12 de noviembre de 2020

MyoBiomark: a novel circulating microRNA for the detection of acute myocarditis

Dr. Pilar Martín

Content

1. The Institution: **Centro Nacional de Investigaciones Cardiovasculares (CNIC)**
2. The Product: **Novel miRNA for the diagnosis of myocarditis**

   a) Target Indications
   b) Innovative mechanisms of action
   c) Differential features facing the market
   d) Current status of development
   e) IPR protection
   f) Pitfalls & Risks to be considered

3. Partnering Opportunities

1. The Institution: Centro Nacional de Investigaciones Cardiovasculares (CNIC)

The Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) is a **leading international research center** dedicated to understanding the basis of cardiovascular health and disease and to translating this knowledge into improved patient care.

The CNIC belongs to the Instituto de Salud Carlos III campus in Madrid. The CNIC adopted its current form after a “relaunch” in 2006, made possible through a partnership between the Spanish government and the Pro CNIC Foundation, a panel of 14 leading Spanish companies and charitable foundations.

Regulatory Molecules of Inflammatory Processes Group

Line of research at the CNIC: T cells in autoimmune and cardiovascular diseases.

. The group is focused on the study of the adaptive immune system and micro-RNAs in the pathophysiology of autoimmunity and cardiovascular diseases such as myocarditis, myocardial infarction or atherosclerosis and in the development of circulating biomarkers for these diseases, both in mice and in patients.

. Contributions to the field (last 5 years): NEJM 2020, Cell & Mol Immunol 2020, Thrombosis Research 2020, Circulation 2019, Mol Cell Biol 2017, J Am Soc Nephrol. 2016, Nature Immunol. 2016, ELife 2016, Sci Transl Med 2016, J Autoimmunity 2015, Mol Cell Biol 2015.

2. The Product: Myobiomark is a novel human microRNA for the diagnosis of acute myocarditis

Myocarditis

THE PROBLEM

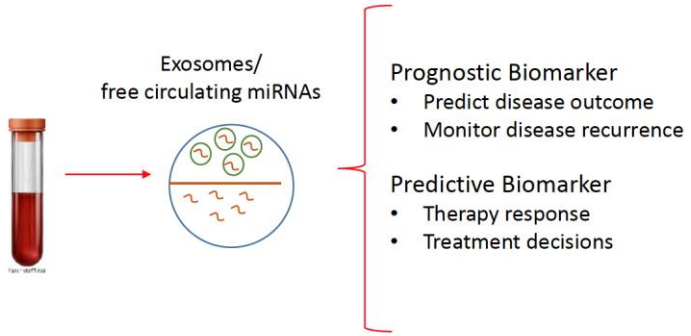
. Prevalence of myocarditis remains underestimated due to difficulties in diagnosing the disease

. 20% of myocarditis develop dilated cardiomyopathy (DCM) with permanent heart failure

. Biopsy-proven myocarditis associates with mortality of 19.2% in 4.7 years

THE HYPOTHESIS

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



THE SOLUTION

The novel miRNA: hsa-miR-Chr8:96 patented by CNIC as the first Biomarker in plasma

specific for the diagnosis of myocarditis patients

New Engl J Med, 202

A clinical need for non-invasive biomarkers for myocarditis

Inflammatory cardiomyopathy

- Acute and chronic inflammation of cardiac tissue.
- Poor outcome of patients if untreated.
- Etiology can be viral, toxins or autoimmune diseases.
- Pro-inflammatory and inflammatory mediators.

Diagnostic procedures

- Important biomarkers such as cardiac troponin, N-terminal pro-B-type natriuretic peptide, interleukins, caspases, macrophages and viral antigens.
- Echocardiography, MRI and positron emission tomography.
- Endomyocardial biopsy, histology and immunohistochemistry.
- Genetic analysis for mutations and polymorphisms.

Personalized treatment possibilities

- Diagnosis based on several parameters such as endomyocardial biopsy, imaging, biomarker and genetic analysis.
- Individualized treatment based on etiology and symptoms.
- Minimal side effects and avoids trial and error methods.
- Future models for personalized drug testing via patient derived cells.
- Next strategy for inflammatory cardiomyopathy definition and classification.

Conclusion

- Proper diagnostic tools needed for personalized medicine for inflammatory cardiomyopathy patients.
- Multiple approach needed – both invasive and noninvasive techniques.
- Establishment of comprehensive and individualized patient treatment.

Neupane B. et al., *Personalized Medicine*, 2018

The problem of diagnosis

Clinically suspected myocarditis with pseudo-infarct presentation: the role of endomyocardial biopsy

Alida L. P. Caforio[1], Giacomo Malipiero[2], Renzo Marcolongo[2], Sabino Iliceto[1]

Submitted Sep 20, 2016. Accepted for publication Sep 20, 2016.
doi: 10.21037/itd.2017.03.103
J Thorac Dis 2017;9(3):423-427

Observational Study

Medicine

OPEN

Clinical characteristics and outcomes of patients with myocarditis mimicking ST-segment elevation myocardial infarction

Analysis of a case series

Shuang Wu, MD, Yan-Min Yang, PhD*, Jun Zhu, MD, Huai-Bin Wan, MD, Juan Wang, PhD, Han Zhang, PhD, Xing-Hui Shao, PhD

Fulminant lymphocytic myocarditis mimicking ST-elevation myocardial infarction FREE

Marco Amoruso ✉, Stefano Muzzarelli, Tiziano Moccetti, Giovanni Pedrazzini

European Heart Journal, Volume 36, Issue 33, 1 September 2015, Pages 2227,

JACC: Cardiovascular Imaging

Volume 3, Issue 8, August 2010
DOI: 10.1016/j.jcmg.2010.05.012

Streptococcal Pharyngitis-Associated Myocarditis Mimicking Acute STEMI

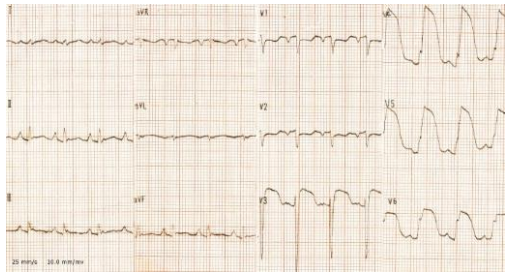
Rasoul Mokabberi, Jamshid Shirani, Afsaneh Haftbaradaran M., B. Dennis Go and William Schiavone

