# *RNA as prognostic biomarkers in patients with acute coronary syndrome*

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## *Introduction and Rational*

Ischemic heart disease is the most frequent cause of death in the world and is the disease. heart disease, which has the highest incidence and prevalence in industrialised countries [1, 2]. It includes several. clinical pictures caused by anatomical and/or functional pathologies of coronary arterial vessels capable of. determine the development of myocardial ischemia. Within ischemic heart disease is possible. distinguish chronic and acute coronary syndromes. Acute coronary syndromes (ACS). include two main categories, distinguished on the basis of the electrocardiographic picture:. - Acute coronary syndromes without persistent overshooting of the ST tract (NSTE. coronary syndromes - not ST elevation), which include myocardial infarction without above the ST tract vellement (NSTEMI) and unstable angina (AU); pathologically the syndromes. Coronary NSTE are characterized by the subocclusion of a vessel, with necrosis or ischemia of the. successor layers in case of NSTEMI or UA respectively.. - Acute coronary syndromes with persistent ST elevation (coronary syndromes. STE - ST elevation); physiopathology, are caused by a complete occlusion of a vessel which, in the absence of reperfusion, causes a full-thickness necrosis. In patients with NSTE coronary syndromes, therapy is guided by stratification of risk and is aimed primarily at facilitating the resolution of the thrombus and avoiding its evolution towards a thrombus occlusive, in addition to the control of the ischemic-anginal picture. It is therefore necessary to set promptly a drug therapy that includes an antiaggregant therapy (single or in association), an anticoagulant therapy and drugs that reduce myocardial oxygen demand (e.g. beta-blockers). Patients should then be subjected, with different timing depending on the estimated ischemic risk, coronarography and possible myocardial revascularization. All patients with STEMI should receive, unless contraindications, therapy Binding antifungal with acetylsalicylic acid and a P2Y12 inhibitor (Prasugrel or Ticagrelor). The addition of an anticoagulant (non-fractionated heparin, enoxaparina, fondaparinux, bivalirudin).The primary therapeutic goal in patients with STEMI, however, is the re-channeling of the vessel occluded as early as possible. This can be achieved by two types of treatment, one pharmacological (fibrinolysis) and one mechanical (primary angioplasty). Primary angioplasty (with stent implant) is the treatment of choice for reperfusion coronary because it is much more effective than thrombolysis in obtaining the reunalization of the vessel occluded.

Despite thrombolytic therapy, angioplasty (PCI) and progress in recent decades have significantly improved the prognosis of patients after a heart attack with or withoutincrease in the ST tract, the overall mortality rate is 8% to one month and 12% a year after discharge [3]. It has also been estimated that, following a myocardial infarction, about 40% of STEMI patients and 30% of NSTEMI patients develop a remodelling of the left ventricle (VS) resulting in contractile dysfunction within 12 months [4, 5].

Previous studies Randomized clinicians identified a left ventricle ejection fraction (FEVS) < 45 as cut-off to define a left ventricular systolic dysfunction [6]. The development of a Left ventricular dysfunction is one of the largest independent predictors of mortality after heart attack myocardial [3, 7]. In contrast to patients with acute myocardial infarction, patients with AU, have a preserved FEVS (>60%), an excellent prognosis and a low rate of evolution towards ventricular dysfunction at one year ( 5-7%) [8]. The AU, characterized by ischemia in the absence of cardiomyocytic necrosis, with the advent of high sensitivity tests for the dosage of troponins thistle accounts for 10% of all ACS [8].

Many circulating proteins, such as Creatine Kinase (CK-MB), cardiac troponin measured with high sensitivity test (hs-cTn), type B Natriuretic Peptide (NT-proBNP), the binding protein fatty acids (H-FABP), lactate dehydrogenase (LDH) have been studied for their potential as biomarkers and used (hs-ctn and NT-proBNP) in the diagnosis and prognosis of dysfunction ventricular [7]. LV pathological remodeling is characterized at the molecular level by a alteration of the expression of different transcripts be they encoding (mRNA) or non-coding (ncRNA) [9, 10]. In particular, circulating RNA, present both in the plasma and within the cells blood, is highly stable and easily measurable even if present at very low concentrations.

