

Background materials for “Prioritizing Candidates of Post–Myocardial Infarction Heart Failure Using Plasma Proteomics and Single-Cell Transcriptomics”.
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1-Heart Failure

Myocardial infarction (MI) is the pathological process due to the occlusion of coronary arterie(s). Reduced or no blood supply to the myocardium resulting in damage leading to apoptosis and/or necrosis of the underlying tissue.

These events are followed by invasion of various immune cells within hours of occlusion which might be responsible for the ensuing inflammation cascade. The important cells known to migrate to the site of injury are the neutrophils, which peak at day one, followed at 3 days by the macrophages. The invasion by these cells is known to influence the local cytokine milieu, which might aggravate or influence the inflammatory events. This inflammatory response is needed to clear up the necrosed heart tissue. Where necrosis occurs, the cardiac tissue become thinner. This weakened area is unable to withstand the pressure and volume load on the heart in the same manner as the other healthy tissue. As a result, there is dilatation of the chamber arising from the infarct region. The initial remodeling phase, after a myocardial infarction, results in repair of the necrotic area and myocardial scarring that may, to some extent, be considered beneficial since there is an improvement in or maintenance of LV function and cardiac output. Over time, however, as the heart undergoes ongoing remodeling, it becomes less elliptical and more spherical. Ventricular mass and volume increase, which together adversely affect cardiac function. Eventually, diastolic function, or the heart's ability to relax between contractions may become impaired, further causing decline. In summary, cardiac remodeling that is defined as a group of molecular, cellular and interstitial changes that manifest clinically as changes in size, mass, geometry and function of the heart after injury could result in poor prognosis because of its association with ventricular dysfunction and malignant arrhythmias. Adverse remodeling leading to heart failure is the primary pathologic process driven by an unbalanced resolution of inflammation. The macrophage cells ($M\phi$) are an important controlling contributor of the inflammation after MI; $M\phi$ subtypes secrete mediators to promote inflammation (M1 phenotype) or to suppress inflammation and promote scar formation (M2 phenotype).

Heart failure occurs when the heart muscle doesn't pump blood as well as it should. Blood often backs up and causes fluid to build up in the lungs (congest) and in the legs. The fluid buildup can cause shortness of breath and swelling of the legs and feet. Poor blood flow may cause the skin to appear blue (cyanotic).

Treatments for HF are limited and heart transplant is not an option for all patients. It is therefore important to find ways to predict whether a patient with myocardial infarction will evolve toward heart failure. Heart failure can't be cured but it can be treated, quite

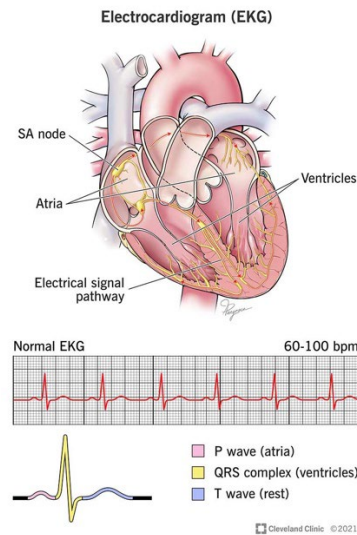
often with strategies involve changes in lifestyle. The sooner the patients manage their symptoms, the better.

2- How to evaluate cardiac function? Electrocardiogram

An electrocardiogram (ECG or EKG) records the electrical signal from the heart to check for different heart conditions. Electrodes are placed on the chest to record the heart's electrical signals, which cause the heart to beat. The signals are shown as waves on an attached computer monitor or printer. (Mayo Clinic).

There are three main components to an ECG: the P wave, which represents depolarization of the atria; the QRS complex, which represents depolarization of the ventricles; and the T wave, which represents repolarization of the ventricles. (Figure 1)

Figure 1: EKG showing heartbeat frequency and duration



Frequently calculated is the ST segment. The ST segment encompasses the region between the end of ventricular depolarization and beginning of ventricular repolarization on the ECG. In other words, it corresponds to the area from the end of the QRS complex to the beginning of the T wave. ST segment depression less than 0.5 mm is accepted in all leads. ST segment depression 0.5 mm or more is considered pathological. Additionally, electrocardiogram can provide evidence of the presence of a T-wave inversion (Figure 2). T-waves that are deep and symmetrically inverted strongly suggest myocardial ischaemia. In some patients with partial thickness ischaemia the T-waves show a biphasic pattern.

