

CHAPTER 3

Immuno-histochemical detection of cardiac troponin I and cardiac troponin T after myocardial infarction in postmortem obtained myocardial tissue.

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Introduction

In postmortem examination of patients with sudden, probable cardiac death a diagnosis of a myocardial infarction is usually based on the finding of severe, occlusive atherosclerotic coronary artery disease. Actual detection of the histological sequelae of infarcted myocardium will develop only after significant time-lag between the onset of the myocardial infarction and death. Signs of irreversible abnormalities can be noticed by electron microscopy already 120 minutes after infarction (1). Using enzyme-histochemical analysis detectable loss of Lactate Dehydrogenase will be recognizable 5 hours after infarction (2).

Histologically however, the presence of neutrophils, initially in small capillaries and subsequently in the interstitium is the first reliable sign of a myocardial infarction and can not be detected in the first 6 to 12 hours after onset of the infarction (3). Nowadays no reliable histological or immunohistological technique is available which allows detection of a myocardial infarction in the first hours after onset.

Recently the presence of an increase in serum concentrations of the cardiac specific isoform of troponin T as well as troponin I early after myocardial infarction have been described (4,5).

Troponin T is a regulatory protein of the thin filaments of striated muscle tissue. It binds troponin I and troponin C to tropomyosin and transfers calcium-induced conformational changes to the thin filaments of striated muscle. Together with troponin I and troponin C it is a part of the troponin complex of muscle. Cardiac specific isoforms of troponin T and of troponin I have been reported (6,7). In addition to an increase in serum concentrations in patients with myocardial infarction an increase in serum cardiac troponin T and cardiac troponin I concentrations have also been described in patients with unstable angina (8,9) as well as in patients with other causes of myocardial damage such as patients after blunt thoracic trauma (10).

In the present study we evaluated immunohistochemically the expression of cardiac troponin T and cardiac troponin I in the myocardium of patients who died due to the complications of a myocardial infarction. More specifically, we evaluated whether in the first hours after a myocardial infarction loss of cardiac troponin T and/or cardiac troponin I expression could be detected which would assist in making a firm diagnosis of a myocardial infarction in these patients.

Patients and Methods

Post mortem cardiac tissue was obtained from 16 Caucasian patients, 13 males and 3 females, aging from 45 to 79 years of age (median 67). All patients died due to the complications of a myocardial infarction associated with occlusive coronary atherosclerosis. The infarction was located in the left ventricle; anteroseptal in 7, posterolateral in 7 and lateral in 2 patients. The primary cause of death was related to cardiac failure in 6, rupture of the ventricular wall in 2, and (presumed) untreatable

ventricular arrhythmia's in 8 patients (3 of these presenting with sudden death).

The time between onset of myocardial infarction and death was primarily determined according to patient history and if necessary corrected according to the presence or absence of classical histological parameters. The time between onset of myocardial infarction and death was estimated to be 0-6 hours in 4 patients, 6 – 24 hours in 5 patients; 1 – 3 days in 4 patients, 3 – 7 days in 2 patients whereas 1 patient died 14 days after the onset of symptomatic myocardial infarction.

In all patients a postmortem examination was performed within 36 hours, median 12 hours. Cardiac tissue was obtained from the posterior wall of the right ventricle as well as from the interventricular septum, anterior wall, lateral wall and posterior wall of the left ventricle. In addition biopsies were taken from intercostal muscle as well as psoas muscle.

All tissue samples were snap frozen in isopentane cooled by liquid nitrogen. In addition tissue samples were fixed in 10 % neutral buffered formalin pH: 7.4 and embedded in paraffin. Frozen as well as paraffin sections were cut at 4 micron for both histological (Haematoxylin and eosin stain (H&E)) and immunohistochemical analysis.

Immunohistochemistry

Monoclonal antibodies directed against cardiac troponin T and cardiac troponin I were kindly donated by Roche™ and Abbott™ respectively. A standard immunohistochemical procedure was performed using a commercially available peroxidase labeled rabbit anti-mouse and a peroxidase labelled goat anti-rabbit antibody. (Dako™, Denmark), with a DAB (diaminobenzidine tetrahydrochloride) as a substrate. (11). Either protease pretreatment or heat induced antigen retrieval (HIAR) using a microwave oven in a Tris buffer pH: 8.0 enhanced the immunohistochemical staining in the formalin-fixed paraffin embedded sections. (12). Non-infarcted myocardium acted as an intrinsic positive control whereas striated muscle derived from the psoas and intercostal muscles served as negative controls.