In addition, the potential of circulating RNA for use as biomarkers has been known for some time cardiovascular [11-13].

## *Objective of the project*

We propose to carry out an observational, prospective study that involves the evaluation of predictive circulating biomarkers of ventricular dysfunction (FE <45%) in patients with ACS.In particular our aim will be to check if there are transcripts circulating in the blood peripheral, coding or not for proteins (mRNAs or ncRNAs), differently modulated in patientswith ACS (ACS_signature) submitted to PCI, distinguished on the basis of the evolution towards dysfunction ventricular. Our study will be based on transcriptomic analysis of the entire genome.

## *Objectives*

Primary objective:

Detect circulating transcripts encoding proteins (mRNAs) or not (ncRNAs) (ACS_signature) predictive ventricular dysfunction in patients with ACS undergoing PCI.

Secondary objective:

Evaluate the association of the ACS_signature with possible adverse events at 12 months and its prognostic ability in the prediction of adverse events additional to the use of standard clinical parameters. Adverse events are defined: death from cardiovascular causes, re infarction, non-fatal stroke, new coronary revascularization, arrhythmias, development of heart failure and new hospitalizations for cardiovascular causes.

## *Study population*

378 patients with a first episode of ACS (STEMI, NSTEMI and UA) will be recruited to PCI.

Criteria for Inclusion:

1. Age >18 years

2. ACS patients (first episode), defined according to ESC 2017 guidelines [14]

3. Indication for treatment of percutaneous revascularization

4. Informed consent to enrol in the study

Exclusion Criteria

1. Severe valvulopathy or other conditions requiring cardiac surgery

2. Previous cardiac surgeries including coronary bypass

3. Total chronic occlusion

4. Patients with known hypersensitivity or contraindication to one of the following drugs:

- heparin

- aspirin,

- clopidogrel,

- ticlopidine,

- sirolimus,

- everolimus.

5. Any contraindication to medicated stent implantation (DES)

6. Patients with previous documented myocardial infarction;

7. FEVS <30% before PCI

8. Patients in cardiogenic shock

9. Patients with evolved STEMI (> 48 h from onset of symptoms/Q-waves to ECG) or undergoing to fibrinolysis

10. Patients with known previous cardiomyopathy with FEVS < 40%

11. Patients with malignant neoplasm or systemic disease with a "quoad vitam" prognosis less than 1 year

12. Patients with known active infectious diseases;

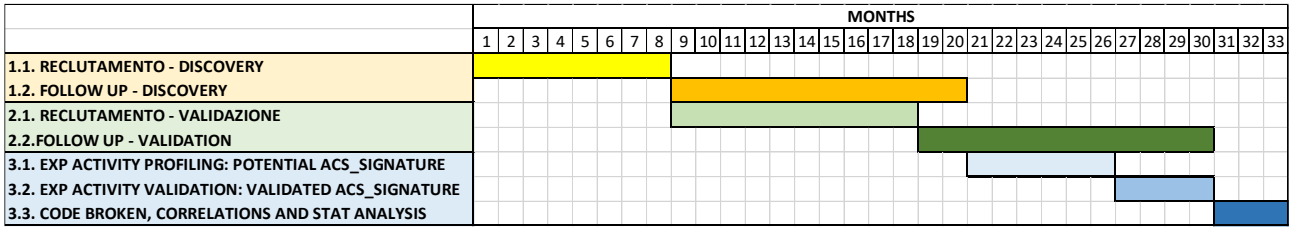
13. Patients who are unable to give valid informed consent at the time enlistment

14. Pregnant women

## *Study design*

This study, observational, prospective, monocentric will rely on the recruitment of patients consecutive with acute coronary syndrome (STEMI, NSTEMI) treated with angioplasty. I am 4 years and 6 months for recruitment and 12 months thereafter for completion of the follow-up, followed by a period of three months to complete the data analysis. Recruitment will be divided into two phases, functional to the subsequent molecular analysis: one of discovery and one of validation, partially overlapped temporally. Based on literature and experience data previous [5], we expect the number of patients who will develop ventricular dysfunction at 12 months from the PCI will be higher among the STEMI than the NSTEMI. A number of NSTEMI patients higher, so the number of patients who will develop ventricular dysfunction

will be similar between these two groups. In addition, 100 patients diagnosed with AU, in which a low rate of evolution towards left ventricular dysfunction, will form a group of further control that will be tested after the validation phase, more relevant and homogeneous compared to healthy patients.



