0±6.43\textdagger</td>
<td>69.7±7.8\textdagger</td>
<td>60.9±15\textdagger</td>
<td>55.7±12\textdagger</td>
<td>0.001</td>
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<tr>
<td>Body surface area, (\text{m}^2)</td>
<td>1.87±0.1</td>
<td>1.83±0.2</td>
<td>1.86±0.18</td>
<td>1.81±0.2</td>
<td>1.80±0.2</td>
<td>1.88±0.2</td>
<td>0.26</td>
</tr>
<tr>
<td>BAV morphotype, RL%/RN%</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>56.4/43.6</td>
<td>67.7/32.3</td>
</tr>
<tr>
<td>Aortic diameter, cm</td>
<td>3.0±0.6</td>
<td>N/A</td>
<td>N/A</td>
<td>3.6±0.6</td>
<td>3.7±0.5</td>
<td>5.0±0.5\textdagger,\textsection</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left ventricle mass index, g/\text{m}^2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>152±43</td>
<td>143±37</td>
<td>125±44\textdagger</td>
<td>0.034</td>
</tr>
<tr>
<td>Aortic valve area index, cm(^2)/\text{m}^2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.39±0.1</td>
<td>0.40±0.1</td>
<td>0.44±0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>22.7</td>
<td>18.2</td>
<td>60\textcenti</td>
<td>82\textcenti,\textsection</td>
<td>69.2\textcenti,\textsection</td>
<td>58.1\textcenti,\textsection</td>
<td>0.009</td>
</tr>
<tr>
<td>Total cholesterol, mg/\text{dL}</td>
<td>N/A</td>
<td>173.2±6.4</td>
<td>190±8\textcenti</td>
<td>178.0±10</td>
<td>176.8±12.0</td>
<td>172.8±7.6</td>
<td>0.044</td>
</tr>
<tr>
<td>Low-density-lipoproteins, mg/dL</td>
<td>N/A</td>
<td>101.2±6.1</td>
<td>137±8\textcenti</td>
<td>107.1±9.6</td>
<td>102.6±10.2</td>
<td>99.2±4.5</td>
<td>0.038</td>
</tr>
<tr>
<td>ARBs, %</td>
<td>0</td>
<td>0</td>
<td>10\textcenti</td>
<td>34\textcenti,\textsection</td>
<td>30.8\textcenti,\textsection</td>
<td>32.3\textcenti,\textsection</td>
<td>0.021</td>
</tr>
<tr>
<td>Statins, %</td>
<td>0</td>
<td>18.2</td>
<td>15</td>
<td>44\textsection</td>
<td>35.9</td>
<td>32.3</td>
<td>0.054</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>0</td>
<td>0</td>
<td>10\textcenti</td>
<td>26\textcenti,\textsection</td>
<td>20.5\textcenti,\textsection</td>
<td>16.1\textcenti,\textsection</td>
<td>0.22</td>
</tr>
</tbody>
</table>

n=41 BAV patients undergoing AVR + ascending replacement for «ascending phenotype» aortic dilatation/aneurysm (>45mm)
Early cell changes and TGFβ pathway alterations in the aortopathy associated with bicuspid aortic valve stenosis

Amalia FORTE*, Alessandro DELLA CORTE†, Mario GROSSI*, Ciro BANCONET, Raffaela PROVENZANO†, Mauro FINICELLI*, Marisa DE FEO†, Luca S. DE SANTO†, Gianantonio NAPPI†, Maurizio COTRUFO†, Umberto GALDERISI* and Marilena CIPOLARO*

Valve-Related Hemodynamics Mediate Human Bicuspid Aortopathy

Insights From Wall Shear Stress Mapping
Gene expression in the aortic wall

- **TGF-β**
- **Endoglin**
- **SOD-3**
- **MMP-2**
- **MMP-14**
- **CTGF**
Confirmation for selected products at protein level (WB and IHC)

**TGF-β at CVX**

- Bar graph showing expression levels of TGF-β with *p* < 0.001 for CVX compared to control.

**Endoglin at CVX**

- Bar graph showing expression levels of Endoglin with *p* < 0.001 for CVX compared to control.