Autopsy Case Reports

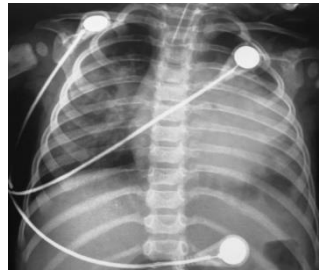
Article / Autopsy Case Report

Infant acute myocarditis mimicking acute myocardial infarction

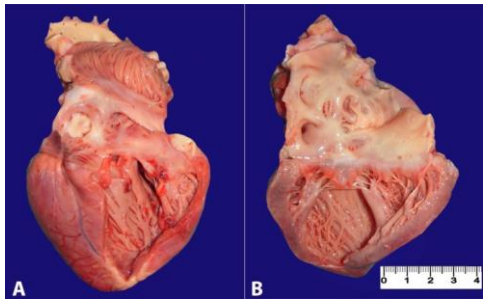
Maher Jedidi[a], Samia Tilouche[b], Tasnim Masmoudi[a], Maha Sahnoun[a], Youssef Chkirbène[a], Sarra Mestiri[c], Lamia Boughamoura[b], Mohamed Ben Dhiab[a], Mohamed Kamel Souguir[a]



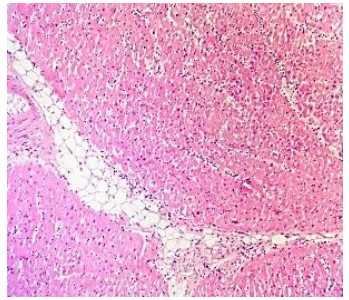
Troponins and ST-segment elevation



Cardiomegaly and pulmonary congestion



Ventricular dilation



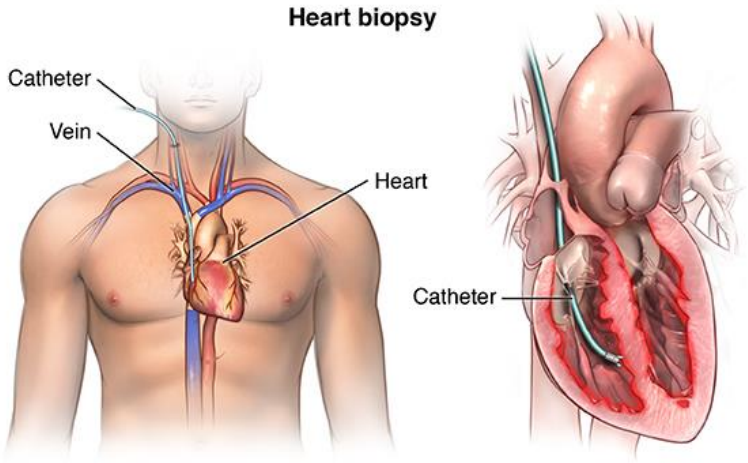
Infiltrating

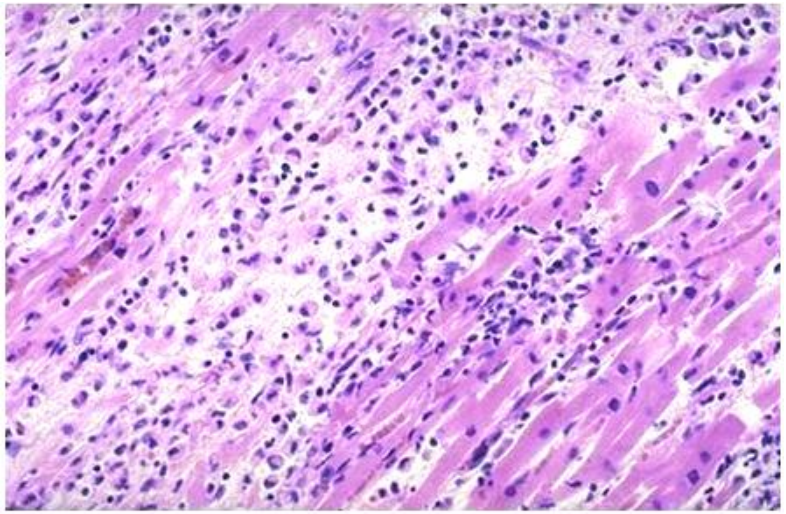
9 months-old girl died due to an underdiagnosed a
mistreated fulminant myocarditis

- Myocarditis mimics acute myocardial infarction in its clinical presentation
- Troponins are not specific for Myocarditis
- Angiography is invasive and needs EMB or CMR for a final recommendation
- CMR not available/adequate for everyone (small villages, pregnant women, etc.)

The problem of diagnosis

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

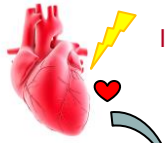




EMB is not commonly performed due to safety reasons:
6% complications
0,4% incidence of death due to perforation

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

Myocarditis



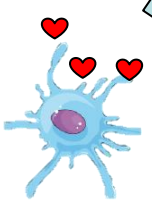
Injury/infection
autoimmunity

Heart-Ag
release

A Th17-mediated disease

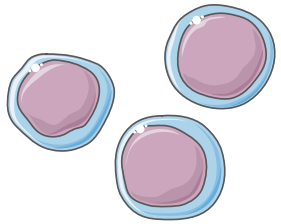
Inflammation → Heart Failure

Myocarditis — Chronic DCM



Dendritic
Cells

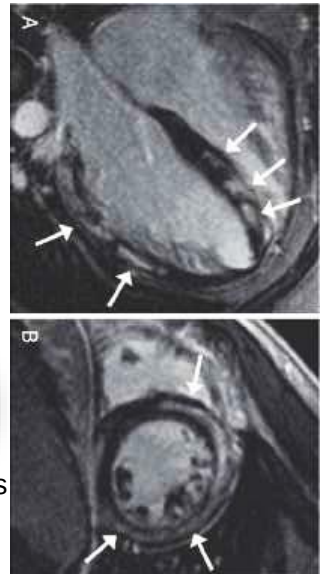
Expansion



Heart-autoantigen
specific CD4+ T cells
(recognize heart antigens)

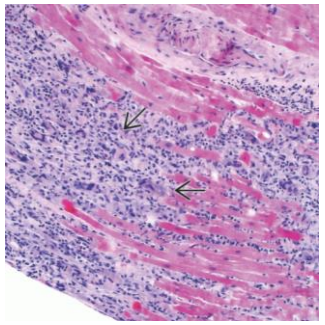
IL-17 production (Th17)
(1-2 weeks)

Immune cell
recruitment
to myocardium
(2-3 weeks)