| *Assessments* | *Pre-procedure* | *Post-procedure (12-24 h)* | *6 months* | *12 months* |
|---|---|---|---|---|
| Medical History* | **x** | | **x** | **x** |
| Recruitment criteria | **x** | | | |
| Blood draw ACS_SIGNATURE | **x** | **x** | **x** | **x** |
| Blood count* | **x** | **x** | **x** | **x** |
| Blood draw Tn-hs e CK-MB* | **x** | **x** | | |
| ECG* | **x** | **x** | **x** | **x** |
| Echocardiography * | **x** | **x** | **x** | **x** |
| Angiography * | **x** | | | |
| Endpoints | | | **x** | **x** |

* routine clinical evaluations

Patients will be enrolled after diagnosis of ACS and before coronary surgery and will be treated according to the guidelines provided for the treatment of acute coronary syndrome and for post-PCI. Unless special contraindications, all patients will receive optimal medical therapy that includes:

- Dual therapy for 12 months

- Statins

- beta-blockers,

- ACE inhibitors,

- Mineralocorticoid receptor antagonists.

Follow-up visits including echocardiographic examination will be performed at 6 and 12 months from myocardial revascularization procedure. The evaluation of the FEVS will be carried out by 3D echocardiography that, as has been documented, shows a proven concordance with the MRI [15]. This method has also shown high reproducibility, with low intra- and inter-operator variability, and increased diagnostic accuracy. During the echocardiographic examination, in addition to FEVS data, other function indices will be evaluated systolic left ventricle such as, for example, telesistolic volumes, telediastolic, parameters tissue Doppler derivatives and myocardial strain indices (global Longitudinal strain).

The study will also need to take samples that will be made with the following scheme: 1) pre operative, before PCI, 2) post-operative 1 taken at 12 - 24h from PCI, 3) post-operative2 -6 months after PCI and 4) post-operative3-12 months after PCI.

Pre-operative withdrawal will be performed in the recovery room of the hemodynamics room after diagnosis of ACS. The post-operative withdrawal.1 will be performed at the Department of Cardiology 12/24 hours after the procedure. Post-operative.2 and post-operative.3 withdrawals will be performed outpatient during the planned echocardiographic checks (6 and 12 months respectively).

In pre-operative and post-operative samples.1 the complete blood count will be performed and Tn-hs, NT-proBNP and LDH will be dosed RNA analysis will be divided into two phases: in the discovery phase, the analysis of the transcriptome by RNA sequencing. The aim is to identify a potentialACS_signature in blood mononucleate cells (PBMCs) of a subset of patients who eventually of the follow up of the discovery phase, show ventricular dysfunction (n= 40) or function conserved ventricular (n= 40). An equal number of STEMI and NSTEMI patients will be analysed.

In the validation phase, the ACS_signature will be measured with independent technique (qPCR) in all patients enrolled in the study, to obtain a Validated ACS-Signature.

## *Study procedures*

They will be taken at each time-point: 1) 3 ml of whole blood in a tube containing stabilizers RNA (type PAX-gene or Tempus), 2) 4 ml in a tube containing EDTA from which the plasma that will be frozen at -80,5° C pending the extraction of RNA, and 3) 4 ml in a tube containing EDTA from which PBMCs will be isolated. The withdrawals in point 2 and 3 must be processed within two hours of the withdrawal, while the withdrawal in tube with RNA stabilizer can be stored at room temperature for up to 3 days, at +4 killed C for 7 days and at -20 killed C/-80 killed C indefinitely without altering the integrity of nucleic acids.Plasma and whole blood RNA will be extracted by silica columns, while RNA from PBMCs will be extracted by Trizol. The integrity of the extracted RNA will be evaluated by running on capillary gel type Bioanalyzer.

On RNA extracted from PBMCs derived from pre-operative sampling in the discovery group, it will be analysis of the entire transcriptome by RNA sequencing (platform Illumina), in order to identify a potential ACS_signature consisting of transcripts differentially expressed in the two groups of patients: those with left ventricular dysfunction and those without ventricular dysfunction.