**Images**

- **A** and **B** show tissue sections labeled as control and TAV, respectively.
- **C** shows a tissue section labeled as BAV.
ELISA for circulating targets (products of the mRNAs analysed in the aortic wall)

Note: MMP-14 was undetectable in almost all patients/controls
$p = 0.802$

serum CTGF

Box plots showing serum CTGF levels across different groups: controls I, controls II, TAV, BAVnon-dil, and BAVdil.
$p = 0.344$

serum SOD-3
$p=0.008$

**serum TGF-β**
The decrease of ENG expression in the BAV aorta (never reported before) as well as the decrease of MMP-14 (consistent with previous findings by others) may explain the decreased levels of sENG.
Interpretation of the findings
serum TGF / serum ENG

$p=0.002$
Serum TGF-β/sENG Ratio

In multivariable linear regression analysis including all patients with BAV ($R^2$ of the model=0.73, F-test=20.9), the serum T/E ratio was predicted by MMP-2 expression at the convexity (standardized β coefficient=0.36, $P=0.003$) and by aortic dilatation (β for BAV$_{dil}$=0.45, $P=0.001$). When considering BAV$_{non-dil}$ patients only, including age as a covariate ($R^2_s=0.84$, F-test=17.2), the expression of MMP-2 at the convexity resulted as an independent predictor of T/E ratio, whereas SOD3 gene expression was an independent inversely associated factor.

Aortic Growth Rate

BAV$_{non-dil}$ patients reached a mean postoperative follow-up time of 38.2±11 months. The median growth rate of the ascending aorta was 0.3 mm/y (interquartile range: 0.0–1.2). Of all the serum proteins considered in the study, only the serum T/E ratio showed a significant correlation with the aortic growth rate ($r=0.66; P<0.001$; Figure 6). Patients with a T/E ≥9 had a median growth of 1.4 mm/y (interquartile range: 0.1–2) versus 0.05 in patients with a T/E <9 (interquartile range: 0.0–0.4; $P<0.001$).

Note: in BAV$_{non-dil}$ patients T/E was not correlated with aortic diameter at baseline

Correlation T/E – aortic growth in prospective follow-up

BAV$_{non-dil}$ patients with T/E >9 displayed a worse gene expression profile (↑MMP-2, ↓SOD-3 etc.)
Conclusions

• The T/E ratio might be a more comprehensive index of pathogenetically relevant changes in the aortic wall, than sTGF-β alone. This is to be considered in the light of the important regulatory functions of the ENG co-receptor.

• A high T/E ratio seems to be indicative of a propensity to aortopathy development/progression in BAV patients without aortic aneurysm (prognostic value).

• The present results apply to the ascending phenotype BAV patients with aortic valve stenosis requiring surgery. In different phenotypes, this and other biomarkers are still to be investigated.