Results

The duration of the myocardial infarction prior to death according to clinical data correlated with the data obtained at classical histopathology on H&E sections.

The expression of both cardiac troponin I and cardiac troponin T expression is still prominent in the first 24 hours after a myocardial infarction. No differences were observed between presumably non-infarcted and infarcted areas of the myocardium. In patients with a myocardial infarction with a duration of several days history,

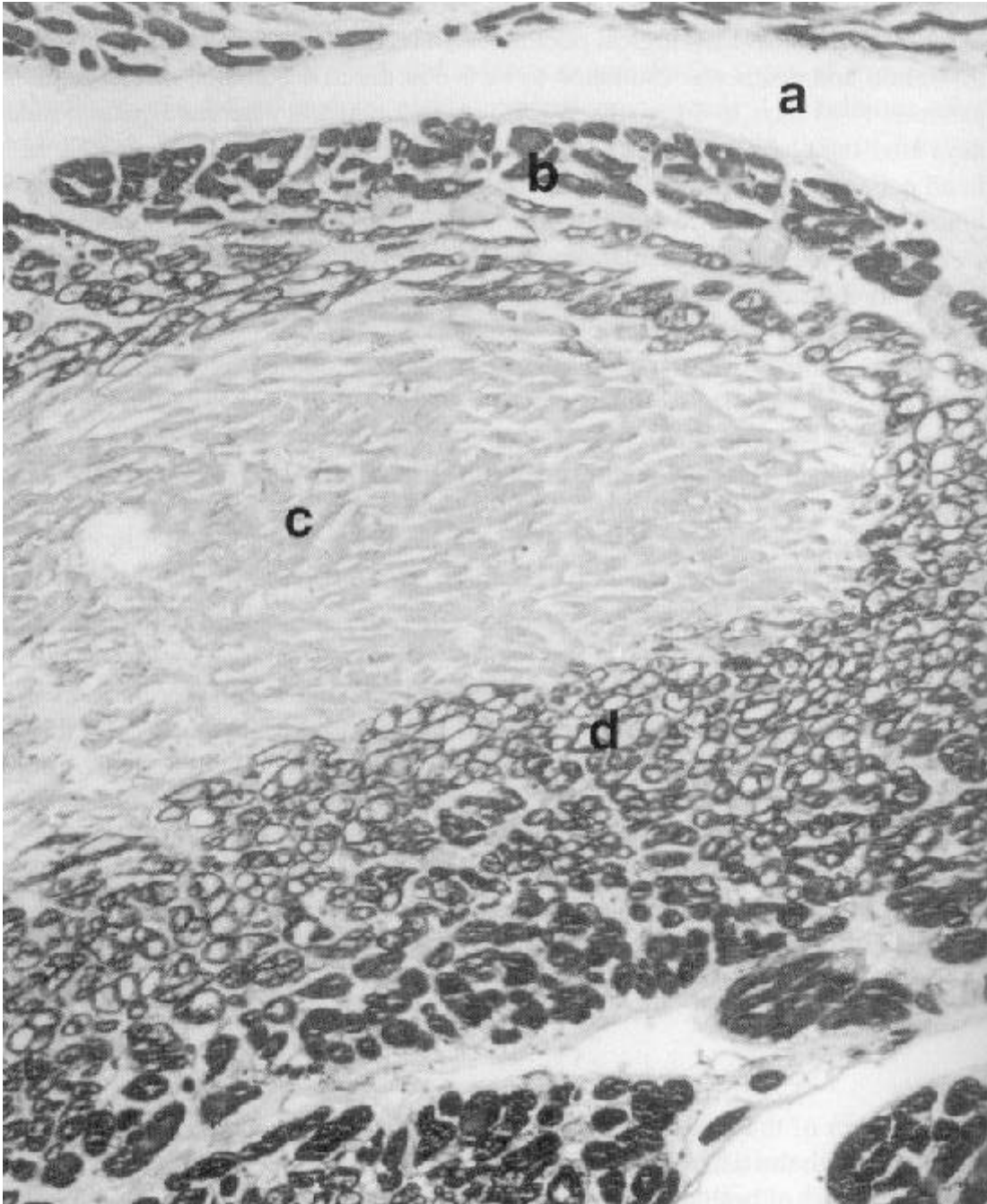


Figure 1. The loss of immunohistochemically detectable cardiac troponin I is shown in infarcted cardiomyocytes 24 hours after a myocardial infarction. Clarification: a is ventricular lumen; b is non-infarcted cardiomyocytes located subendocardially; c is infarcted cardiomyocytes; and d is non-infarcted cardiomyocytes. Original magnification x 100.

