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Servicio de Inmunología

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Collaboration with Cardiology Units:

Hospital de la Princesa
(Drs. Jiménez-Borreguero/ Fernando Alfonso)

Fundación Jiménez Díaz
(Dr. Borja Ibáñez)

Hospital 12 de Octubre
(Dr. Héctor Bueno)
hsa-miRNA-Chr8.96: First Plasma Biomarker for Diagnosis of Myocarditis

- **EX-microRNAs as biomarkers in MYOCARDITIS**
  - (Th17-mediated conditions)

Exosomes/free circulating miRNAs

Prognostic Biomarker
- Predict disease outcome
- Monitor disease recurrence

Predictive Biomarker
- Therapy response
- Treatment decisions

Francisco Sánchez-Madrid and Pilar Martin
Servicio de Inmunología, Hospital de la Princesa, IIS-IP
Universidad Autónoma de Madrid
Centro Nacional Investigaciones Cardiovasculares
Improving the diagnosis of MINOCA patients

- A liquid biopsy for myocarditis

MINOCA: Myocardial Infarction with Non-Obstructive Coronary Arteries
Endomyocardial Biopsy (EMB) remains the gold standard in diagnosis of myocarditis.

EMB is not commonly performed due to safety reasons:
- 6% complications
- 0.4% incidence of death due to perforation

Nowadays there is a lack of early and non-invasive methods to diagnose myocarditis.
Myocarditis is an underdiagnosed cause of acute heart failure, sudden death, and chronic DCM. However, the correct diagnosis remains challenging.

The clinical course may range from completely recovery to end-stage heart failure.

Approx. 5-20% of all cases of sudden death in young adults (< 35 years of age)

Myocarditis is present in 10-50% of heart biopsy samples from patients with acute DCM, with a prevalence of 36.5 per 100,000 in U.S.

Myocarditis and IDCM are cause of approx. 45% of heart transplants in the U.S
Angiography is invasive and not available/adequate for everyone (small villages, pregnant women, etc.).

The problem of diagnosis...myocarditis mimicks acute myocardial infarction

Troponins and ST-segment elevation

Infant acute myocarditis mimicking acute myocardial infarction.

9 months-old girl died due to an underdiagnosed and mistreated fulminant myocarditis

Cardiomegaly and pulmonary congestion
Two mouse models of heart diseases

Myocardial Infarction:
- LAD: Left Anterior Descending artery ligation
- cTnI: Chronic
- CK-MB: Acute
- LDH: Ad. exp.

Myocarditis:
- EAM: Experimental Autoimmune Myocarditis
- Immunization s.c. MyHC
- Ac-RSLKLMATLFSTYASADRH-OH

**Different kinetics of Myocardial Infarction and Myocarditis**

**Th17 cells**
- Myocarditis (EAM)
- Myocardial Infarction (LAD)

**Heart damage (CK-MB)**
- Acute phase
miR-721 is overexpressed in Th17 cells

EAM: Experimental Autoimmune Myocarditis

miR-721 is highly expressed in Th17 and in plasma obtained from the acute phase of Myocarditis

DE of Th17-derived miRNAs from severe Myo vs mild Myo

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Fold change (TH17Ag-KO vs TH17Ag-WT)</th>
<th>Fold change</th>
<th>p-value</th>
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<tbody>
<tr>
<td>mmu-miR-721</td>
<td>7.71026</td>
<td>189.6731</td>
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<tr>
<td>mmu-miR-483*</td>
<td>7.170248</td>
<td>130.45058</td>
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<td>mmu-miR-18a</td>
<td>4.012081</td>
<td>1.9583826</td>
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<tr>
<td>mmu-let-7d*</td>
<td>7.515896</td>
<td>1.5813458</td>
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<tr>
<td>mmu-let-7l*</td>
<td>8.906817</td>
<td>1.4830275</td>
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</table>

miR-721 kinetic in BALB/c

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>CD4% Cells</th>
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<tr>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>21</td>
<td>80</td>
</tr>
<tr>
<td>28</td>
<td>100</td>
</tr>
</tbody>
</table>

Cells

Plasma

mmu-miR-721
miR-721 is contained into extracellular vesicles

Draining Lymph Nodes (day 6 of EAM) cultured 48h +MyHCα +IL-23 (20M cells/ml)

miR-721 expression in plasma correlates with % Th17 in blood

The size of microvesicles
Elucidating the homologous miR-721 in human (chr8:96)

Cloning the human miRNA from myocarditis patient plasma
Peripheral blood samples from:

- Healthy donors (80)
- Patients with Acute Myocarditis (39)
- Patients with Acute Myocardial Infarction (STEMI: 40, NSTEMI: 45)

