Hospital Universitario
de la Princesa
Servicio de Inmunología

-Francisco Sánchez-Madrid
-Hortensia de la Fuente

Centro Nacional de Investigaciones
Cardiovasculares Carlos III (CNIC)

- Group: Pilar Martín

- Raquel Sánchez-Díaz
- Rafael Blanco

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)

UNIVERSIDAD AUTONOMA DE MADRID

INSTITUTO DE INVESTIGACIÓN SANITARIA
HOSPITAL UNIVERSITARIO DE LA PRINCESA
SaludMadrid

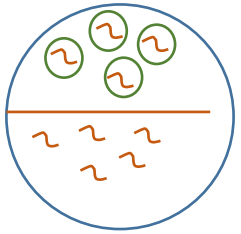
cnic
Centro Nacional de Investigaciones Cardiovasculares Carlos III
Desde la investigación a la salud

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN
cnic

hsa-miRNA-Chr8.96: First Plasma Biomarker for Diagnosis of Myocarditis

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

Exosomes/
free circulating miRNAs



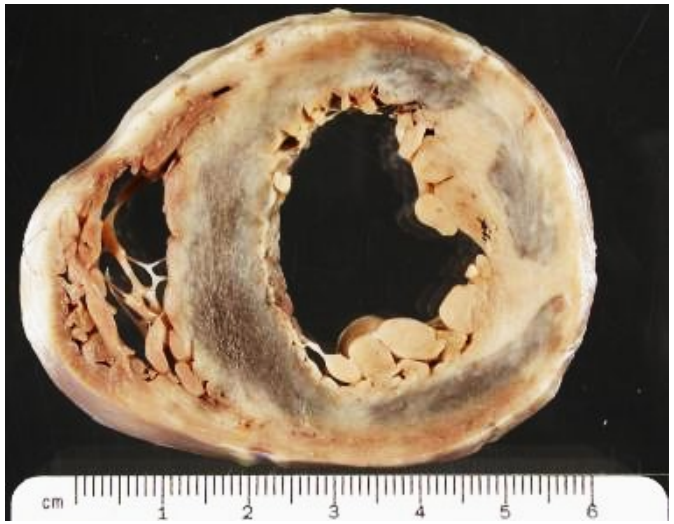
Prognostic Biomarker
- Predict disease outcome
- Monitor disease recurrence

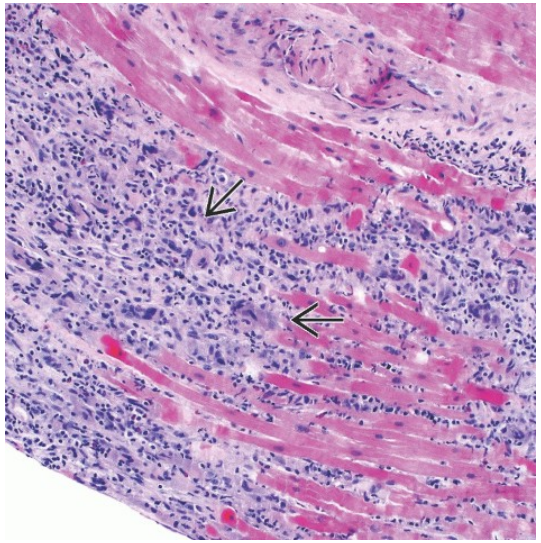
Predictive Biomarker
- Therapy response
- Treatment decisions

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**

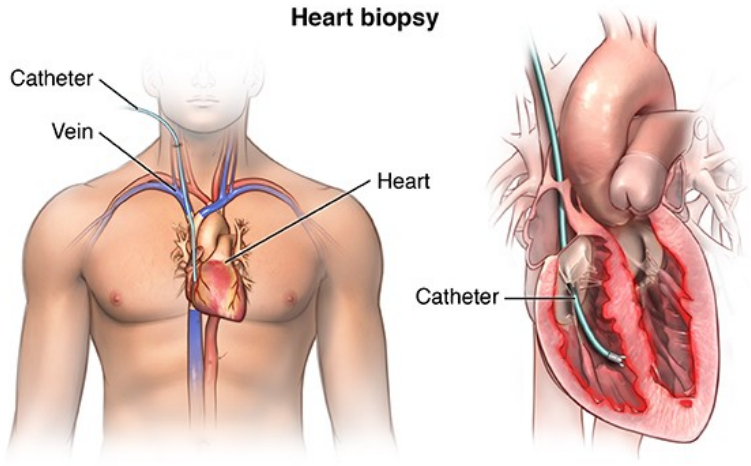


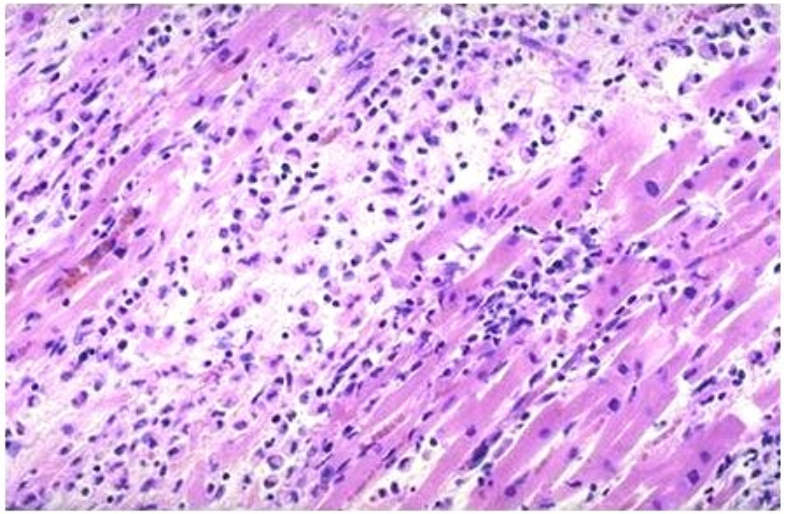


MINOCA: Myocardial **I**nfarction with **N**on-**O**bstructive **C**oronary **A**rteries

The problem of diagnosis

Endomyocardial Biopsy (EMB) remains the gold standard in diagnosis of myocarditis



