Circulating anti-Heart Autoantibodies are Non-invasive Markers of high Cellular Rejection Burden in Heart Transplantation


E-mail: alida.caforio@unipd.it

*Dept of Cardiological, Thoracic and Vascular Sciences **Cardiac Pathology, Padua University, Padua, Italy
Autoimmune responses may occur after solid organ transplantation regardless of immunosuppression.

In autoimmune disease autoantibodies provide non-invasive early markers for active phases of immune-mediated inflammation in the target organ.

Serum anti-heart autoantibodies (AHA) of the organ-specific (O-s) and cross-reactive (Cr) types detected by indirect immunofluorescence (IFL) are autoimmune markers in immune-mediated inflammatory cardiomyopathy.

Non invasive early markers for acute rejection (AR) are lacking in human heart transplantation (Htx).
<table>
<thead>
<tr>
<th></th>
<th>Organ-specific n (%)</th>
<th>Cross-reactive-1 n (%)</th>
<th>Cross-reactive-2 n (%)</th>
<th>Neg n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM (n=327)</td>
<td>83 (25)*^</td>
<td>35 (11)*^^</td>
<td>3 (1)</td>
<td>206 (63)</td>
</tr>
<tr>
<td>Myocarditis (n=130)</td>
<td>73 (56) *^</td>
<td>19 (15)**^</td>
<td>0 (0)</td>
<td>38 (29)</td>
</tr>
<tr>
<td>Relatives of DCM (n=567)</td>
<td>176 (31)*^</td>
<td>7 (1)</td>
<td>12 (2)</td>
<td>372 (66)</td>
</tr>
<tr>
<td>Other heart disease (OCD) (n=160)</td>
<td>1 (1)</td>
<td>7 (4)</td>
<td>5 (3)</td>
<td>147 (92)</td>
</tr>
<tr>
<td>Ischemic heart failure (IHF) (n=141)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>8 (6)</td>
<td>131 (92)</td>
</tr>
<tr>
<td>Normals (n=270)</td>
<td>7 (2.5)</td>
<td>8 (3)</td>
<td>9 (3)</td>
<td>246 (91)</td>
</tr>
</tbody>
</table>

* p=0.0001 vs OCD or IHF; **p=0.0002 vs OCD
^ p=0.0001 and ^^p =0.0003 vs normals
Survival free from DCM by cardiac antibody status at diagnosis in relatives classified as normal, LVE or DFS at baseline

\[ p(\text{log-rank}) = 0.0086 \]

Caforio et al 2007
Circulation
Aim

• To assess the frequency and potential predictive role of AHA for acute rejection (AR) after heart transplantation (HTx).
HTx patients - demographics

- HTx patients (n=44, 32 M, aged 51 ± 16 years, f-u 100 ± 72 months post-HTx)

- Pre Htx dgn
  - DCM 24%
  - Other 76%

- Post Htx immunosuppressive therapy
  - Triple 30%
  - Mono or dual 70%

- Presence of angiographic cardiac allograft vasculopathy (CAV)
  - yes 46%
  - no 54%
AHA control groups - demographics

• AHA control patients:

Non-inflammatory heart disease (NICD) (n=160, 80 M, aged 37±17)
  – Valvular heart disease (n=55, 18 M, age 59±13 yrs)
  – Hypertrophic cardiomyopathy (n=67, 37 M, age 44±14 yrs)
  – Congenital heart defects (n=38, 25 M, median age 11, range 1-67)

Ischaemic heart disease (IHD) (n=141, 131 M, aged 51 ±12 yrs)

Normal blood donors (N) (n=270, 123 M, aged 35 ±11 yrs)
Methods

• Circulating anti-heart autoantibody (AHA) screening test
  – Sera from ARVC and controls tested by standard indirect immunofluorescence (s-IFL) on cryostat sections of normal human O blood group myocardium. Normal human skeletal muscle used to detect cross-reactive AHA(type 1 and 2).