- Clinical parameters:
  • Heart function (ECG & Echocardiography)
  • Heart damage markers (TPI, CK-MB...)
  • Magnetic Resonance Imaging (Gadolinium enhancement)
  • Dyslipidemia
  • others

To study:

➢ Th17 cells and Biomarker in peripheral blood: plasma and circulating cells.
### Phase I clinical trial

Baseline characteristic of the Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAD Patients with Available Data</th>
<th>TFOS, Time from the Onset of Symptoms</th>
<th>LV EF, Left Ventricle Ejection Fraction</th>
<th>CMR, Cardiovascular Magnetic Resonance</th>
<th>LGE, Late Gadolinium Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>37</td>
<td>41</td>
<td>45</td>
<td>66.2±14.43</td>
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<tr>
<td>Age, y</td>
<td>42.3±1.20</td>
<td>37.1±1.61</td>
<td>41.0±2.22</td>
<td>45.0±10.27</td>
<td>&lt;0.0001</td>
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<tr>
<td>Sex (men/women)</td>
<td>80/41</td>
<td>9.28</td>
<td>11.90</td>
<td>45.0±16.92</td>
<td>16/29</td>
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<tr>
<td>TFOS, d</td>
<td>4.16±1.68</td>
<td>4.37±2.05</td>
<td>41.0±10.27</td>
<td>45.0±10.27</td>
<td>1.5±2.076</td>
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<tr>
<td>Echocardiography at Admission, %</td>
<td>80/100</td>
<td>66.50</td>
<td>100</td>
<td>80.00</td>
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<tr>
<td>LV EF, %</td>
<td>63.8±10.55</td>
<td>54.7±11.32</td>
<td>60.2±12.01</td>
<td>57.6±10.55</td>
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<td>Segmental Contraction Abnormalities, %</td>
<td>80/0</td>
<td>60.50</td>
<td>100</td>
<td>67.74</td>
<td>22.22</td>
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<td>ECG Alterations, %</td>
<td>80/100</td>
<td>76.50</td>
<td>100</td>
<td>77.77</td>
<td>22.22</td>
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<tr>
<td>ST Segment Elevation, %</td>
<td>80/0</td>
<td>76.50</td>
<td>100</td>
<td>77.77</td>
<td>22.22</td>
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<tr>
<td>Q-wave, %</td>
<td>80/0</td>
<td>76.50</td>
<td>100</td>
<td>77.77</td>
<td>22.22</td>
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<tr>
<td>Coronary Angiography or CT Angiography Performed, %</td>
<td>80/0</td>
<td>76.50</td>
<td>100</td>
<td>77.77</td>
<td>22.22</td>
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<tr>
<td>Coronary Artery Disease, %</td>
<td>80/0</td>
<td>76.50</td>
<td>100</td>
<td>77.77</td>
<td>22.22</td>
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<tr>
<td>Laboratory Findings</td>
<td>80/0</td>
<td>76.50</td>
<td>100</td>
<td>77.77</td>
<td>22.22</td>
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<tr>
<td>Peak Troponin 1</td>
<td>8.74±6.20</td>
<td>42.0±13.16</td>
<td>18.0±13.16</td>
<td>13.0±10.27</td>
<td>0.0018</td>
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<tr>
<td>Peak Troponin T</td>
<td>129±139</td>
<td>402±139</td>
<td>1227±2340</td>
<td>399±58.7</td>
<td>0.0019</td>
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<tr>
<td>Peak Creatine kinase, U/L</td>
<td>502±318.7</td>
<td>1766±1854</td>
<td>399±58.7</td>
<td>607±26.67</td>
<td>0.0191</td>
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<tr>
<td>Peak Creatine kinase MB, U/L</td>
<td>413±233.2</td>
<td>964±57.7</td>
<td>607±26.67</td>
<td>50.4±26.67</td>
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<tr>
<td>CMR Performed, %</td>
<td>11.25</td>
<td>75.68</td>
<td>14.63</td>
<td>2.43</td>
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<tr>
<td>LGE, %</td>
<td>80/0</td>
<td>78.57</td>
<td>100</td>
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<td>Cardiovascular Risk Factors, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
<td></td>
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<tr>
<td>Dyslipidemia, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
<td></td>
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<tr>
<td>Smoker or Former Smoker, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
<td></td>
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<tr>
<td>Arterial Hypertension, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
<td></td>
</tr>
<tr>
<td>Diabetic, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
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</tr>
<tr>
<td>Renal Insufficiency, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
<td></td>
</tr>
<tr>
<td>Peripheral Artery Disease, %</td>
<td>37.0</td>
<td>36.58</td>
<td>45.0±10.27</td>
<td>45.0±10.27</td>
<td></td>
</tr>
</tbody>
</table>

A MINOCA case report with late diagnosis of myocarditis

**Arteriogram**

No occlusion of coronary arteries

**CMR**

Gadolinium enhancement (inflammatory edema)
hsa-miR-chr8:96 is enriched in EV from human plasma of myocarditis patients