Monocytes

Neutrophils



Normal

Dilated Cardiomyopathy

Clinical management

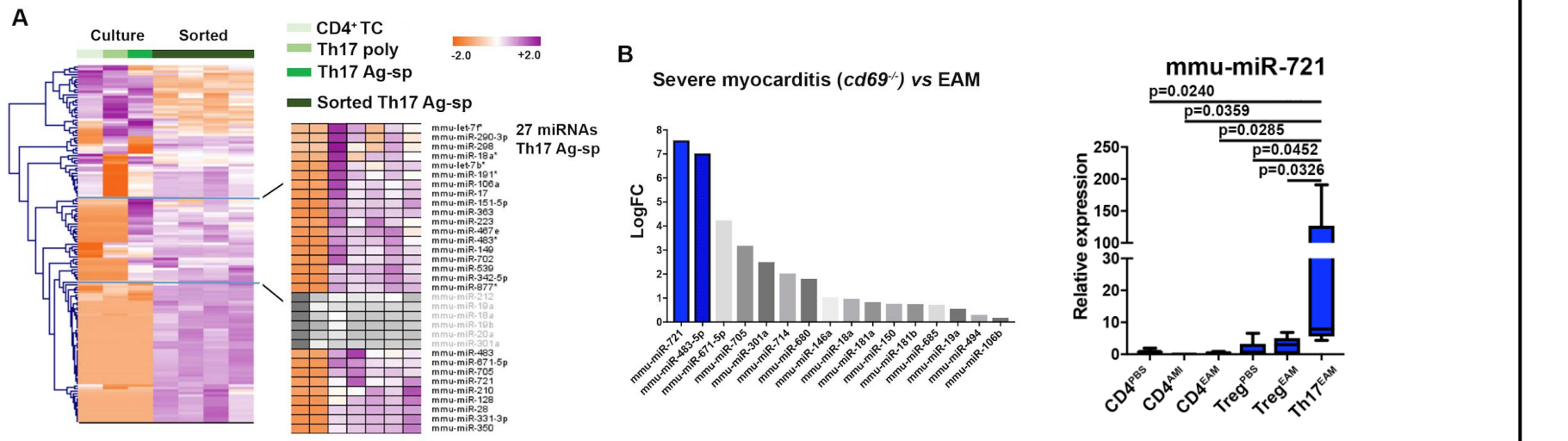
- Immunosuppressors
- Drugs to improve heart function
- Mechanical assistance
- Cardiac transplantation

…lack of specific treatment
…lack of specific diagnostic test

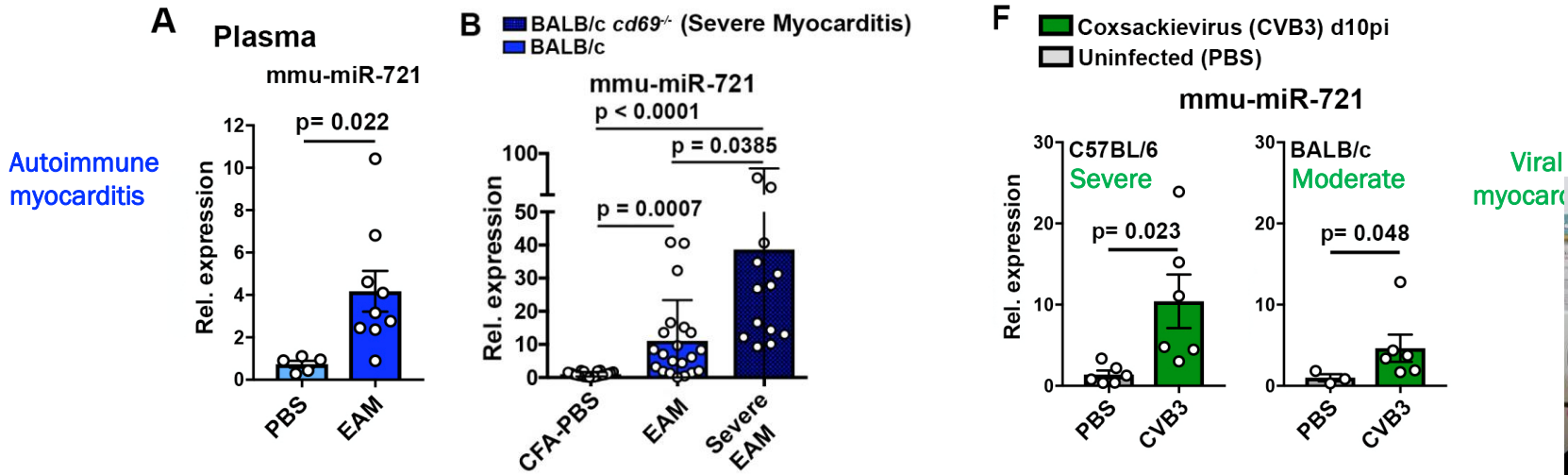
Caforio AL. *European Heart Journal* 2013

miRNA profiling of EAM-Th17 cells

miR-721 is synthetized by Ag-specific Th17 cells from mice with myocarditis

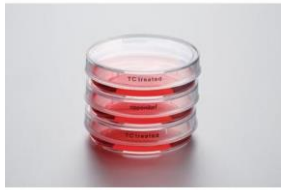


miR-721 is abundant in plasma from mice with autoimmune or viral myocarditis and its expression increases in severe forms of disease