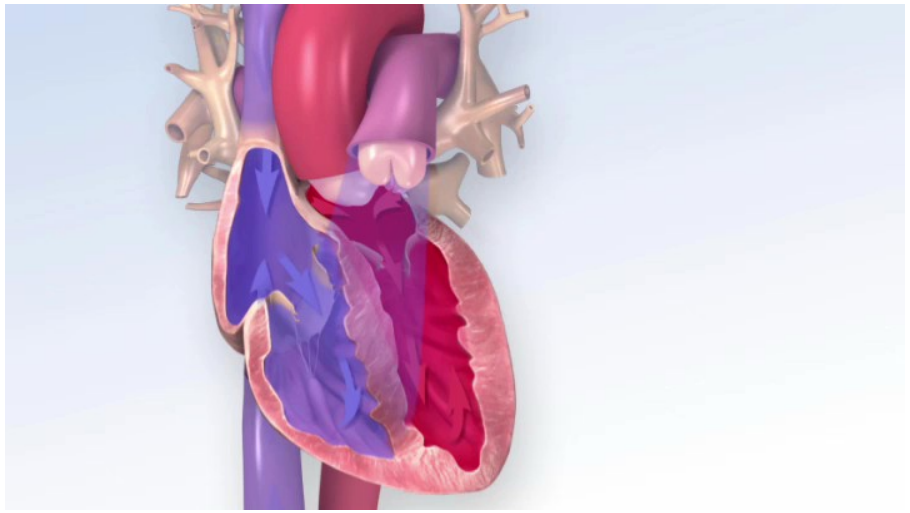


EMB is not commonly performed due to safety reasons:
6% complications
0,4% incidence of death due to perforation

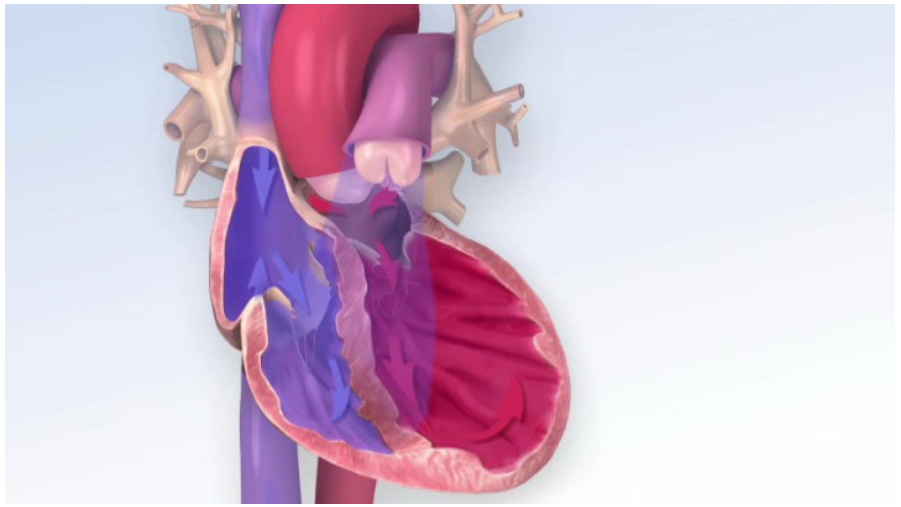
Nowadays there is a lack of early and non-invasive methods to diagnose myocarditis

The numbers…

- 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



Healthy



DCM

The problem of diagnosis…myocarditis mimicks acute myocardial infarction

Angiography is invasive and not available/adequate for everyone (small villages, pregnant women, etc.)

South Med J. 2010 Sep;103(9):936-9. doi: 10.1097/SMJ.0b013e3181eb3443.

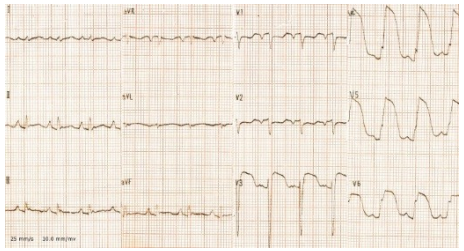
Myocarditis masquerading as a ST elevation myocardial infarction (STEMI).

Shah A[1].

Am J Med. 2012 Dec;125(12):1230-3. doi: 10.1016/j.amjmed.2012.06.016. Epub 2012 Oct 9.

Acute nonrheumatic streptococcal myocarditis: STEMI mimic in young adults.

Upadhyay GA[1], Gainor JF, Stamm LM, Weinberg AN, Dec GW, Ruskin JN.



Pediatr Emerg Care. 2014 Jul;30(7):493-5. doi: 10.1097/PEC.0000000000000170.

Chest pain in two athletic male adolescents mimicking myocardial infarction.

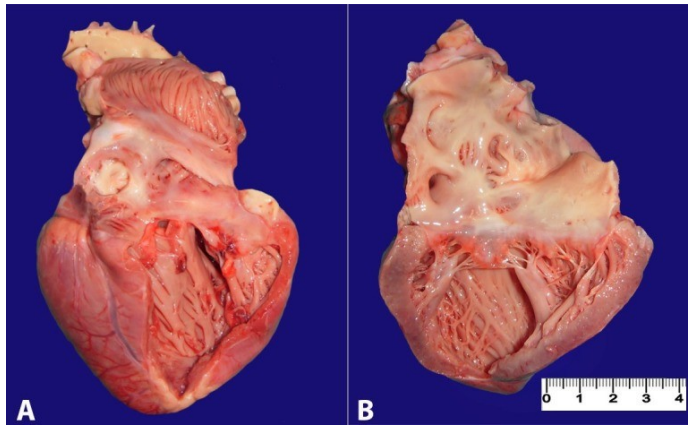
Gupta SK[1], Naheed Z.

Am J Case Rep. 2016 Jan 4;17:1-5.

A Rare Case of Toxic Myocarditis Caused by Bacterial Liver Abscess Mimicking Acute Myocardial Infarction.

Zou Y[1], Lin L[1], Xiao H[1], Xiang D[1].

Troponins and ST-segment elevation

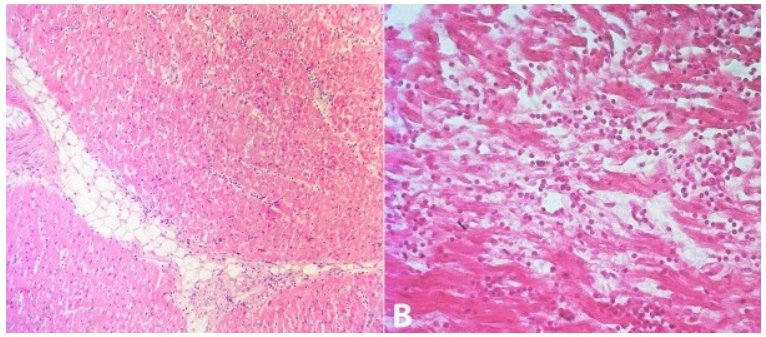


Ventricular dilation

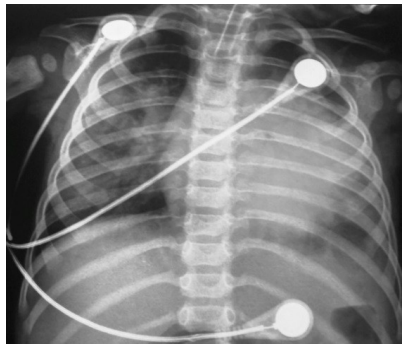
Autops Case Rep. 2016 Dec 30;6(4):15-19. doi: 10.4322/acr.2016.052. eCollection 2016 Oct-Dec.