In the validation phase, the potential ACS_signature will then be validated by qPCR in all STEMI and NSTEMI patients enrolled in the study, including those who will submit a degree intermediate ventricular dysfunction (LVEF 45-49%). They will also be analysed separately two subgroups of patients diagnosed with STEMI and NSTEMI.

RNA that will pass validation (Validated ACS_Signature), will be further tested also in the AU control group and all other timepoints, to assess the time trend potential biomarkers. In addition, the expression of the Validated ACS-Signature will be evaluated in the plasma and whole blood, to assess biodistribution and translational potential. Patient recruitment, withdrawals, patient data collection and all clinical procedures requests will be made by the Clinical, Interventional and Intensive Care Cardiology Unit Coronary Surgery (UTIC) of the IRCCS Policlinico San Donato. The collection and conservation of blood and pre-analytic treatment will be carried out at the BioCor Biobank of the IRCCSPoliclinico San Donato, while RNA measurements will be carried out in the Laboratory of Molecular Cardiology of IRCCS Policlinico San Donato. The study of modulated transcripts by RNA Sequencing with library preparation, sequencing and analysis bioinformatics will be carried out at the Center for Translational Genomics and bioinformatics, of the San Raffaele Hospital.

## *Sample size*

Based on literature [4, 5] it is expected that about 40% of STEMI patients and 30% of STEMI patients NSTEMI patients, after a first episode of myocardial infarction, experience dysfunction of the left ventricle.

Based on these data, we expect recruiting in 18 months 278 patients with ACS performing PCI, 100 will experience ventricular dysfunction (Table 1).

**Table 1. Patient recruitment and follow up**

| Study phase | Total number of patients | | Dysfunction LV expected | |
|---|---|---|---|---|
| | STEMI | NSTEMI | STEMI | NSTEMI |
| DISCOVERY | 49 | 75 | 20 | 22 |
| VALIDATION | 61 | 93 | 31 | 28 |
| TOTAL STEMI + NSTEMI | 110 | 168 | 50 | 50 |

The number of patients to be recruited will be 378, of which 110 STEMI, 168 NSTEMI and 100 AU, as control. By preventing 100 events in total, respectively 50 per group, you can enterabout 6/7 variables for each model of multivariate logistic regression (STEMI NSTEMI).Experimental RNA measurement activities shall follow the distribution shown in Table 2.

**Table 2. Experimental activity**

| | DISCOVERY | | VALIDATION | |
|---|---|---|---|---|
| | STEMI | NSTEMI | STEMI | NSTEMI |
| LV DYSFUNCTION | 20 | 20 | 50 | 50 |
| NO LV DYSFUNCTION | 20 | 20 | 60 | 118 |
| TOTAL | 40 | 40 | 110 | 168 |

For discovery activities using RNA-sequencing, based on literature data [16], we assume a sample size of 40 patients (20 per group) to evaluate the 40 transcriptsthat differentiate patients with ventricular dysfunction and not ventricular dysfunction, out of a total of 17,500 tests, considering a power of 94.0 %, a value of FDR equal to 0.02. For the activities of validation using qPCR, 95% power is obtained by analyzing 50 samples per group evaluating differences between the averages of 2,2 times, with $\alpha$= 0,01 and $\sigma$= 1,4.

## *Statistical methods*

The characteristics of patients will be presented with the average, median, standard deviation and range interquartile for continuous and frequency variables and % for nominal and categorical variables. The Shapiro-Wilks will be used to check if distributions are Gaussian. For distributions normal, statistical analysis will be carried out with parametric tests while for non-normal distributions a non-parametric statistical analysis will be carried out. Logistic regression models will be used to assess the association between gene characteristics and clinical outcome. In particular, the power discriminatory (c statistics) will be expressed in terms of area under the curve in a Receiver Operating Characteristics (ROC) analysis. Different cut-off levels will be explored for each of which sensitivity, specificity, negative predictive power and positive predictive power will be evaluated. The added prognostic value of ACS_signature in relation to variables will also be assessed traditional clinics including NTproBNP, Tn-hs and CK-MB using the prediction curve.

## *Ethical aspects*

The study will be conducted according to GCP, local laws and rules of the World Medical Association Declaration of Helsinki.