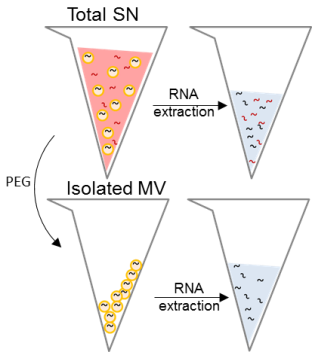


miR-721 is secreted into plasma extracellular vesicles by Th17 cells

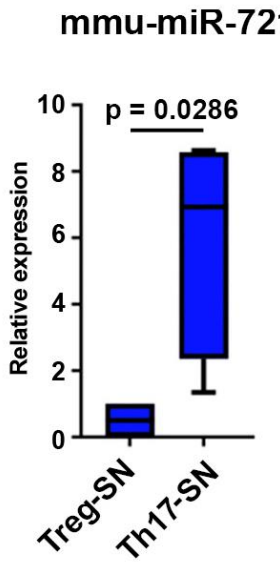
Draining-Lymph Nodes from EAM



Culture of isolated Th17 from EAM d-LNs

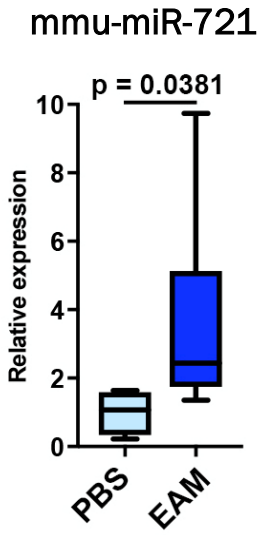


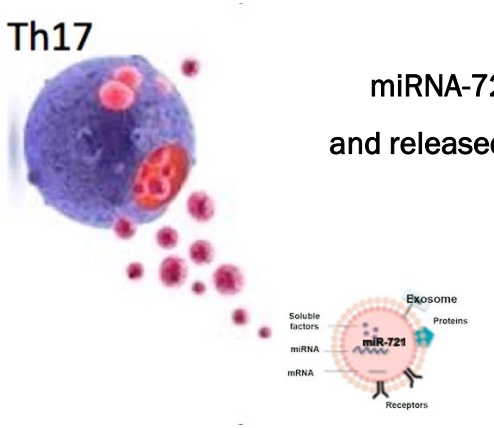
Andreu Z., J Extracell Vesicles 2016



Mmu-miRNA-721 is selectively secreted in extracellular vesicles (EV) from supernatants (SN) of Th17 cells

Plasma from EAM

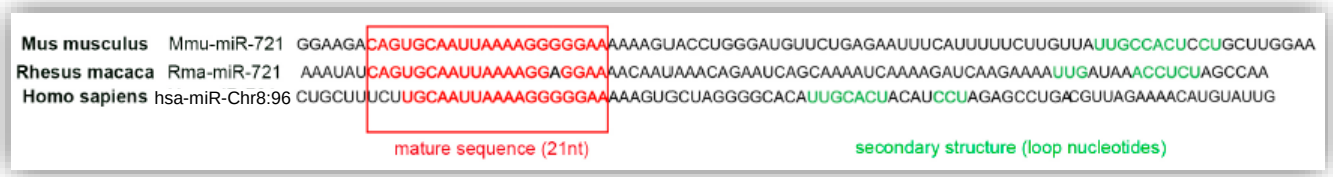




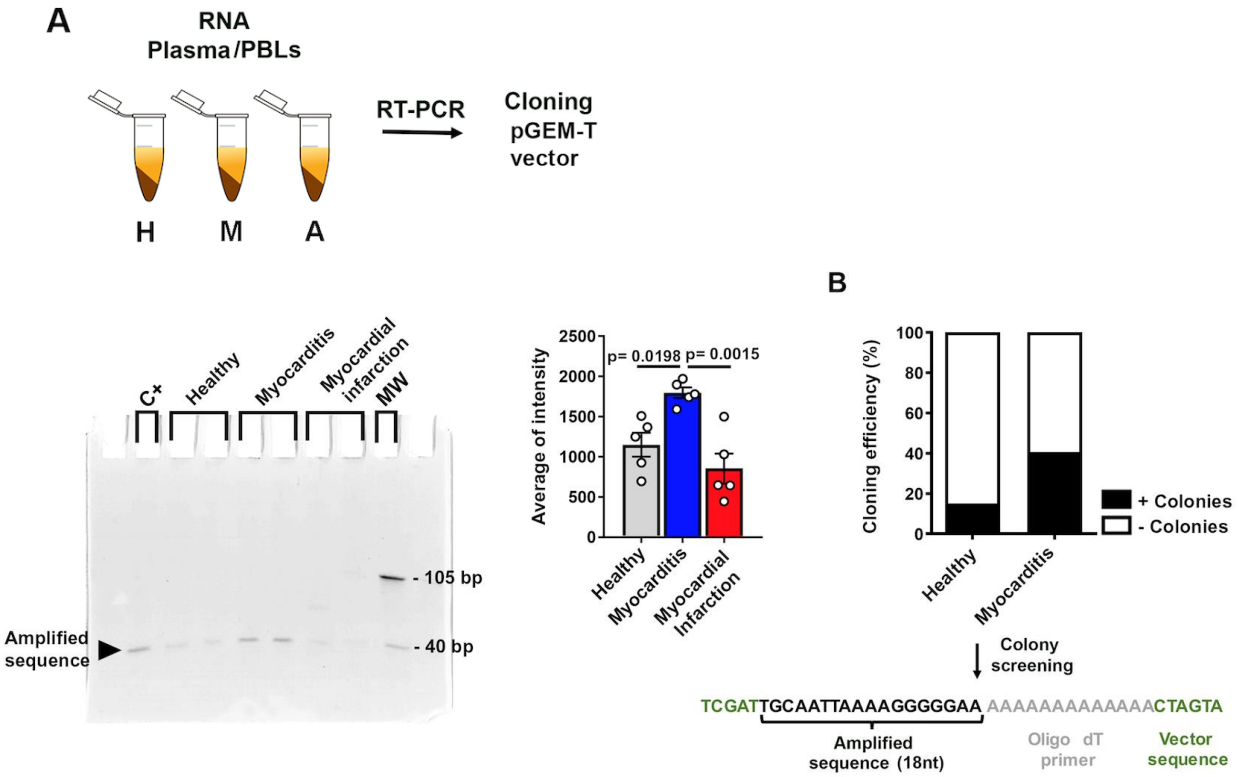
miRNA-721 is encapsulated in EVs and released by Th17 cells to the plasma

Identification, cloning and validation of the mmu-miR-721 human homolog: hsa-miR-Chr8:96

Conserved sequences in the genome:



Identification



Cloning of the human homologue

A national multicenter study

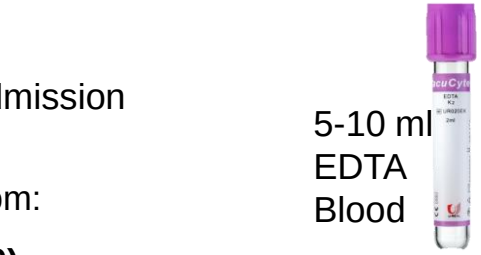
cnic Centro Nacional de Investigaciones Cardiovasculares Carlos III
Desde la investigación a la salud

Recruitment of patients in
5 hospitals from Spain

Hospital Universitario de La Princesa
Hospital Universitario Fundación Jiménez Díaz
HOSPITAL UNIVERSITARIO hm hospitales monteprincipe
SERVICIO DE SALUD DEL PRINCIPADO DE ASTURIAS | Hospital Universitario Central de Asturias
Hospital Universitario 12 de Octubre

within 24h from hospital admission

5-10 ml EDTA Blood



Peripheral blood samples from:

- ✓ **Healthy donors (80)**
- ✓ Patients with **Acute Myocarditis (151)**
- ✓ Patients with **Acute Myocardial Infarction (150)**
- ✓ MINOCA **(20)**