Infant acute myocarditis mimicking acute myocardial infarction.

Jedidi M[1], Tilouche S[2], Masmoudi T[1], Sahnoun M[1], Chkirbène Y[1], Mestiri S[3], Boughamoura L[2], Ben Dhiab M[1], Souguir MK[1].



Infiltrating cells



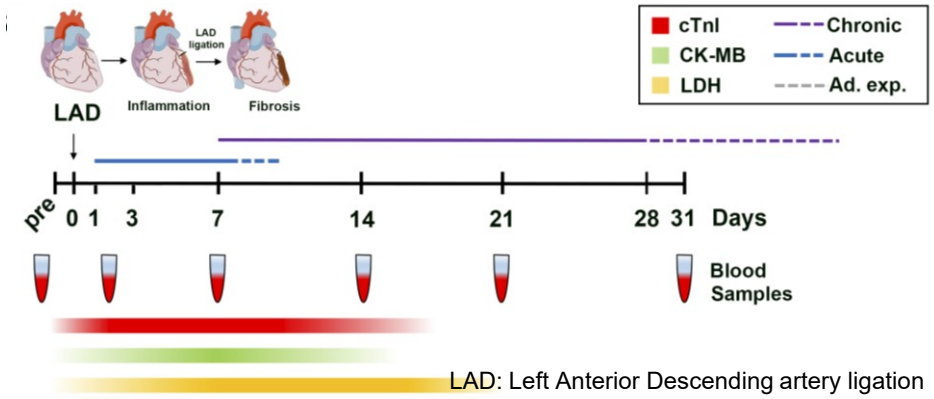
9 months-old girl died due to an underdiagnosed and mistreated fulminant myocarditis