• Further clinical validation of the T/E (identification of a non-arbitrary cut-off, correlation with aortic growth, association with aortic events?) is warranted.
Thank you
<table>
<thead>
<tr>
<th></th>
<th>TAV Concavity</th>
<th>TAV Convexity</th>
<th>BAV&lt;sub&gt;non-&lt;em&gt;dil&lt;/em&gt;**</th>
<th>BAV&lt;sub&gt;non-&lt;em&gt;dil&lt;/em&gt;**</th>
<th>BAV&lt;sub&gt;dil**&lt;em&gt;Concavity&lt;/em&gt;&lt;/sub&gt;</th>
<th>BAV&lt;sub&gt;dil**&lt;em&gt;Convexity&lt;/em&gt;&lt;/sub&gt;</th>
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<td><strong>MMP-2</strong></td>
<td></td>
<td></td>
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<tr>
<td>MMP-14</td>
<td>$r=0.45$</td>
<td>$r=0.71^*$</td>
<td>$r=0.28$</td>
<td>$r=0.17$</td>
<td>$r=0.95^{**}$</td>
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<td>ENG</td>
<td>$r=0.61^*$</td>
<td>$r=0.63^*$</td>
<td>$r=0.14$</td>
<td>$r=-0.03$</td>
<td>$r=0.56$</td>
<td>$r=-0.79^*$</td>
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<td><strong>TGF-β</strong></td>
<td><strong>$r=0.94^{</strong>}$**</td>
<td><strong>$r=0.61^*$</strong></td>
<td><strong>$r=0.86^{</strong>}$**</td>
<td><strong>$r=0.93^{</strong>}$**</td>
<td><strong>$r=0.91^{</strong>}$**</td>
<td><strong>$r=0.80^*$</strong></td>
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<td>CTGF</td>
<td>$r=0.76^{**}$</td>
<td>$r=0.35$</td>
<td>$r=0.33$</td>
<td>$r=0.36$</td>
<td>$r=0.84^*$</td>
<td>$r=-0.48$</td>
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<td>SOD3</td>
<td>$r=0.67^*$</td>
<td>$r=0.44$</td>
<td>$r=0.92^{**}$</td>
<td>$r=-0.15$</td>
<td>$r=0.88^{**}$</td>
<td>$r=-0.77^*$</td>
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<td><strong>MMP-14</strong></td>
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<tr>
<td>ENG</td>
<td>$r=0.39$</td>
<td><strong>$r=0.89^{</strong>}$**</td>
<td>$r=0.58^*$</td>
<td>$r=0.54^*$</td>
<td>$r=0.67^*$</td>
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<td>TGF-β</td>
<td>$r=0.39$</td>
<td>$r=0.51$</td>
<td>$r=0.32$</td>
<td>$r=0.30$</td>
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<td>$r=0.58^*$</td>
<td>$r=0.11$</td>
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<td>SOD3</td>
<td>$r=0.34$</td>
<td>$r=0.47$</td>
<td>$r=0.27$</td>
<td>$r=0.14$</td>
<td>$r=0.83^*$</td>
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<td><strong>ENG</strong></td>
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<tr>
<td>TGF-β</td>
<td>$r=0.49$</td>
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<td>$r=-0.19$</td>
<td>$r=-0.22$</td>
<td>$r=0.12$</td>
<td>$r=0.48$</td>
<td>$r=0.63$</td>
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<tr>
<td>SOD3</td>
<td>$r=0.45$</td>
<td>$r=0.31$</td>
<td>$r=0.01$</td>
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<tr>
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<td><strong>$r=0.67^*$</strong></td>
<td><strong>$r=0.74^*$</strong></td>
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<td>$r=0.42$</td>
<td><strong>$r=0.86^{</strong>}$**</td>
<td>$r=-0.01$</td>
<td><strong>$r=0.91^{</strong>}$**</td>
<td>$r=-0.45$</td>
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<tr>
<td><strong>CTGF</strong></td>
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</tr>
<tr>
<td>SOD3</td>
<td>$r=0.60^*$</td>
<td>$r=0.21$</td>
<td>$r=0.32$</td>
<td>$r=0.14$</td>
<td>$r=0.73^*$</td>
<td>$r=0.53$</td>
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</table>
Only TGF-β and (more weakly) MMP-2 showed a significant correlation between gene expression at tissue level (convexity) and circulating levels of respective product:

- TGF-β mRNA correlation with sTGDF-β: $r=0.61$; $p=0.006$
- MMP-2 mRNA correlation with sMMP-2: $r=0.49$; $p=0.04$
MicroRNA-29 in aortic dilation: implications for aneurysm formation.  

Progressive Aortic Dilation Is Regulated by miR-17-Associated miRNAs.  

miRNAome Profiling in Bicuspid Aortic Valve-Associated Aortopathy by Next-Generation Sequencing.  

Specific circulating microRNA signature of bicuspid aortic valve disease.  

Evaluation of micrornucleic acids as potential biomarkers in the bicuspid aortic valve-associated aortopathy.
miRs, miRs in the Wall, Who Is the Most Causative of Them All?*

Mary Sheppard, MD, a,b,c Debra L. Rateri, BSc, a Alan Daugherty, PhD, DSc a,d

Given the prevalence of BAV-dependent ascending aortic aneurysms and lack of validated medical therapy, such studies highlight the need to develop appropriate animal models relevant to human disease. Determining the importance of miRNAs in