Recopilation of possible **common clinical parameters:**

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

To study:

➢**Biomarker in peripheral blood:** plasma and circulating cells

Validation in three independent cohorts:

- Massachusetts General Hospital, Boston, USA
- Center for Molecular Cardiology, Zürich, Switzerland
- Hospital of Padua , Italy. (Biopsy-proven cohort)

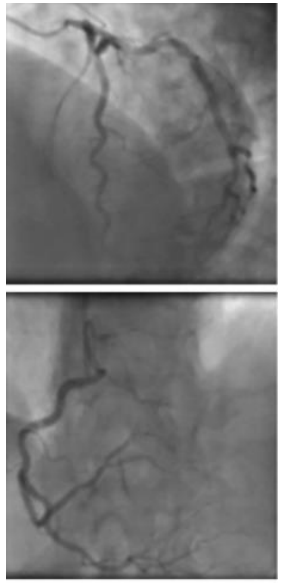
A multicenter study

Parameter	AD	Control Group	AD	Acute Myocarditis	AD	STEMI	AD	NSTEMI	*P* Value
N		80		41		41		45	
Age, y	80	42.31±1.2	41	37.00±16.07	41	60.32±13.22	45	66.21±14.43	<0.0001
Sex (women/men)	80	41/39	41	10/31	41	11/30	45	16/29	
TFOS, d	-	-	39	6.028±0.9652	40	0.875±1.453	38	1.500±2.076	0.0006
CRF, %		-	39	48.65	41	87.80	45	88.89	
Dyslipidemia, %		-	39	17.94	41	36.58	45	64.44	
Smoker or former-smoker, %		-	39	28.20	41	53.66	45	44.44	
AH, %		-	39	5.13	41	39.02	45	66.67	
Diabetic, %		-	39	2.56	41	17.07	45	28.89	
Renal Insufficiency, %		-	39	0	41	4.88	45	6.67	
PAD, %		-	39	0	41	4.88	45	4.44	
Laboratory findings			38		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		-	25	1283±1373	29	4025±3374	26	1227±2340	<0.0001
Peak CK, U/L		-	21	532.2±339.0	41	1766±1854	40	399±516.7	<0.0001
Peak CK-MB, U/L		-	12	48.33±32.82	9	96.44±57.72	5	60.74±26.67	0.0514
ECG alterations. %	80	100	39	66.67	41	100		77.77	
ST segment elevation, %	80	0	39	69.23	41	100	45	0	
Q-wave, %	80	0	39	17.94	41	12.20	45	22.22	
CT or coronary angiography performed, %		0	39	43.58	41	100	45	100	
CAD, %		-	17	0	41	97.56	45	88.89	
Echocardiography at admission, %	80	100	41	97.56	41	97.56	45	80.00	
LV EF, %	80	63.81±0.55	40	54.50±11.12	40	51.21±12.01	34	57.65±10.55	0.0070
Segmental contraction abnormalities, %	80	0	38	42.10	40	82.15	34	67.64	
CMR performed, %		11.25		68.29		14.63		2.43	
LGE, %	9	0	28	78.57	6	100	1	100	

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.

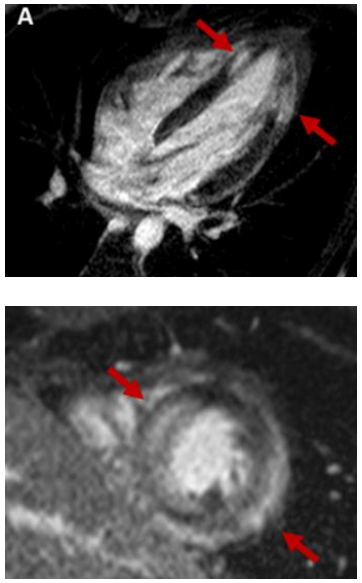
A MINOCA case with late diagnosis of myocarditis

Arteriogram



No occlusion of coronary arteries

CMR

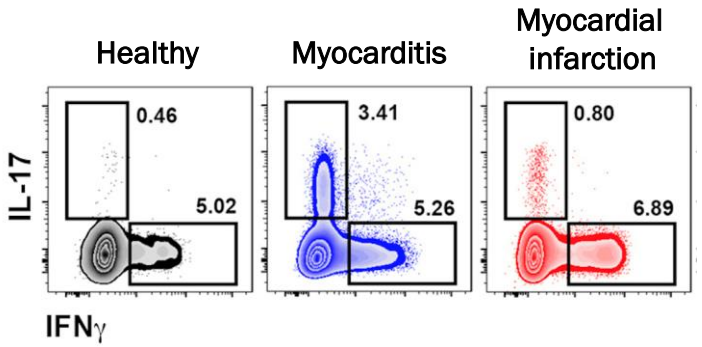


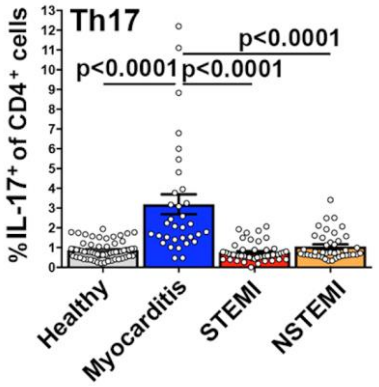
Gadolinium enhancement (inflammatory edema)

Plasma hsa-miR-Chr8:96 expression is a specific biomarker for acute myocarditis patients

Blood
EDTA tube

PBMCs





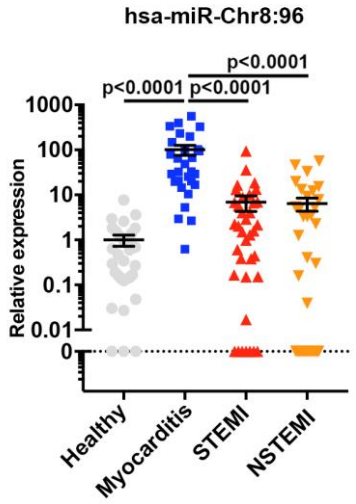
Th17 cell responses in blood are exclusive of Myocarditis

STEMI: ST-elevation Myocardial Infarction
NSTEMI: non-ST-elevation Myocardial Infarction