Cardiomegaly and pulmonary congestion

Two mouse models of heart diseases

Different kinetics of Myocardial Infarction and Myocarditis

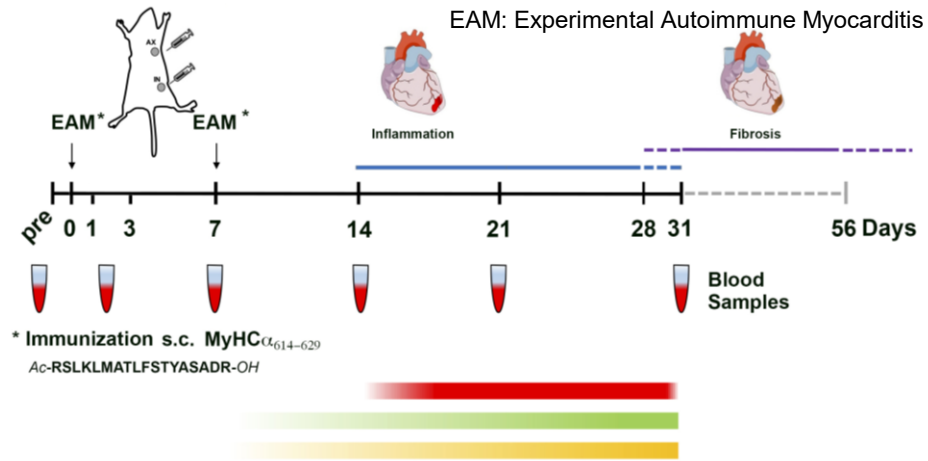
Myocardial Infarction

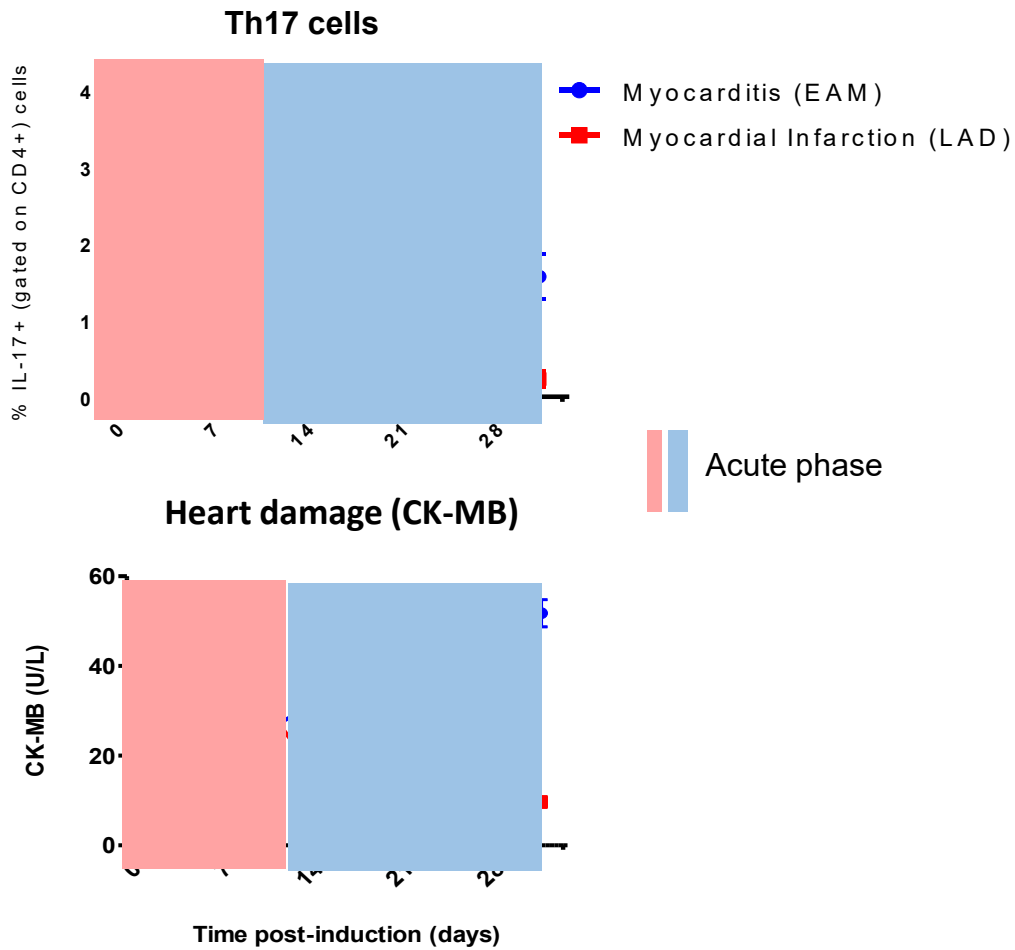


LAD: Left Anterior Descending artery ligation

EAM: Experimental Autoimmune Myocarditis

Myocarditis

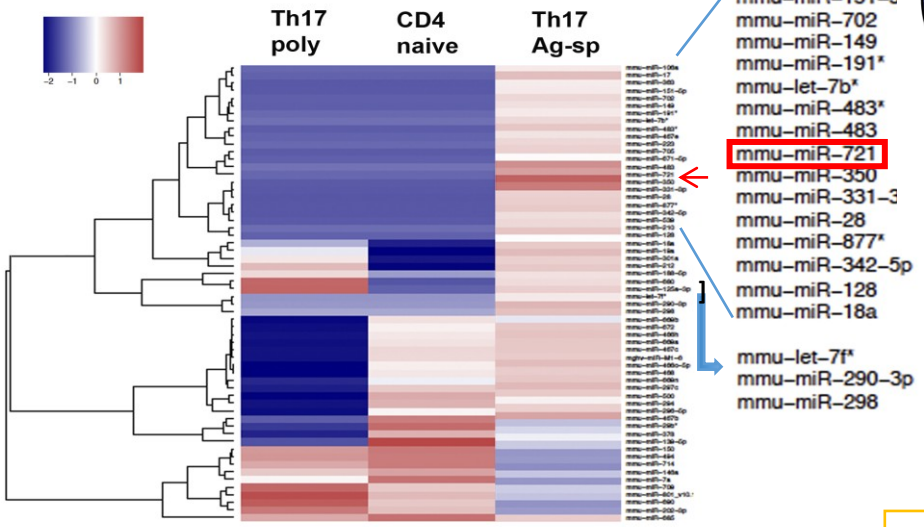


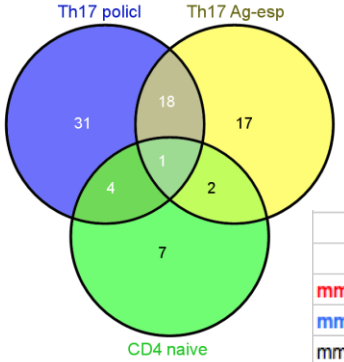


miR-721 is overexpressed in Th17 cells

EAM: Experimental Autoimmune Myocarditis

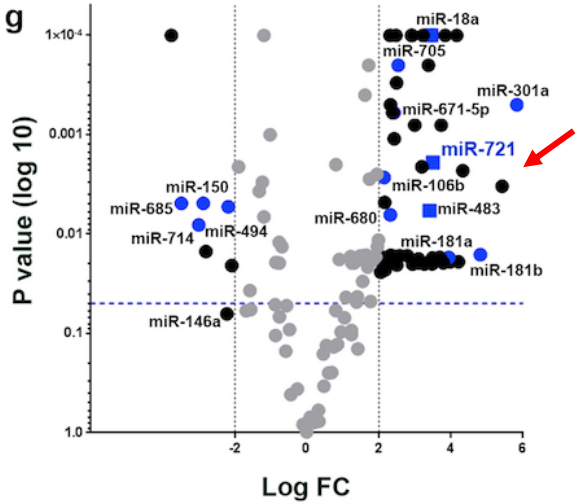
microRNA microarray





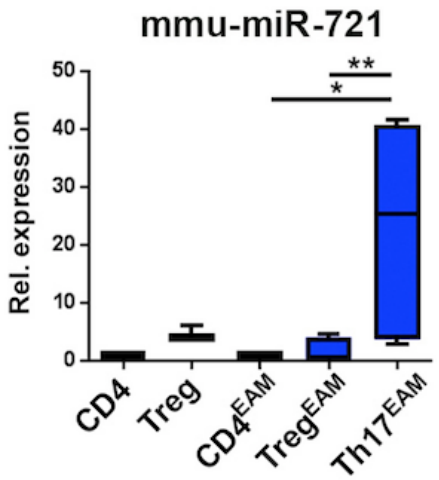
DE of Th17-derived miRNAs from **severe Myo vs mild Myo**

		Fold change > 1.8
	TH17Ag_WT.txt:gTotalGeneSignal (normalized)	Fold change([TH17Ag-KO] vs [TH17Ag-WT])
mmu-miR-721	7,71026	189,67311
mmu-miR-483	7,170248	130,45058
mmu-miR-18a	4,012081	1,9583826
		Fold change < 1.8
mmu-let-7b*	7,515896	1,5813458
mmu-let-7f*	8,906817	1,4830275

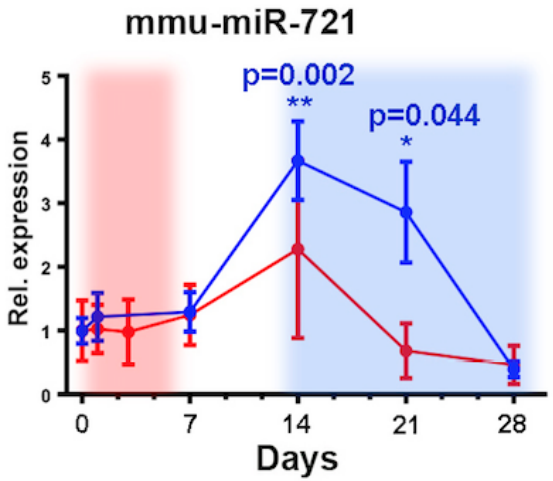


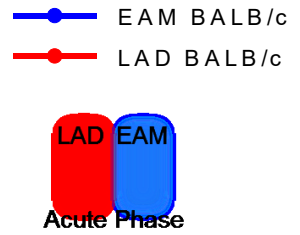
miR-721 is highly expressed in Th17 and in plasma obtained from the acute phase of Myocarditis

Cells



Plasma

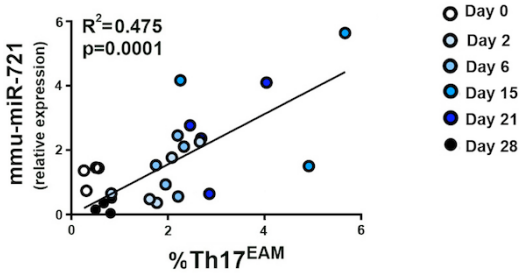




miR-721 is contained into extracellular vesicles

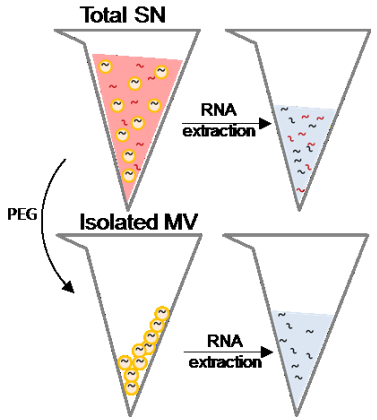
EAM: Experimental Autoimmune Myocarditis