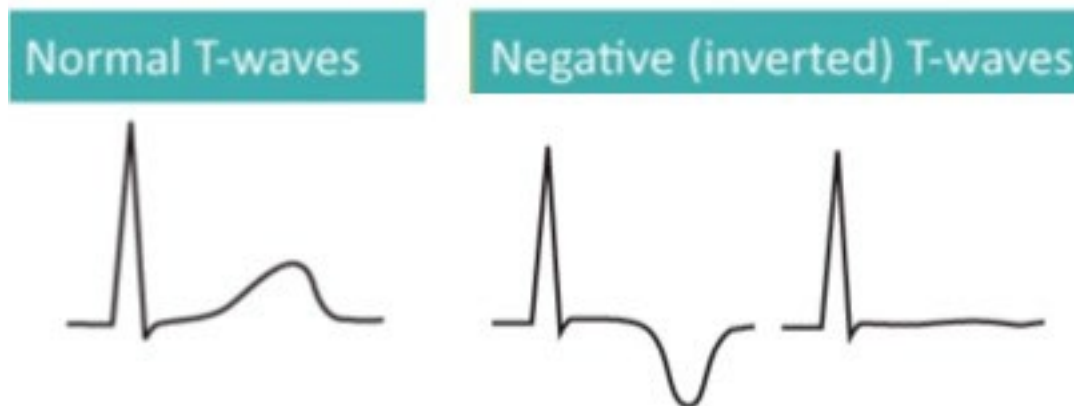


Figure 2: Normal and Inverted T-wave examples.

During each heartbeat, a healthy heart has an orderly progression of depolarization that starts with pacemaker cells in the sinoatrial node, spreads throughout the atrium, and passes through the atrioventricular node down into the bundle of His and into the Purkinje fibers, spreading down and to the left throughout the ventricles. This orderly pattern of depolarization gives rise to the characteristic ECG tracing. To the trained clinician, an ECG conveys a large amount of information about the structure of the heart and the function of its electrical conduction system. Among other things, an ECG can be used to measure the rate and rhythm of heartbeats, the size and position of the heart chambers, the presence of any damage to the heart's muscle cells or conduction system, the effects of heart drugs, and the function of implanted pacemakers.

3- Cardiac markers following Myocardial infarction

Several cardiac markers or biomarkers can be measured to evaluate heart function. They can be useful in the early prediction or diagnosis of disease, specifically after a myocardial infarction.

Most of the early markers identified are enzymes. However, not all of the markers currently used are enzymes. For example, in formal usage, troponin would not be listed as a cardiac enzyme. Troponin is released during MI from the cytosolic pool of the myocytes. Its subsequent release is prolonged with degradation of actin and myosin filaments. Isoforms of the protein, T and I, are specific to myocardium. Differential diagnosis of troponin elevation includes acute infarction, severe pulmonary embolism causing acute right heart overload, heart failure, myocarditis. Troponins can also calculate infarct size but the peak must be measured in the 3rd day. After myocyte injury, troponin is released in 2–4 hours and persists for up to 7 days. The normal value is for Troponin I <0.3 ng/ml and Troponin T <0.2 ng/ml. Other biomarkers are also used such as Creatine Kinase (CK-MB), or Lactate dehydrogenase. Depending on the marker, it can take between 2 and 24 hours for the level to increase in the blood. Additionally, determining the levels of cardiac markers in the laboratory - like many other lab measurements - takes substantial time. Cardiac markers are therefore not useful in diagnosing a myocardial infarction in the acute phase. The clinical presentation and results from an ECG are more appropriate in the acute situation.

Heart failure (HF) and mortality after myocardial infarction remains elevated despite advances in medical and revascularization therapies. To these days, prediction on who will recover from MI or who will progress to HF lacks biomarkers to make a prognosis.

4- What is a biomarker and how to determine its validity as a diagnosis or prognosis tool?

A biomarker is a measurable indicator of the severity or presence of some disease state. For example, body temperature is a well-known biomarker for fever. Blood

pressure is used to determine the risk of stroke. It is also widely known that cholesterol values are a biomarker and risk indicator for coronary and vascular disease, and that C-reactive protein (CRP) is a marker for inflammation. Biomarkers help in early diagnosis, disease prevention, drug target identification, drug response etc. Biomarkers need to also be detectable in a rapid manner and from accessible tissue or fluid. Blood or urine are primary choices. When biomarkers are measured in blood/urine, they are usually secreted proteins with a known function associated for the relevant diseases. There are no validated and accepted biomarkers to predict the progression from Myocardial Infarction to Heart Failure. The proposed article addressed this unmet need.