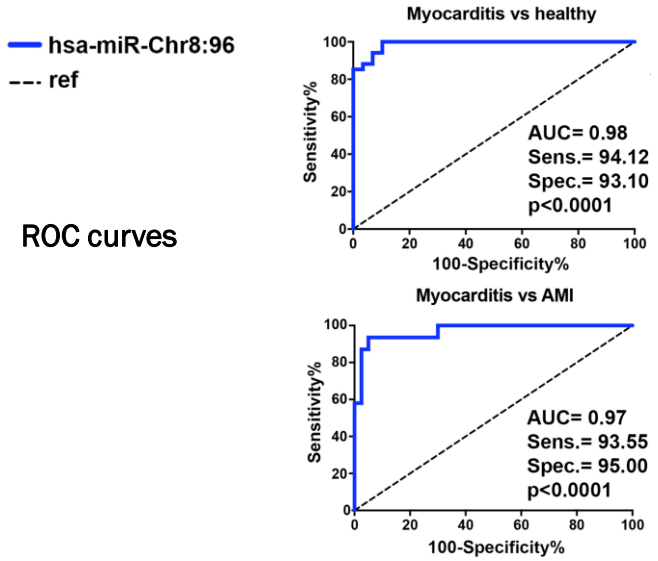
Blood
EDTA tube

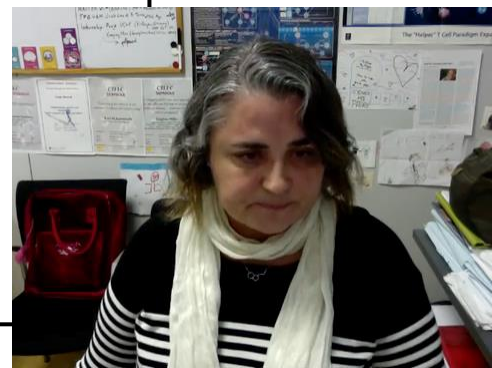
Plasma

qPCR
custom probe



ROC curves





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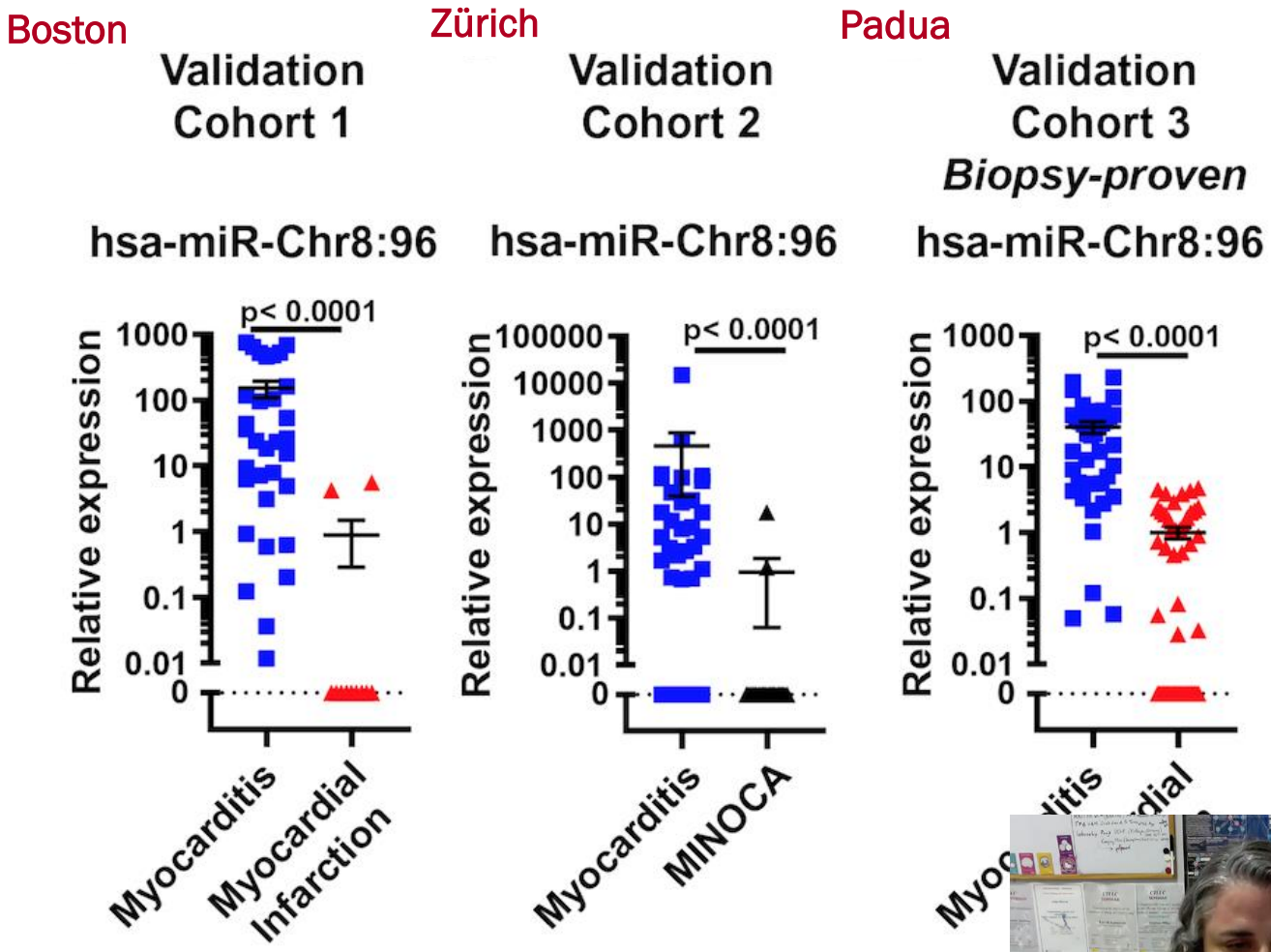
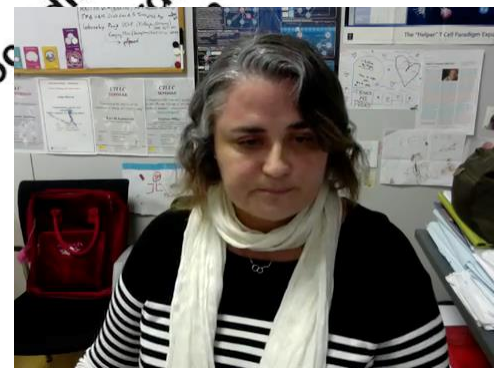
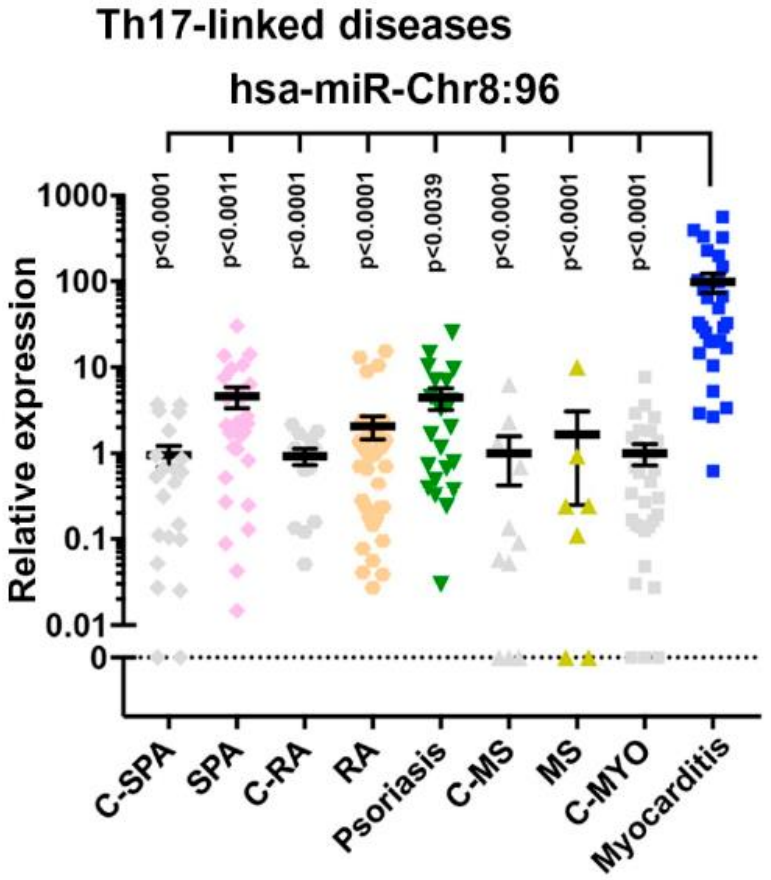