Blood

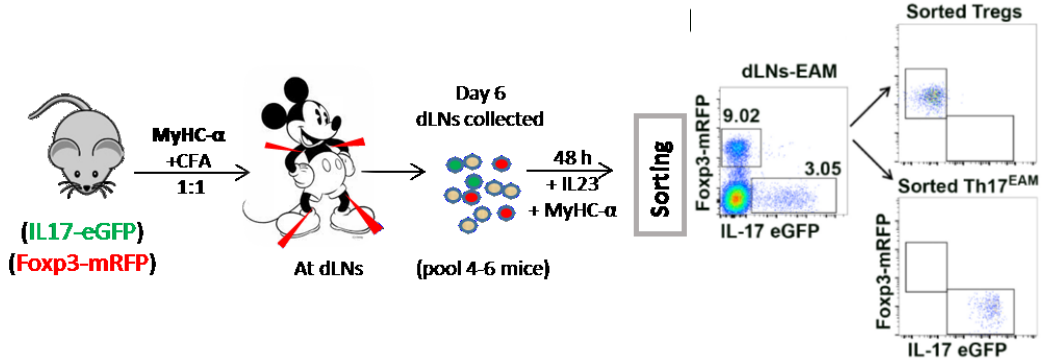


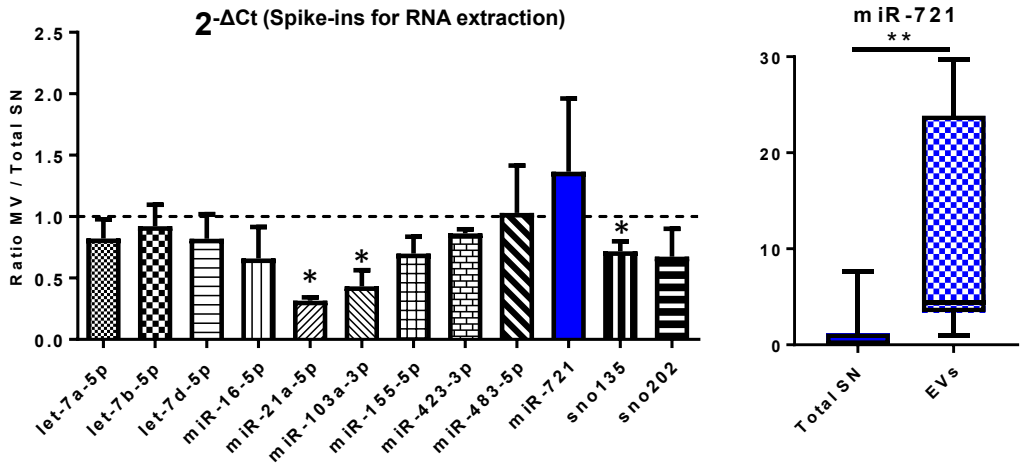
miR-721 expression in plasma correlates with % Th17 in blood

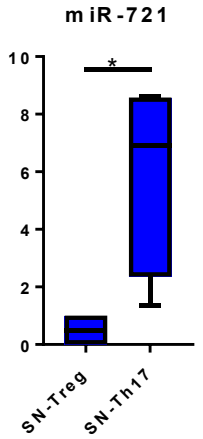
Draining Lymph Nodes (day 6 of EAM) cultured 48h +MyHCα +IL-23 (20M cells/ml)

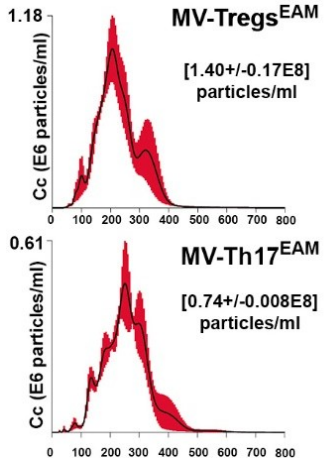


Andreu Z., J Extracell Vesicles 2016









The size of microvesicles

Elucidating the homologous miR-721 in human (*chr8:96*)

Homo sapiens chromosome 8, GRCh38.p7 Primary Assembly

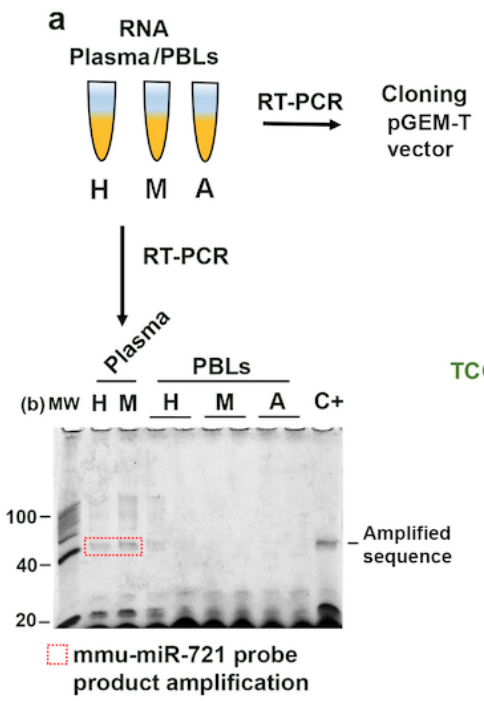
Sequence ID: NC_000008.11 Length: 145138636 Number of Matches: 73

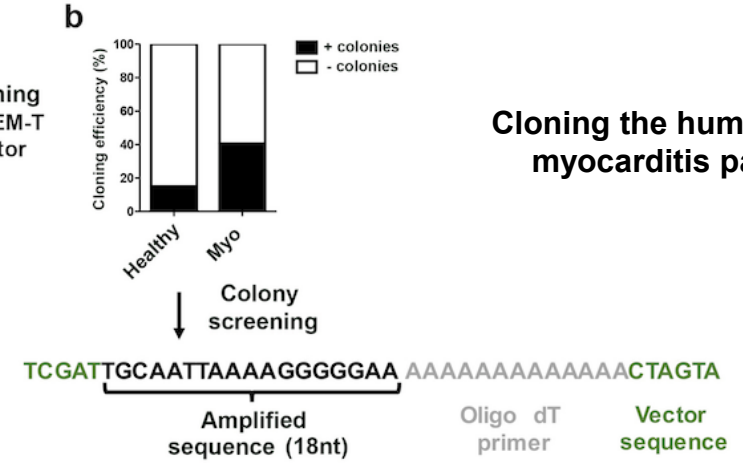
Range 1: 95353471 to 95353488 GenBank Graphics ▼ Next Match ▲ Previous M

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	0.35	18/18(100%)	0/18(0%)	Plus/Minus

Features: 84282 bp at 5' side: protein C8orf37
791075 bp at 3' side: growth/differentiation factor 6 precursor

```
Query  4         TGCAATTAAAAGGGGGAA  21
                 ||||||||||||||||||
Sbjct  95353488  TGCAATTAAAAGGGGGAA  95353471
```





