Post-Mortem Infarction Diagnosis Using Molecular Techniques

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Abstract

The cardiovascular system plays a major role in life. From the past up to now, one of the main causes of death is cardiac infarction. Heart is the most important organ of this system and its function is to transfer blood and its contents to the whole body. There is a direct line through pulmonary artery to the lungs, so blood can be flushed out with oxygen.

Infarction is a severe damage in a localized site of the heart, which leads to that part’s necrosis. There are symptoms reported, such as discomfort, ache and others that are analysed below.

Post-mortem examination of the cardiac muscle is a basic way to find out information about infarction. There are alterations, depended on the time between the infarction and the observation of the heart. Nowadays, science and technology progress allows us to use molecular biology techniques as a tool to be accurate and detailed about diagnosis. There are dilutions and machinery which can save time and provide results beyond a doubt. Immunohistochemistry and Real Time Polymerase Chain Reaction are such techniques, which can detect and quantify the class of infarction. These techniques are analysed below, including with some subcategories of the ways we can use them.

Keywords: Post mortem; Infarction; Real time PCR; Cardiac; Heart

Introduction

In order to understand, it is important to know a few things about heart, such as the anatomy, the physiology and the histology of the cardiac muscle. It is the main organ of the cardiovascular system and it works as a pump. It is surrounded by pericardium and its inner part (myocardium) has four cavities (atria and ventricles), covered by endocardium. These cavities are located on the right and left sight of the myocardium. Cardiac muscle is externally divided into anterior (or lateral), diaphragmatic and posterior, which is also called “base of the heart” [1-6].

According to physiology, heart is the main organ of the circular process of transferring blood and its contents. Blood enters the cardiac muscle from the right atrium, from where it moves to the right ventricle, and then it moves to lungs, where it is enriched with oxygen. After this process, blood enters the left atrium and it moves to the left ventricle, from where it enters the circulation. The prevention of blood reflux is achieved by the valves. This whole process is coordinated by myocardial contractions, which are the heart rate, determined by electrical currents produced by specialized cells in specialized tissues and transported through a network of specialized fibers, which is called "system of production and stimulation". This system works through dynamic action [7-12].

The histologic view of the heart is more microscopic. During fetal age, a blood vessel shaper maintains three tunics that enclose, resulting three layers. Mesothelial cells create a smooth surface that forms the epicardium. Muscle cells form muscle fibres, forming the myocardial and endothelial cells and endothelial cells form the endocardium in the form of a smooth lining. Cardiac muscle is a striated muscle, composed by muscle fibres, myofibrils, muscle filaments and transverse striations. Monocytes have a nucleus, they are branched out and their final parts join together to create elongated cardiac fibers. Their lateral sides are joined together at specific locations. Myocardial cells are sited as a layer composite formed and three-dimensional spiral shape to form an anastomotic communication network from relevant neighbouring cells. The proper blood circulation is achieved by the synchronized contraction of these adjacent cells [13-17].

Infarction

Myocardial infarction is a worldwide leading death among all cardiovascular diseases. It reflects a prolonged localized ischemic necrosis site, an irreparable myocardial damage usually due to atherosclerotic stenosis of a large coronary artery. As a result of imbalance between supply and demand deaths of myocardial cells caused. The range and the position of thrombus varies. Possible ischemic symptoms include diffuse discomfort, dispone, diaphoresis, nausea, arrhythmia or syncope. Myocardial infarction may be defined from clinical, electrocardiographic, biochemical, imaging and pathological characteristics. Nevertheless asymptomatic cases can also occur [18-27].

Universal classification of myocardial infarction consists of five types: Spontaneous myocardial infarction (Type 1), Myocardial infarction secondary to an ischemic imbalance (Type 2), Myocardial...
Immunohistochemistry

Immunohistochemistry is a technique based on antigen-antibody binding and on the principles of immunology and chemistry. It relies on the process of identifying antigenic epitopes from monoclonal or polyclonal ant bodies [30-46,50]. The biochemical reactions that take place render the result visible. Different antigens correspond in different cell lines and tissues [51-60]. In conclusion it is a specialized technique by which a distinction is made between physiological and pathological parts of tissues and questions with a genetic background can be answered [47-49,57,58,61].

The method is sensible and specific and can be used in formalin-fixed, paraffin-embedded tissues. The above features give a competitive advantage over the other diagnostic methods. Particular attention needs to be paid to the correct stabilization of the tissues, the markers used and the staining. In this way, the sample remains unspoken and the results are interpreted with greater precision. Chromogens are used to quantitate the enzyme reaction and the concentration of products [47,48,51,52,59,60].

Better analysis of post-mortem diagnosis is a primary goal. It is necessary to understand and evaluate the myocardial infarction and ischemic heart disease. In specimens corresponding to cases of myocardial infarction, there are differences in tissue’s pathophysiology and in the form of muscle fibres as it emerged from the cell death. These changes can be evaluated by the immunohistochemistry method, highlighting the different ways of reacting multiple cell antibodies. It is also possible to place the myocardial infarction in a timeline according to the time that has occurred [49,50,54,58].

In addition cardiac proteins released into the circulation of blood can be characterized as cellular antigens. A combination of markers and antibodies is needed for adequate evidence of myocardial necrosis and final diagnosis. Loss of staining reflects an early stage. Myocardial infarction is better characterized by immunohistochemistry as the latter facilitates precise analysis of structural organization in connection with the chemistry of cells and tissue engineering. With the use of sensitive, specific markers, controls and antibodies against proteins and in coordination with the intensity of staining myocardial areas are outlined and determined [53,55,56].

Real-Time Polymerase Chain Reaction

Real Time PCR is used for fast and accurate quantification of RNA and DNA. This technique allows the detection of sequence and point mutations, the measurement of products’ quantity and the monitoring of the rate at which a target molecule is amplified in real time. Initially, the product is not detectable, because it is in very small quantities. This stage is followed by the multiplication phase of the product (nearly doubled in each signal). The more molecular targets are initially available, the fewer cycles will be required to start the exponential phase [57-62].

If we compare the number of cycles that needed more than one reaction to reach their exponential phase, we can determine how many molecules were used as a template initially. To determine each cycle’s product there are several approaches, but all based on the detection of fluorescent label (tag), which binds to each synthesized molecule [57-62].

Taq-man

This method is based on probe hydrolysis from Taq-polymerase. Firstly, two probes are made, one for each SNP (A/T or G/C). Then PCR is applied and depending and depending on the color we conclude: The probe having mismatch, is not cleaved by the exonuclease property of polymerase. The probe who sticks is cleaved [57-62].

SYBR-green

Fluorescent dye is used, which inserts the double strand of the produced PCR product. When DNA is decomposed, a pigment is released. When DNA is composed, it binds and phosphoresces [57-62].

Fluorescence resonance energy transfer

This method uses two probes, each hybridized very close to the other. The upstream probe is labeled at the 3’ end with a fluorescent donor-molecule and the downstream at the 5’end with a fluorescent acceptor-molecule [57-62].
Conclusions

To sum up, cardiac muscle is one of the most important organs of human body and its function sometimes can be unbalanced due to some disruptions. This fact can cause problems which may even lead to death. For example, cardiac infarction is a major cause of death and it has many details and factors which determine both the cause and the time that happened. The five types of infarction which are described above have specific symptoms, but if it comes to death, there are details which make the difference and they have to be examined further. Most of the times a macroscopic post-mortem examination is not enough to give answers. Molecular biology and molecular techniques can help to find these details. Technology and science progress are able to combine methods, techniques and protocols to provide accurate and specific answers about infarction.

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