AMI:
acute myocardial infarction

MINOCA:
AMI with non-obstructive coronary arteries



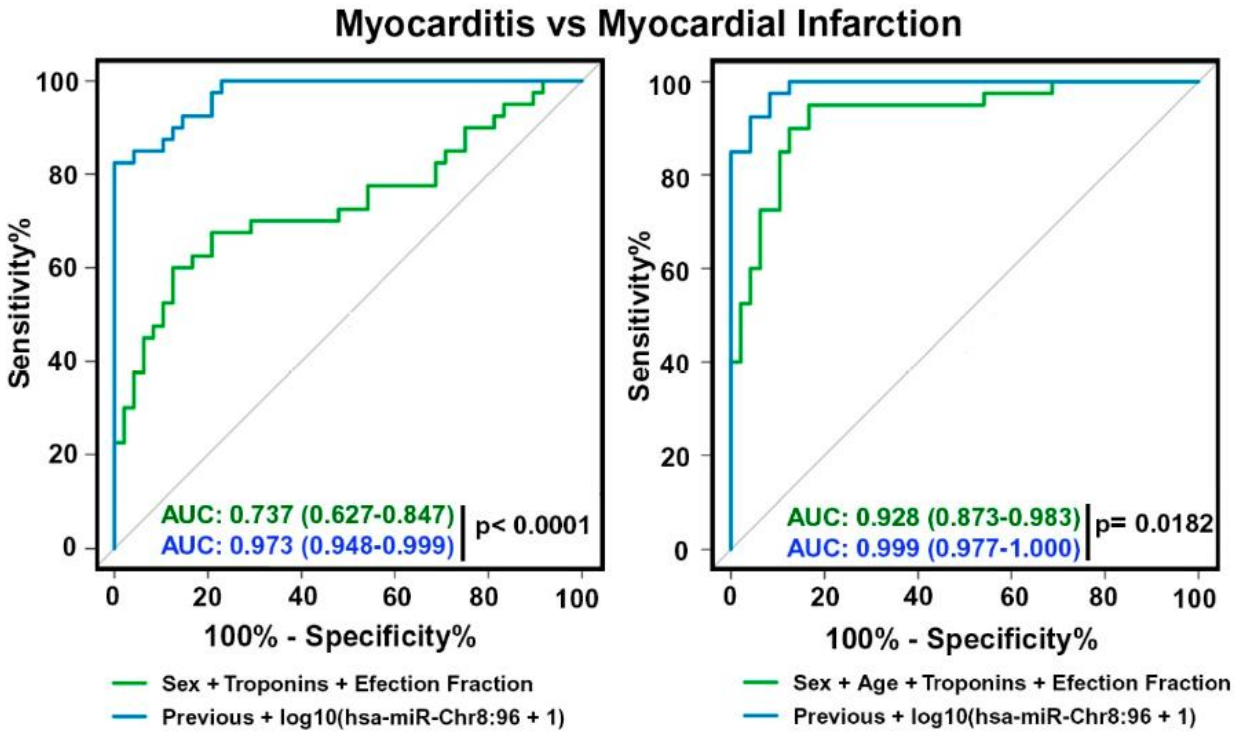
The novel miRNA is specifically expressed in plasma from patients with myocarditis, compared to myocardial infarction and with patients diagnosed of other Th17-related diseases: rheumatoid-arthritis (RA), spondylo-arthritis (SPA), psoriasis and multiple sclerosis (MS).



Multiparametric analysis

	Odds Ratio (95% CI)	P-value
(Intercept)	130.967 (0.732, 46741.671)	0.080
Sex (women)	0.858 (0.189, 3.869)	0.840
Age (years)	0.902 (0.86, 0.937)	<0.001
Troponins (Normalized)	0.998 (0.994, 1.001)	0.282
Ejection Fraction %	1.004 (0.933, 1.083)	0.907

	Odds Ratio (95% CI)	P-value
(Intercept)	1.417 (0.003, 643.699)	0.909
Sex (women)	1.562 (0.268, 10.2)	0.624
Age (years)	0.901 (0.848, 0.943)	<0.001
Troponins (Normalized)	0.998 (0.993, 1.002)	0.404
Ejection Fraction %	1.031 (0.943, 1.13)	0.505
Log10 (hsa-miR-Chr8:96 + 1)	16.659 (4.716, 92.949)	<0.001



. The diagnostic performance of this novel microRNA yielded a robust area under the curve of 0.927 (95% CI, 0.879-0.975; p<0.0001) for discrimina myocarditis from myocardial infarction pat

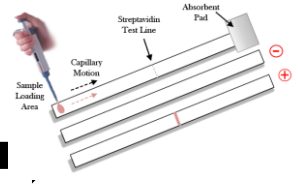
. The microRNA retained its diagnostic va adjusted by age, gender, ejection fraction troponins.