Cloning the human miRNA from myocarditis patient plasma

c

Homo sapiens chromosome 8: 96405821...96405842 (hsa-Chr8:96)

```
96406003 CCTTGGAGGG CGCACAATCA CTCAGCTGAT TTGATTTCGT ATACATGTAC GTAGGGCAGG
96405943 GATTCGTTAG AAAACATGTA TTGAGTCTAA TCATTGTGCA TATCACAGAG CTGTTTTTCT
96405883 GCCATATATT GGGGGTTAGG GAAAATGCAG GGGAACTGCT TTCTTGCAAT TAAAAGGGGG
96405823 AAAAAGTGCT AGGGGCACAT TGCACTACAT CCTAGAGCCT GACCAGAAGA CATCCTCATC
96405763 TTGCTTGTAT CCCTACCCCT CTTGTTCCTC TACTAGCACA AACAAGGCCT CCAAAGCCCA
96405703 CCCAGGACCC TTCCCCTGCA CCAGCACTGC CCTCTAGGAA GGAACTAGGA
```

Mus musculus	Mmu-miR-721	GGAAGACAGUGCAAUUAAAAGGGGGAAAAAAGUACCUGGGAUGUUCUGAGAAUUUCAUUUUUCUUGUUAUUGCCACUCCUGCUUGGAA
Rhesus macaca	Rma-miR-721	AAAUAUCAGUGCAAUUAAAAGGAGGAAAACAAUAAACAGAAUCAGCAAAAUCAAAAGAUCAAGAAAAUUGAUAAACCUCUAGCCAA
Homo sapiens	Hsa-miR-721	CUGCUUUCUUGCAAUUAAAAGGGGGAAAAAGUGCUAGGGGCACAUUGCACUACAUCCUAGAGCCUGACGUUAGAAAACAUGUAUUG

Chr8:96

mature sequence (21nt)

secondary structure (loop nucleotides)

Phase I clinical trial / *PoC*

MULTICENTER STUDY (3 HOSPITALS)

5-10
EDTA
Blood

within 24h from the onset of symptoms

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.

Collaboration with Cardiology Units:

Hospital de la Princesa
Jiménez-Borreguero/ Fernando Alfonso)

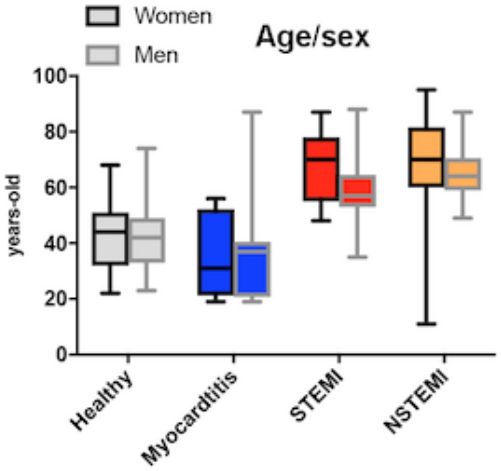
Fundación Jiménez Díaz
(Dr. Borja Ibáñez)

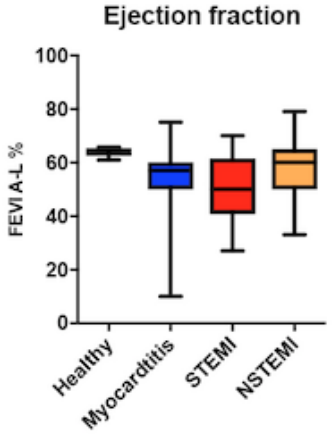
Hospital 12 de Octubre
(Dr. Héctor Bueno)

Phase I clinical trial Baseline characteristic of the Study Population

Parameter	PAD	Control Group	PAD	Acute Myocarditis	PAD	STEMI	PAD	NSTEMI	*P* Value
N		80		37		41		45	
Age, y	80	42.31±1.20	37	37.11±16	41	60.32±13.22	45	66.21±14.43	<0.0001
Sex (women/men)	80	41/39	37	9/28	41	11/30	45	16/29	
TFOS, d	-	-	37	4.162±6.18	41	0.875±1.453	41	1.5±2.076	0.0006
Echocardiography at admission, %	80	100	37	96.29	41	97.56	45	80.00	
LV EF, %	80	63.81±0.55	36	54.72±11.32	40	51.21±12.01	34	57.65±10.55	0.0076
Segmental contraction abnormalities, %	80	0	36	40.50	40	82.15	34	67.64	
ECG alterations. %	80	100	37	65.50	41	100		77.77	
ST segment elevation, %	80	0	37	67.58	41	100	45	0	
Q-wave, %	80	0	37	18.98	41	12.20	45	22.22	
Coronary angiography or CT angiography performed, %		0	37	37.83	41	100	45	100	
Coronary Artery Disease, %		-	37	0	41	97.56	45	88.89	
Laboratory findings			36		41		44		
Peak Troponin I		-	14	8.746±8.206	11	42.02±35.16	18	13.16±20.59	0.0014
Peak Troponin T		-	23	1286±1399	29	4025±3374	26	1227±2340	<0.0001
Peak creatine kinase, U/L		-	20	502.6±318.7	41	1766±1854	40	399±516.7	<0.0001
Peak creatine kinase MB, U/L		-	11	41.36±23.32	9	96.44±57.72	5	60.74±26.67	0.0191
CMR performed, %		11.25		75.68		14.63		2.43	
LGE, %	9	0	28	78.57	6	100	1	100	
Cardiovascular risk factors, %		-	37	48.65	41	87.80	45	88.89	
Dyslipidemia, %		-	37	18.92	41	36.58	45	64.44	
Smoker or former-smoker, %		-	37	26.67	41	53.66	45	44.44	
Arterial hypertension, %		-	37	5.41	41	39.02	45	66.67	
Diabetic, %			37	2.70	41	17.07	45	28.89	
Renal Insufficiency, %			37	0	41	4.88	45	6.67	
Peripheral Artery Disease, %			37	0	41	4.88	45	4.44	

PAD, patients with available data;
TFOS, time from the onset of symptoms;
LV EF, left ventricle ejection fraction;
CMR, cardiovascular magnetic resonance;
LGE, late gadolinium enhancement.