» Caforio et al. Circulation 2007;115: 76-83
Acute rejection scores and cumulative immunosuppressive load

- Post-HT rejection scores (RS)/biopsy number:
  ŠISHLT grade 0=0; 1A=1; 1B=2; 2=3; 3A=4; 3B=5; 4=6
  ŠRS in the whole F/U (TRS) summing up all the scores
  ŠRS in the 1st year (RS 1yr); TRS including only severe (>=3A) grades (sev TRS); RS 1yr including only severe grades (sev RS 1yr)

- Cumulative PDN load at 1 year post-HT (PDN1yr) and methyprednisolone load at 1 year post-HT (MethPD1yr) in mg/Kg

- Cumulative total steroid load at 1 year (TOTCORT1yr=PDN1yr+MethPD1yr) in mg/Kg, following conversion of each MethPD dose to an equivalent PDN dose (4mg of MethPD=5mg PDN)

- Cumulative dose (mg/Kg) of CsA and Aza at 3,6,12 mo

Caforio et al Am J Transplant 2004
Statistical analysis

• Comparison of AHA frequency in HTx versus controls and of clinical and diagnostic features in HTx patients with and without AHA:

  – Univariate analysis ($\chi^2$ test, Student’s t test or ANOVA, log-rank test)
Example of anti-heart antibody (AHA) negative (neg) serum pattern

AHA negative pattern on human miocardium (X100)

AHA negative pattern on human skeletal muscle (X400)

Example of Cross-Reactive-2 antiheart antibody (AHA) serum pattern


Positive broad cross-striational pattern on human myocardium (X400)

Positive broad cross-striational pattern on human skeletal muscle (X400)
Example of cross-reactive-1 (Cr-1) anti-heart antibody (AHA) positive serum pattern

Fine striational Cr-1 pattern on human myocardium (X400)  
Fine striational Cr-1 weak positive pattern on human skeletal muscle (X400)

Example of organ-specific (O-S) anti-heart antibody (AHA) positive serum pattern


Positive diffuse pattern on human myocardium (X40)

Negative pattern on human skeletal muscle (X40)
**Frequency of the anti-heart AAbs (AHA) by s-IIFL in HTx**

<table>
<thead>
<tr>
<th></th>
<th>Organ-specific n (%)</th>
<th>Cross-reactive-1 n (%)</th>
<th>Cross-reactive-2 n (%)</th>
<th>Neg n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Htx pts (n=44)</strong></td>
<td>12 (27) *^</td>
<td>3 (7)</td>
<td>0 (0)</td>
<td>29 (59)</td>
</tr>
<tr>
<td><strong>Non inflammatory heart disease (NICD) (n=160)</strong></td>
<td>1 (1)</td>
<td>6 (4)</td>
<td>5 (3)</td>
<td>148 (92)</td>
</tr>
<tr>
<td><strong>Ischemic heart disease (IHD) (n=141)</strong></td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>8 (6)</td>
<td>131 (92)</td>
</tr>
<tr>
<td><strong>Normals (n=270)</strong></td>
<td>7 (2.5)</td>
<td>8 (3)</td>
<td>8 (3)</td>
<td>247 (91)</td>
</tr>
</tbody>
</table>

* p=0.0001 vs NICD or IHF; ^ p=0.0001 vs normals
Associations of AHA status with clinical and diagnostic features in HTx

<table>
<thead>
<tr>
<th></th>
<th>AHA positive (n=15)</th>
<th>AHA negative (n=29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Htx (yrs)</td>
<td>51 ± 17</td>
<td>51 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>73</td>
<td>72</td>
<td>NS</td>
</tr>
<tr>
<td>DCM/Other dgn pre HTx (%)</td>
<td>27/73</td>
<td>22/78</td>
<td>NS</td>
</tr>
<tr>
<td>Triple IS/non triple IS (%)</td>
<td>25/75</td>
<td>33/67</td>
<td>NS</td>
</tr>
<tr>
<td>Time from Htx (mo)</td>
<td>113 ± 66</td>
<td>94 ± 75</td>
<td>NS</td>
</tr>
<tr>
<td>Rejection score in the 1st yr</td>
<td>1.7 ± 0.1</td>
<td>1.1 ± 0.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Severe total rejection score</td>
<td>1.3 ± 0.1</td>
<td>0.46 ± 0.4</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Conclusions

• Patients post-HTx have a higher frequency of serum organ-specific AHA than disease and normal control subjects.

• Overall 34% of pts were AHA positive, similar to DCM. AHA positive status in HTx pts was associated with a higher total rejection score during f-u.

• The finding of AHA may provide a non-invasive predictor of high rejection burden after HTx.