Blanco-Domínguez R, et al. Martín, P

PROSPECTIVE REGISTRY FOR THE VALIDATION OF A NEW DIAGNOSTIC MARKER IN PATIENTS WITH A SUSPECTED CLINIC OF MYOCARDITIS (MIOCARDITIS-CNIC) CEI PI 23_2020

1. Validation and prototype development

1. Preparation of nanoparticle-based miRNA sensors (IMDEA-Nanociencia).
2. Validate prototype results with qPCR



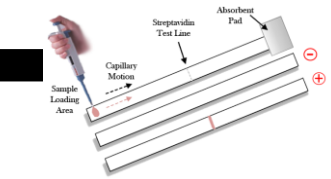
Prototype

2. Clinical Registry

Coordinator: CNIC
(Clinical Trial Coordination Unit)

HOSPITAL VIRGEN DE LA ARRIXACA, MURCIA
HOSPITAL CLINICO SAN CARLOS, MADRID
HOSPITAL CLINICO DE SALAMANCA
HOSPITAL GENERAL DE VALENCIA
HOSPITAL UNIVERISTARIO DE LA PRINCESA
HOSPITAL MONTEPRÍNCIPE
HOSPITAL DE VIGO
HOSPITAL DE VALLADOLID

1. Inclusion criteria: Patients who consult and present a clinical suspicion of acute myocarditis, based on the presence of compatible clinical findings.
2. Registration of procedures (T
   analysis and regulatory dossi
3. 5ml sample serum/plasma (in
   months)



Prototype

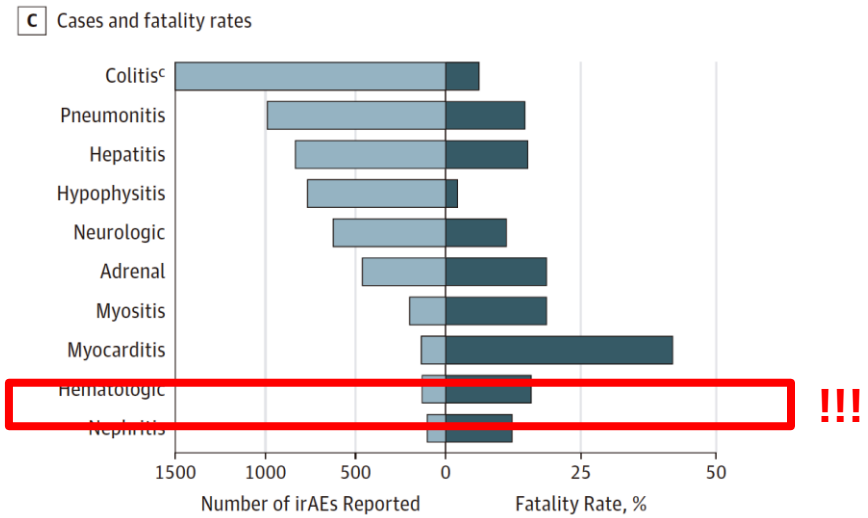
3. Technology transfer

1. Marketing and commercialization activities
2. Patent, Publication (open access), dissemination

"Method for diagnosing cardiomyopathies"
European patent (EP3384043B1) granted on January 23rd 2020.
USA application (US15/780,888) pending.

Colección de mu
C.0000800 en el
Biobancos

Secondary application under development: Early Diagnosis of Immune checkpoint (ICI) -myocarditis

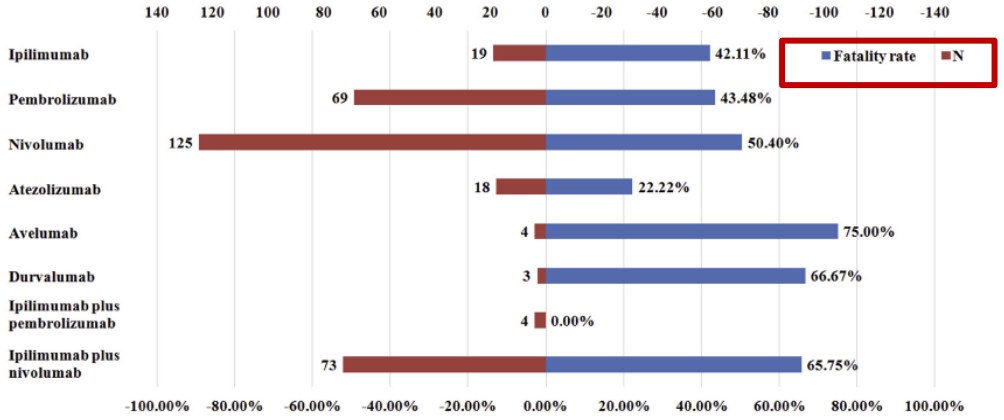


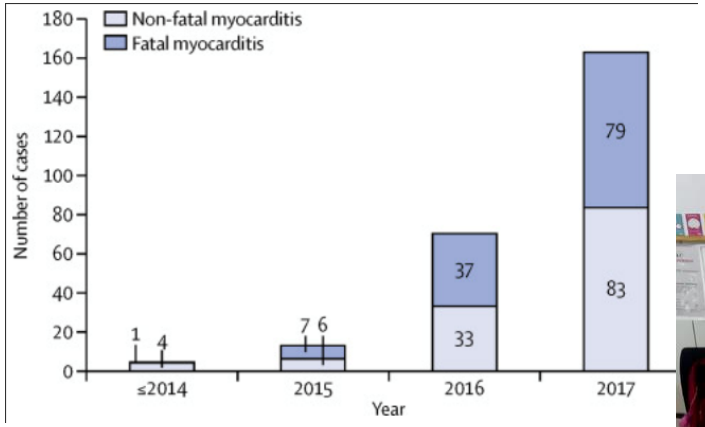
Prevalence of ICI-myocarditis 1.14%

ICI-Myocarditis have the highest fatality rate
50.4% monotherapy
65.% combination

84% of ICI-myocarditis were classified as severe

Number of reports and fatality rates for ICI-myocarditis





JAMA Oncology 2018, doi:10.1001/jamaoncol.2018.3923

The Lancet 2018, 392:382-38

3. Partnering Opportunities

European Patent application EP15382596.3 entitled “Method for diagnosing cardiomyopathies” on December 12th 2015, granted on January 23rd 2020.

USA application (US15/780,888) pending

The CNIC is the only applicant in the patent family.

The CNIC is looking for an industrial partner interested in licensing the patent family and completing the necessary steps for the diagnostic kit to reach the market.

If the company needs it, the CNIC is open to collaborate through a Research and Development Contract.

thank you for your attention