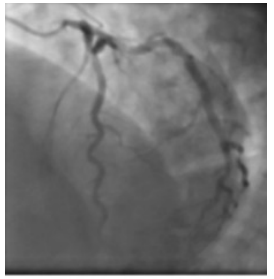


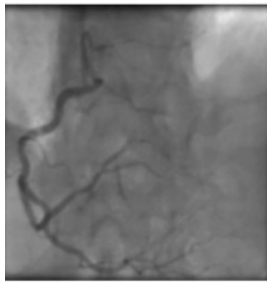


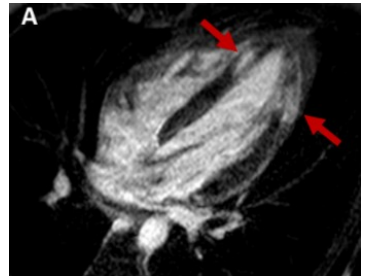
A MINOCA case report with late diagnosis of myocarditis

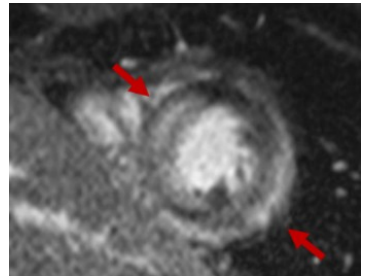
Arteriogram

CMR







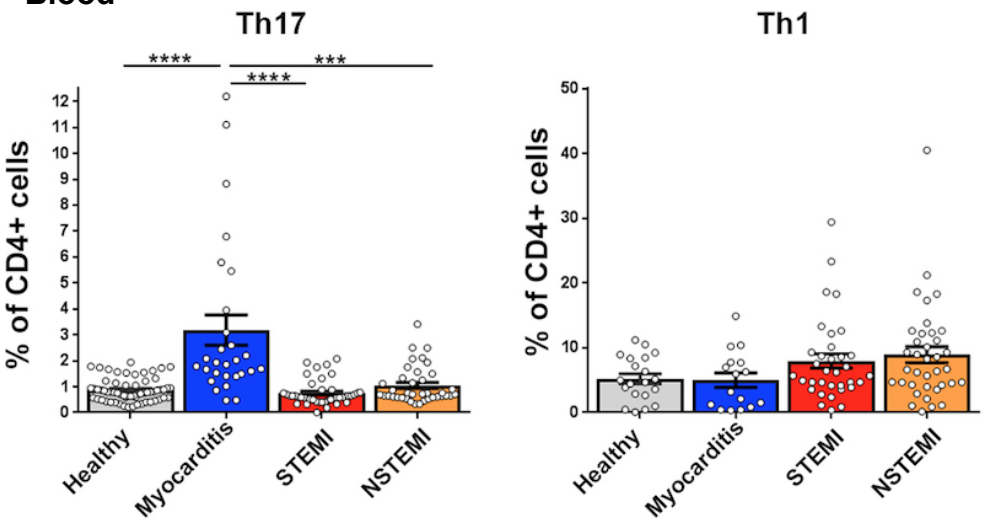


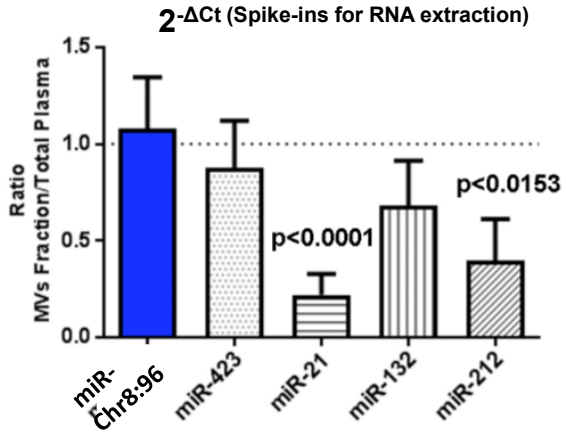
No occlusion of coronary arteries

Gadolinium enhancement (inflammatory edema)

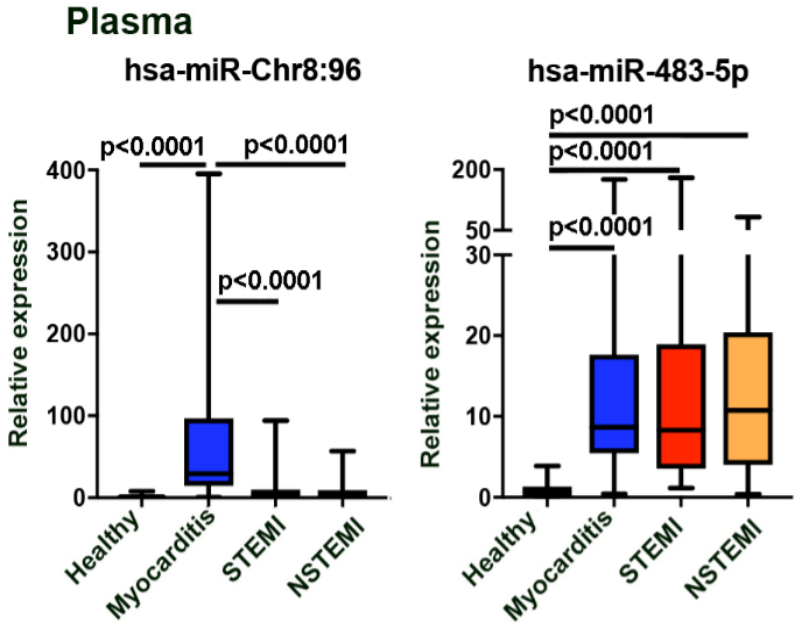
hsa-miR-chr8:96 is enriched in EV from human plasma of myocarditis patients

