Abstract: **P5455**

**Serum microRNA-181a-5p expression pattern correlates with acute cellular rejection in heart transplantation**

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**Topic(s):**
Heart Transplantation

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**Background:** Acute Cellular Rejection (ACR) remains a major complication in heart transplantation (HT). Since 1970, endomyocardial biopsy (EMB) has been the gold standard for ACR early detection. However, this invasive procedure has several limitations such as risk of complications, interobserver variability, false negative results and high cost. Therefore, a new non-invasive ACR biomarker is needed. Potential candidates could be microRNAs (miRs), small regulatory RNAs whose serum expression profiling could be modified in ACR patients. The aim of this study is the identification of differentially serum miRs expression patterns in ACR after HT.

**Methods:** Among HT performed in our Hospital (2013-2018), we selected patients with following criteria: i) "0R ? 2R ? 0R" EMB pattern for ACR (0R=no ACR, 2R=moderate ACR); ii) no antibody mediated rejection; iii) serum collected the same day as the EMBs. The 3 serum samples per patient matched in time with EMB were called "0Rs₁ ? 2Rs₂ ? 0Rs₃". In each of these serum samples, 179 miRs expression profile according manufacturer’s instructions were analysed. Global Mean (GM) method was used to normalize our results expressed as miRs relative expression \[2^{(-\Delta\text{Cq})}\]. Expression pattern was defined as a 2 steps process: a significative rise (0Rs₁ ? 2Rs₂) followed by a significative fall (2Rs₂ ? 0Rs₃), or vice versa, in miR expression. Analysis of differences between 3 groups were calculated using one-way ANOVA with matched data and post-hoc Tukey test. ROC curve was generated for selected miRNA. A two-sided p<0.05 was considered statistically significant.

**Results:** 21 patients and their serum "0Rs₁ ? 2Rs₂ ? 0Rs₃" samples were included in the study. Among 179 miRs analysed, 5 miRs showed significative difference between 0Rs₁ and 2Rs₂, 3 miRs were over-expressed (miR-181a-5p, miR-339-3p, let-7f-5p) and 2 under-expressed (miR-505-3p, miR-2110). Only miR-181a-5p met significative difference between 2Rs₂ and 0Rs₃ showing a statistical significative rise/fall pattern. Serum miR-181a-5p ROC analysis demonstrated significant discrimination between biopsy proven ACR from no-ACR biopsy, AUC=0.692 (p=0.0335).

**Conclusion:** Our findings suggest miR-181a-5p as a novel serological biomarker for detecting ACR after HT. After this preliminary discovery phase, miR-181a-5p is currently in a new validation phase to demonstrate its diagnostic performance.

<table>
<thead>
<tr>
<th>mean relative expression [2^{(-\Delta\text{Cq})}] (95%CI)</th>
<th>Adjusted p-value (ANOVA)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80 (0.60-1.02)</td>
<td>1.11 (0.90-1.31)</td>
<td>0.86 (0.71-1.01)</td>
</tr>
</tbody>
</table>
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Results: 21 patients and their serum "0Rs1 ? 2Rs2 ? 0Rs3" samples were included in the study. Among 179 miRs analysed, 5 miRs showed significative difference between 0Rs1 and 2Rs2, 3 miRs were over-expressed (miR-181a-5p, miR-339-3p, let-7f-5p) and 2 under-expressed (miR-505-3p, miR-2110). Only miR-181a-5p met significative difference between 2Rs2 and 0Rs3 showing a statistical significative rise/fall pattern. Serum miR-181a-5p ROC analysis demonstrated significant discrimination between biopsy proven ACR from no-ACR biopsy, AUC=0.692 (p=0.0335).

Conclusion: Our findings suggest miR-181a-5p as a novel serological biomarker for detecting ACR after HT. After this preliminary discovery phase, miR-181a-5p is currently in a new validation phase to demonstrate its diagnostic performance.

Relative Expression \(2^{(-\Delta Cq)}\) and AUC expressed as mean, (95% Confident Interval). NS: not significative

<table>
<thead>
<tr>
<th></th>
<th>0R_s1</th>
<th>2R_s2</th>
<th>0R_s3</th>
<th>0R_s1 vs 2R_s2</th>
<th>2R_s2 vs 0R_s3</th>
<th>0R_s1 vs 0R_s3</th>
<th>(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-181a-5p</td>
<td>0,80</td>
<td>1,11</td>
<td>0,86</td>
<td>0,0194</td>
<td>0,0225</td>
<td>NS</td>
<td>0,692 (0,529-0,855)</td>
</tr>
</tbody>
</table>

Relative Expression \(2^{(-\Delta Cq)}\) and AUC expressed as mean, (95% Confident Interval). NS: not significative

AUC=0.692