expression of both cardiac troponins in the infarcted myocardial cells is gradually diminishing until neither cardiac troponin I nor cardiac troponin T can be detected at 3 days post-myocardial infarction. In all patients both cardiac troponin I and cardiac troponin T can still be detected within the myocardial cells surrounding the infarcted area, as well as in individual surviving myocardial cells within the infarction (see figure 1).

Expression of neither cardiac troponin I nor troponin T was seen in the skeletal muscle biopsies of intercostal and psoas muscle.

Discussion

In the present study we evaluated in patients who died due to a myocardial infarction immunohistochemically the loss of intracellular cardiac troponin I and cardiac troponin T. Our results show that infarcted myocardial cells show a gradual decrease in both cardiac troponin I and cardiac troponin T expression cytoplasmatically, resulting in a total loss of detectable cardiac troponin at day 3 post-infarction. In the first 24 hours after myocardial infarction no decrease in cardiac troponin could be detected immunohistochemically.

Using lightmicroscopy on routine H&E stained sections, histological detection of infarcted cardiomyocytes by examination of cardiac tissue post-mortem is rather difficult. The earliest reliable histological sign at 8 to 12 hours post infarction, is increased eosinophilia of the cardiomyocytes with subsequent loss of cross-striations and degradation of the nucleus, which is established at 8 to 12 hours after the onset of the infarction. This is followed by the inflammatory response characterized by influx of neutrophils at 12 to 16 hours post-infarction. Subsequently, degradation of these neutrophils results in the histological appearance of "nuclear dust" which reaches its maximum at 24 hours post-infarction. In the next days, there is an increasing proliferation of fibroblasts which changes into myofibroblasts immunophenotypically. In addition, there is proliferation of capillaries, influx of lymphocytes and macrophages in the next days post-infarction leading to degradation and phagocytosis of the necrotic cardiomyocytes, finally resulting in scar tissue (3).

Consequently, early detection of ischemic or infarcted cardiomyocytes in the first hours after infarction by routine histological analysis is not reliable.

Using electronmicroscopy, infarcted cardiomyocytes can be detected as early as 2 hours after a myocardial infarction, since only small tissue samples can be examined with this technique, clinical application of electronmicroscopy is not possible.

Enzyme immunohistochemical analysis using Nitro-blue-tetrazolium (NBT) has also been used during the last 20 years. In this procedure the integrity of the cardiomyocyte cell membrane is tested through its ability to retain lactate dehydrogenase intracytoplasmatically. The NBT is not reduced in the absence of the enzyme and consequently, in the infarcted area the (blue) staining will not develop. This technique

can be used both microscopically as well as macroscopically using a cross-section of the heart (13). It has been established that, using the NBT enzyme stain, infarcted myocardial cells can be detected as early as 4 to 8 hours after the onset of the infarction (13).

Recently, studies indicate that the presence of the membrane attack complex of the complement system (C5b-C9) and or complement component C9 at the cell membranes of myocardial cells detected immunohistochemically is a parameter indicating myocardial cell necrosis (14,15). Upregulation of genes of the complement components can be detected as early as 1 hour after ischemia (16).

From the present study we conclude that loss of cardiac troponin I and cardiac troponin T can be immunohistochemically observed 3 days after the onset of a myocardial infarction. In the first 24 hours immunohistochemically detectable loss of cardiac troponin I as well as of cardiac troponin T are not present. Their gradual decline correlate with the increased serum levels of detectable cardiac troponin after a myocardial infarction. There is a slow increase in serum levels with a maximum at 24 hours followed by a slow decrease to normal levels in the subsequent days. This indicates that the cardiac troponins are relatively firm bound to other structures in the myocardial cells, e.g. myosins, resulting in a rather slow but longstanding release in the circulation after loss of cellular integrity.

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