Th17 cells are over-represented in the blood of myocarditis patients

2-ΔCt (Spike-ins for RNA extraction)

Hsa-miR-Chr8:96 is preferentially secreted into EVs

Hsa-miR-Chr8:96 is expressed exclusively in myocarditis patients plasma

Hsa-miR-483-5p is a general biomarker for myocardial damage

STEMI: ST-elevation Myocardial Infarction
NSTEMI: non-ST-elevation Myocardial Infarction
Circulating hsa-miR-Chr8:96 is an efficient biomarker for myocarditis

**Receiver operating characteristic (ROC) analysis** of each evaluated miRNA to analyze their diagnostic power to discriminate myocarditis patients from healthy controls, from acute myocardial infarction STEMI and NSTEMI patients, or from all the non-myocarditis samples analyzed.

The ROC plots represent sensitivity (i.e., true positive rate) versus 1 – specificity (i.e., false positive rate).

**Hsa-miR-Chr8:96 Sensitivity and Specificity > 90% guarantees the potential as a Biomarker for acute myocarditis**
Achy feeling in the chest

Hsa-miR-Chr8:96, expressed by Th17 cells, and released to plasma in EVs

Biomarker for differential diagnosis of Myocarditis versus Acute Myocardial Infarction

Goal: Biosensor For miRNA detection
- 30 min test!
- Total RNA (500ng)
- Detection at levels of pM
New microRNAs for the diagnosis of cardiomyopathies (Number EP15382596)
Priority date: December 2015. PCT: presentada en 2016

The present invention provides an in vitro method for obtaining data useful for diagnosing a cardiomyopathy, measuring the expression levels of two microRNAs in the blood plasma of patients.

Given the absence of methods capable of assuring a degree of acceptable specificity and sensitivity for the diagnosis of myocarditis, particularly during an acute episode, the present invention solves this problem by providing a specific and sensitive assay for diagnosing myocarditis by using blood samples.

Electrochemical biosensors for diagnosing acute myocarditis (Number EP17382324)
Priority Date: 31/05/2017

Detection and/or quantification of miRNA, RNA or DNA molecules related to the inflammation of the myocardium or myocarditis in at least one isolated complex clinical sample of different types of specimens by using a electrochemical sensor.

Business Plan

Business Plan and business Model: Business Plan (BP) will be finely designed and deployed once the feasibility of successful development of a Diagnostic tool and commercialization of the modular biosensing platform will be characterized.

The biosensing+biomarker platform, a business case including CAPEX-OPEX and funding opportunities will be driven with the support of innovation business private consultancy.

Potential UE Market
Market penetration
Units to be sold
ExWorks price
New microRNAs for the diagnosis of cardiomyopathies

The present invention provides an *in vitro* method for obtaining data useful for diagnosing a cardiomyopathy, measuring the expression levels of two microRNAs in the blood plasma of patients.

Given the absence of methods capable of assuring a degree of acceptable specificity and sensitivity for the diagnosis of myocarditis, particularly during an acute episode, the present invention solves this problem by providing a specific and sensitive assay for diagnosing myocarditis by using blood samples.

- **Number** EP1382296
- **Priority date** 01/12/2015
- **Applicants** CNIC
- **Inventors** Pilar Martín Fernández, Raquel Sánchez Díaz, Adela Mateosan Martín, Jesús Jiménez Borreguero, Francisco Sánchez Madrid

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**Electrochemical biosensors for diagnosing acute myocarditis**

**Priority Date:** 31/05/2017

**Titulares:** Canaan R&D and CNIC

**Inventors:** Pilar Martín, Rafael Blanco Domínguez, Raquel Sánchez Díaz, Francisco Sánchez-Madrid

*Detection and/or quantification of miRNA, RNA or DNA molecules related to the inflammation of the myocardium or myocarditis in at least one isolated complex clinical sample of different types of specimens by using an electrochemical sensor*
Diagnostic approach (a *Cardiovascular Liquid Biopsy*)

**Myocarditis**

- **1 day**: Onset of symptoms
- **1-2 week**: Adaptive immune response (Th17/Tregs)
- **4 weeks**: Acute myocarditis, myocardial inflammation
- **2 week**: miR-721
- **4 weeks**: Fibrosis & HF

**AMI**

- **1 day**: Chest pain (Hospital)
- **2 week**: miR-Chr8:96
- **4 weeks**: Chest pain (Hospital)
- **2 week**: miR-721
- **4 weeks**: Th17

**I/R**

- **1 day**: Innate immune response
- **2 week**: Adaptive immune response (Th1/Th17)
- **4 weeks**: Myocardial inflammation/remodeling and necrosis/fibrosis