## *Publication policy*

The data collected are the property of IRCCS Policlinico San Donato and will be the subject of publication.

## *Bibliography*

1. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M: Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J 2016, 37(42):3232-3245.

2. Timmis A, Townsend N, Gale C, Grobbee R, Maniadakis N, Flather M, Wilkins E, Wright L, Vos R, Bax J, Blum M, Pinto F, Vardas P, ESC Scientific Document Group: European Society of Cardiology: Cardiovascular Disease Statistics 2017. Eur Heart J 2018, 39(7):508-579.

3. Pedersen F, Butrymovich V, Kelbaek H, Wachtell K, Helqvist S, Kastrup J, Holmvang L, Clemmensen P, Engstrom T, Grande P, Saunamaki K, Jorgensen E: Short- and long-term cause of death in patients treated with primary PCI for STEMI. J Am Coll Cardiol 2014, 64(20):2101-2108.

4. Cleland JG, Torabi A, Khan NK: Epidemiology and management of heart failure and left ventricular systolic dysfunction in the aftermath of a myocardial infarction. Heart 2005, 91 Suppl 2:ii7-13; discussion ii31, ii43-8.

5. Hanania G, Cambou JP, Gueret P, Vaur L, Blanchard D, Lablanche JM, Boutalbi Y, Humbert R, Clerson P, Genes N, Danchin N, USIC 2000 Investigators: Management and in-hospital outcome of patients with acute myocardial infarction admitted to intensive care units at the turn of the century: results from the French nationwide USIC 2000 registry. Heart 2004, 90(12):1404-1410.

6. Tsao CW, Lyass A, Larson MG, Cheng S, Lam CS, Aragam JR, Benjamin EJ, Vasan RS: Prognosis of Adults With Borderline Left Ventricular Ejection Fraction. JACC Heart Fail 2016, 4(6):502-510.

7. Reinstadler SJ, Feistritzer HJ, Klug G, Mair J, Tu AM, Kofler M, Henninger B, Franz WM, Metzler B: High-sensitivity troponin T for prediction of left ventricular function and infarct size one year following ST-elevation myocardial infarction. Int J Cardiol 2016, 202:188-193.

8. D'Souza M, Sarkisian L, Saaby L, Poulsen TS, Gerke O, Larsen TB, Diederichsen AC, Jangaard N, Diederichsen SZ, Hosbond S, Hove J, Thygesen K, Mickley H: Diagnosis of unstable angina pectoris has declined markedly with the advent of more sensitive troponin assays. Am J Med 2015, 128(8):852-860.

9. Creemers EE, Wilde AA, Pinto YM: Heart failure: advances through genomics. Nat Rev Genet 2011, 12(5):357-362.

10. Margulies KB, Bednarik DP, Dries DL: Genomics, transcriptional profiling, and heart failure. J Am Coll Cardiol 2009, 53(19):1752-1759

11. Beermann J, Piccoli MT, Viereck J, Thum T: Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiol Rev 2016, 96(4):1297-1325.

12. Devaux Y, Stammet P, Friberg H, Hassager C, Kuiper MA, Wise MP, Nielsen N, Biomarker subcommittee of TTM trial (Target Temperature Management After Cardiac Arrest, NCT01020916): MicroRNAs: new biomarkers and therapeutic targets after cardiac arrest? Crit Care 2015, 19:54-015-0767-2.

13. Busch A, Eken SM, Maegdefessel L: Prospective and therapeutic screening value of non coding RNA as biomarkers in cardiovascular disease. Ann Transl Med 2016, 4(12):236

14. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P, ESC Scientific Document Group: 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute

myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2018, 39(2):119-177.

15. Feistritzer HJ, Reinstadler SJ, Klug G, Reindl M, Wohrer S, Brenner C, Mayr A, Mair J, Metzler B: Multimarker approach for the prediction of microvascular obstruction after acute ST-segment elevation myocardial infarction: a prospective, observational study. BMC Cardiovasc Disord 2016, 16(1):239-016-0415-z.

16. Zhao S, Li CI, Guo Y, Sheng Q, Shyr Y: RnaSeqSampleSize: real data based sample size estimation for RNA sequencing. BMC Bioinformatics 2018, 19(1):191-018-2191-5.