A Targeted Proteomic Approach Identifies Novel Biomarkers of Arterial Thromboembolic Risk in ENGAGE AF-TIMI 48

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Background

• Assessing risk of stroke or systemic embolism is a cornerstone of atrial fibrillation (AF) management

• Although guidelines recommend clinical risk scores (CHA$_2$DS$_2$-VASc score) to estimate thromboembolic risk, there is mounting evidence that circulating biomarkers can substantially improve risk stratification$^1$

Biomarkers and IS/SEE Risk

**hsTnT (ng/ml)**
- **<7**: 0.71
- **7-<14**: 1.40
- **≥14**: 2.18

**NT-proBNP (pg/ml)**
- **<450**: 1.03
- **450-<950**: 1.05
- **≥900**: 2.55

**GDF-15 (pg/ml)**
- **<1200**: 0.92
- **1200-<1800**: 1.57
- **≥1800**: 2.28

Annualized Event Rate (%)

Objective

To leverage the power of highly multiplex biomarker testing to identify novel biological pathways associated with arterial thromboembolic risk in a well-characterized cohort of patients with AF
Methods

• Study Population
  • ENGAGE AF-TIMI 48 was a multinational, randomized, double-blind trial of the oral Factor Xa inhibitor edoxaban vs warfarin for the prevention of stroke/systemic embolism in patients with AF and CHADS$_2$ score ≥2 (median f/u = 2.8 years)$^1$

• Biomarkers
  • Blood samples prospectively collected at randomization
  • 184 biomarkers tested using Olink proteomic panels (CVD II & CVD III)
    • Panels selected to include some biomarkers with established associations in AF

• Clinical Outcome
  • Ischemic stroke or systemic embolic event (IS/SEE)
    • Outcomes adjudicated centrally by Clinical Events Committee (CEC)

Olink Proteomic Panels

- Multiplex assays simultaneously test 92 biomarkers per plate using oligonucleotide-labeled antibodies for protein detection

• Nested case-control study (1:1 selection) of 184 candidate biomarkers
  • Cases → patients who experienced IS/SEE during follow-up (n=188)
  • Controls matched on age, sex, hx stroke/TIA, CrCl (n=188)

• Evaluated associations between biomarkers and IS/SEE using conditional logistic regression with FDR threshold (q<0.05) for statistical significance
  • Biomarkers modeled as continuous and categorical (quartiles) variables

• ORs adjusted for components of CHA$_2$DS$_2$-VASc score (HF, HTN, ASCVD, and DM)

• Evaluated biomarker correlations among significant biomarkers

• Included significant biomarkers & clinical covariates in a multi-marker model
Baseline Biomarker Concentrations and IS/SEE Risk

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>OR per 1-SD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L2*</td>
<td>1.62 (1.26-2.10)</td>
</tr>
<tr>
<td>GDF-15</td>
<td>1.55 (1.21-1.99)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>1.54 (1.20-1.97)</td>
</tr>
</tbody>
</table>

*PD-L2 = programmed cell death 1 ligand 2
Adjusted Associations with IS/SEE

**PD-L2**
- Quartile 1 (Reference)
- Quartile 2: 2.75 (1.44, 5.27)
- Quartile 3: 3.15 (1.62, 6.13)
- Quartile 4: 4.38 (2.11, 9.10)

**GDF-15**
- Quartile 1 (Reference)
- Quartile 2: 3.14 (1.49, 6.60)
- Quartile 3: 2.28 (1.07, 4.85)
- Quartile 4: 4.29 (1.96, 9.39)

**NT-proBNP**
- Quartile 1 (Reference)
- Quartile 2: 1.19 (0.64, 2.22)
- Quartile 3: 1.52 (0.81, 2.86)
- Quartile 4: 3.02 (1.53, 5.97)
### Biomarker Relationships

- PD-L2 less correlated with NT-proBNP or GDF-15 than NT-proBNP and GDF-15 are with each other.

<table>
<thead>
<tr>
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<th>PD-L2</th>
<th>NT-proBNP</th>
<th>GDF-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L2</td>
<td>1.00</td>
<td>0.18 (p&lt;0.001)</td>
<td>0.27 (p&lt;0.001)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>1.00</td>
<td>0.50 (p&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>GDF-15</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

- Multi-marker model:

```
<table>
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<tr>
<th>Biomarker</th>
<th>Adj-OR* per 1-SD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L2</td>
<td>1.54 (1.18-2.02)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>1.28 (0.95-1.72)</td>
</tr>
<tr>
<td>GDF-15</td>
<td>1.29 (0.93-1.77)</td>
</tr>
</tbody>
</table>
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*Also adjusted for components of CHA$_2$DS$_2$-VASc score

**PD-L2 independently associated with IS/SEE**
• PD-L2 is a ligand for programmed cell death protein 1 (PD-1), a transmembrane receptor protein expressed on T lymphocytes that plays an important role in suppressing immune activation

• Impaired PD-1 signaling has been implicated in atherogenesis, but a relationship between PD-1 axis and arterial thromboembolism has not been described

Conclusions

• A targeted proteomic approach identified PD-L2 as a potential novel biomarker of IS/SEE risk in patients with AF

• Further investigation of PD-1 signaling may elucidate new mechanisms of ischemic stroke risk in patients with AF and other cardiovascular diseases