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



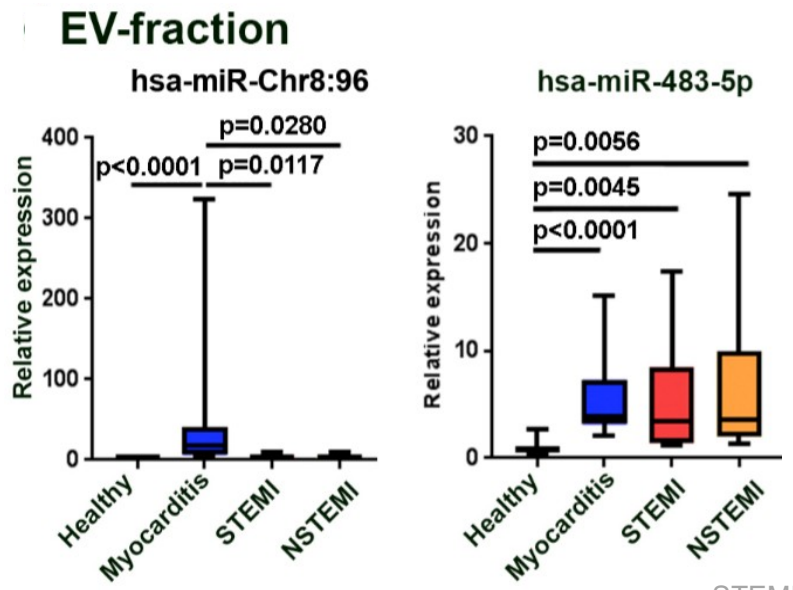


Hsa-miR-Chr8:96 is preferentially secreted into EVs



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

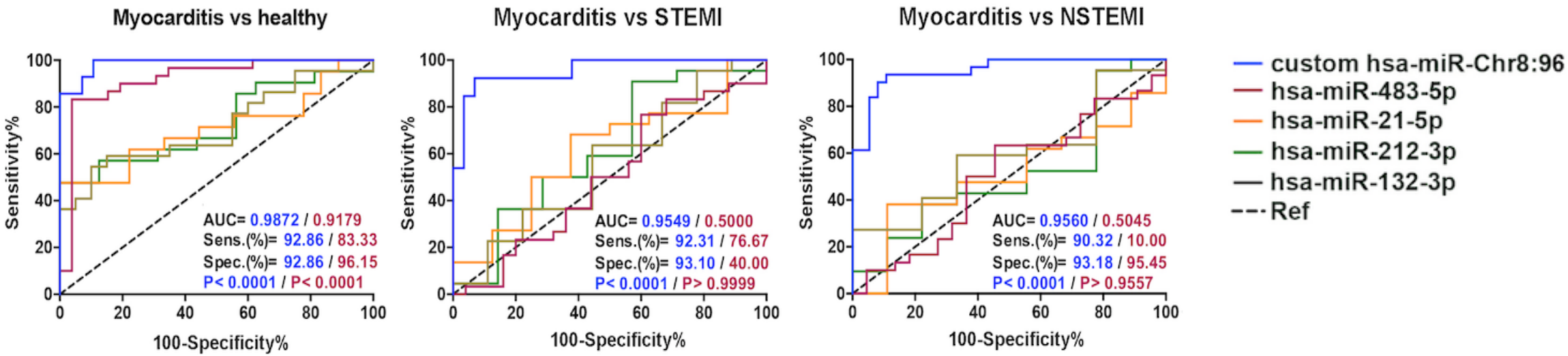
Hsa-miR-483-5p is a general biomarker for myocardial damage



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 sentitivity (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

NON-INVASIVE, ACCURATE AND FAST METHOD OF DIAGNOSIS: Plasma Hsa-miR-chr8:96/miR-721

Achy feeling in the chest

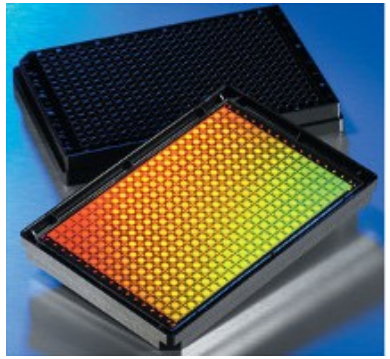
miRNA 721

Acute myocardial infarction

Acute Myocarditis

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

European patents and business plan

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

2 European patents

December 21st, 2015

Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	300177863
Application number	EP15382596.3
File No. to be used for priority declarations	EP15382596
Date of receipt	01 December 2015
Your reference	901 190 sp
Applicant	Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)
Country	ES
Title	Process to obtain useful data for the diagnosis of cardiomy-opathies.

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** EP15382596
- **Priority date** 01/12/2015
- **Aplicants** CNIC
- **Inventors** Pilar Martín Fernández; Raquel Sánchez Díaz; Adela Matesanz Marín; Jesús Jiménez Borreguero; Francisco Sánchez Madrid

May 31st, 2017

Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	300235810
Application number	EP17382324.6
File No. to be used for priority declarations	EP17382324
Date of receipt	31 May 2017
Your reference	902 239
Applicant	Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III
Country	ES
Title	Electrochemical biosensors for diagnosing acute myocarditis

Electrochemical biosensors for diagnosing acute myocarditis

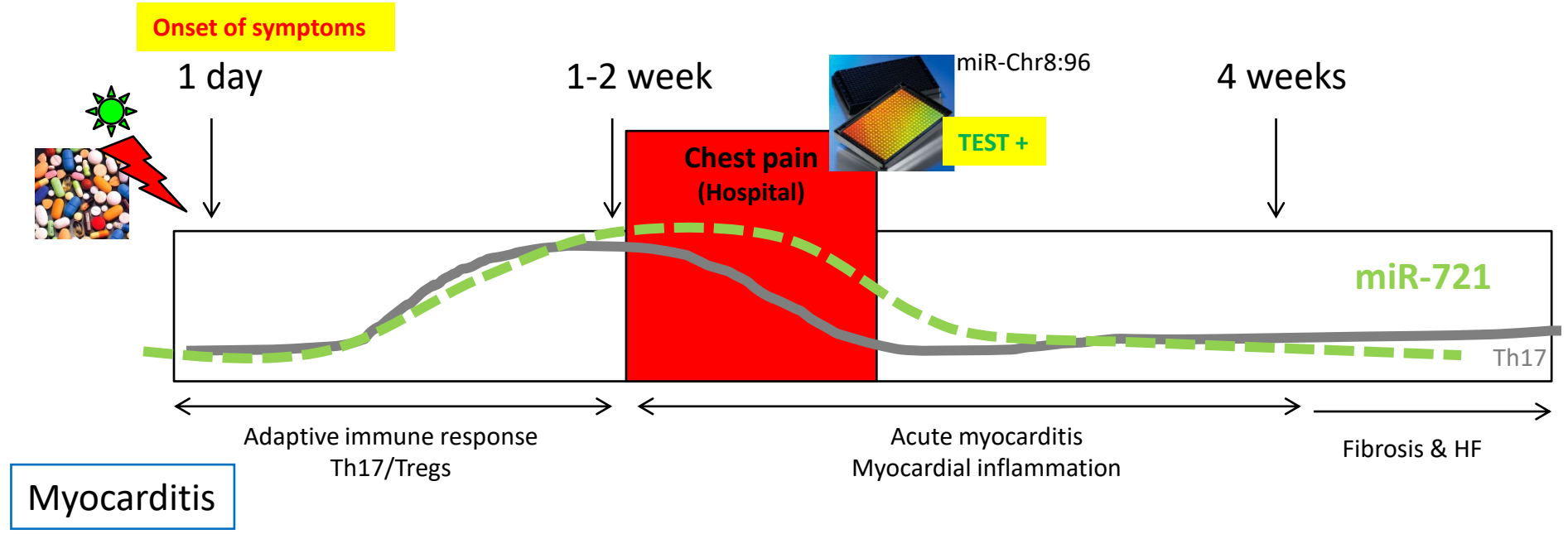
Priority Date: 31/05/2017

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 a electrochemical sensor

Diagnostic approach (a *Cardiovascular Liquid Biopsy*)



Myocarditis

AMI