A valid biomarker is defined as per USDA as follow: “a biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test results”. The validity of a biomarker is closely linked to what we think we can do with it. This biomarker context drives not only how we define a biomarker but also the complexity of its qualification. It is important that once a biomarker is identified it will be confirmed in additional cohorts of patients.

5-Proteomics as a method to identify biomarkers

Proteomics is defined as a large-scale study of proteins. After genomics and transcriptomics, proteomics is the next step in the study of biological systems. It is more complicated than genomics because an organism's genome is more or less constant, whereas proteomes differ from cell to cell and from time to time. Distinct genes are expressed in different cell types, which means that even the basic set of proteins produced in a cell must be identified.

In the past, this phenomenon was assessed by RNA analysis, which was found to lack correlation with protein content. It is known that mRNA is not always translated into protein, and the amount of protein produced for a given amount of mRNA depends on the gene it is transcribed from and on the cell's physiological state. Proteomics confirms the presence of the protein and provides a direct measure of its quantity.

Not only does the translation from mRNA cause differences, but many proteins also are subjected to a wide variety of chemical modifications after translation. The most common and widely studied post-translational modifications include phosphorylation and glycosylation. Many of these post-translational modifications are critical to the protein's function.

Phosphorylation: It happens to many enzymes and structural proteins in the process of cell signaling. The addition of a phosphate to particular amino acids—most commonly serine and threonine mediated by serine-threonine kinases, or more rarely tyrosine mediated by tyrosine kinases—causes a protein to become a target for binding or interacting with a distinct set of other proteins that recognize the phosphorylated domain. Because protein phosphorylation is one of the most studied protein

modifications, many "proteomic" efforts are geared to determining the set of phosphorylated proteins in a particular sample.

Ubiquitination: Ubiquitin is a small protein that may be affixed to certain protein substrates by enzymes called E3 ubiquitin ligases. Determining which proteins are poly-ubiquitinated helps understand how protein pathways are regulated. This is, therefore, an additional legitimate "proteomic" study.

Additional modifications: In addition to phosphorylation and ubiquitination, proteins may be subjected to (among others) methylation, acetylation, glycosylation, oxidation, and nitrosylation. Some proteins undergo all these modifications, often in time-dependent combinations. This illustrates the potential complexity of studying protein structure and function.

6- Additional information on the proteomics used in the paper: Slow Off-rate Modified Aptamer (SOMAmer)-based capture array

The SOMAmer-based method is a highly multiplexed assay for the purpose of biomarker identification (discovery). However, this method is not adapted to a format suitable and convenient for diagnostic applications.

For more information, please read PLoS One. 2011; 6(10): e26332.
Published online 2011 Oct 17. doi: 10.1371/journal.pone.0026332

Extracted text from above reference:

"Current proteomic measurement methods tend to be limited with respect to throughput, sensitivity, or multiplicity [2]. The authors developed a proteomic platform that promises to surpass current technologies with respect to these limitations. The assay is highly multiplexed, sensitive (sub-picomolar), reproducible, and quantitative [3]. It is based on affinity capture, and therefore has some parallels with antibody-based methods. The assay utilizes synthetic DNA SOMAmers (Slow Off-rate Modified Aptamers) as protein capture reagents rather than antibodies.

SOMAmers are short, single stranded deoxyoligonucleotides. Like aptamers, they are selected in vitro from large random libraries for their ability to bind to discrete molecular targets, which can be small molecules, peptides, or proteins [4], [5]. SOMAmers are unlike aptamers in that they bear dU residues that are uniformly functionalized at the 5-position with moieties (e.g. benzyl, 2-naphthyl, or 3-indolyl-carboxamide) that can participate in interactions with target molecules as well as form novel secondary and tertiary structural motifs within the SOMAmer itself ([6] data not shown). Nuclease resistance and selection success rates [3] are greatly improved over aptamers, and affinities are comparable to antibodies.

Hence, SOMAmers bear significant promise as synthetic protein-binding reagents [5], [6], [7]. In particular, we find that problems of capture reagent cross-reactivity and non-

specific adsorption to surfaces are diminished or absent in our SOMAmer-based protein measurement assays. These are issues that limit the intrinsic multiplexing capability of antibody-based proteomic assays to 30–50 analytes [2]. In contrast, at the time of this writing, our SOMAmer-based, high-content biomarker discovery platform reliably measures more than one thousand protein analytes in a single sample ([3] data not shown).

Principle of Slow Off-rate Modified Aptamers assay:

