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REVIEW

Immunohistochemical detection of early myocardial infarction: a systematic review

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Abstract The postmortem diagnosis of early myocardial infarction is a challenge for forensic pathologists because the routine histology is neither specific. Many authors have suggested the use of the immunohistochemistry to fill the gaps in the histological diagnosis of early myocardial infarction. This review aims to analyse advances of immunohistochemical detection of early cardiac damage due to ischaemia. To this purpose, we reviewed experimental studies that investigated immunohistochemical markers and their estimated timing of expression. The review was performed according to specific inclusion and exclusion criteria, and a total of 23 studies assessing the immunohistochemical markers for the diagnosis and timing of early myocardial infarction were identified. The literature review highlights that the analysed markers are complement components, others being inflammatory mediators, cardiac cell proteins, plasma proteins, stress or hypoxia-induced factors and proteins associated with heart failure. All studies demonstrate the effectiveness of the tested markers in the early detection of myocardial infarction in both animal and human samples.

Keywords Early myocardial infarction · Immunohistochemistry · Sudden cardiac death · Systematic review

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Introduction

Sudden cardiac death (SCD) represents an unexpected natural death from a cardiac cause that occurs within a short period of time, generally within the first hour from the onset of symptoms, in a subject without any prior condition that would appear fatal [1]. The annual incidence of SCD in Europe and North America is below 50–100/100,000 persons [2–5]. In Asia, the reported value is 40/100,000 persons [6].

The most common cause of sudden cardiac death is coronary atherosclerotic disease (CHD), followed by cardiomyopathies, myocarditis, valvular disease and channelopathies. Generally, the accepted proportion of all SCDs resulting from CHD is ≈80 %, and SCD accounts for ≈50 % of all CHD-related deaths. Considering these data, during the past years, many studies explaining the mechanism of SCD due to CHD were carried out. Knowledge about the pathophysiology of SCD, which can be differentiate into vascular pathophysiology, myocardial pathophysiology and systemic modulations, have allowed understanding the causes of CHD-related SCDs in relation to different factors such as the severity, distribution and characteristics of the anatomic substrate of coronary atherosclerotic and myocardial tissue alterations. Therefore, in the cases of SCDs following coronary atherosclerotic disease, the cause of death can be a transient or acute myocardial ischaemia, an arrhythmia due to myocardial scar and remodelling, and a sudden cardiac arrest due to an arrhythmia followed by acute or subacute changes in coronary plaque and/or transient coronary artery spasm [7]. Therefore, understanding the pathophysiology of CHD-related SCD is critical in postmortem diagnosis.

In some cases, the postmortem diagnosis of sudden cardiac death due to myocardial ischaemia is a major concern in forensic autopsy cases. This represents a challenge for forensic pathologists when death occurs within minutes to a few hours

after an ischaemic insult. In fact, myocardial cell death does not occur instantaneously at the onset of ischaemia, and, at least, several hours are required before the myocardial necrosis can be identified with standard macroscopic or microscopic examination. Recognition of early myocardial damage using routine haematoxylin and eosin (H&E) staining is neither specific nor sensitive enough if the death of the patient occurred shortly (<6 h) after the onset of the ischaemic injury [8].

In this regard, the usefulness of histochemical (i.e. chromotrope aniline blue) and immunohistochemical methods for the diagnosis of early myocardial damage following ischaemia has been suggested. In particular, many studies have been carried out about markers to use with immunohistochemistry in the diagnosis of early myocardial infarction. In fact, before the influx of inflammatory molecules and cells become histologically detectable, the presence of cellular and humoral mediators can be evaluated by immunohistochemistry, and many studies demonstrate that this method may be an excellent tool for the postmortem diagnosis and timing of cardiac damage induced by ischaemia.

In light of the several evidences about the usefulness of immunohistochemistry, this review aims to analyse advances of immunohistochemical detection of early cardiac damage due to ischaemia. For this purpose, we reviewed experimental studies that investigated immunohistochemical markers and their estimated timing of expression.

Methods

This systematic review has been conducted employing the PubMed database. On this website, we searched for articles between 1990 and September 2016 using the key terms "immunohistochemistry" AND "early myocardial infarction".

We decided to use as a rule of thumb the fact that the abstracts of those articles that indicated in the title that they might have evaluated the use of immunohistochemical markers for the diagnosis and timing of early myocardial ischaemia were to be read. The entire article was read if the abstract indicated that the article potentially met the inclusion criteria.

Articles were included in the following review according to the following inclusion criteria: English language, year of publication from 1990 and positive results within 6 h from the onset of ischaemic damage.

Articles were excluded by title, abstract or full text for irrelevance to the topic in question. Further exclusion criteria were article reviews and editorial comments. Furthermore, we decided to start our research from 1990 considering that the most relevant studies were published after this date.

Data derived from our research of articles include authors, publication dates, type of samples, type of markers, their expression and timing.

Results

In Fig. 1, the flow of articles retrieved for the review is reported. As summarised in Table 1, a total of 27 studies assessing the immunohistochemical markers for the diagnosis and timing of early myocardial infarction were identified.

According to Table 1, Casscells et al. studied the expression of fibronectin in a sample of rats killed after left coronary ligation at different time points. The study showed an increase in the intensity of staining at 4 h after coronary artery ligation in the cytoplasm, Z bands and interstitial space of some myocytes located in the area supplied by the ligated vessels. Moreover, fibronectin staining in the cytoplasm and matrix of capillary, venous and arterial endothelial cells of the infarct centre and border zone was found. At 24 h, similar patterns about fibronectin localization were observed, but there was an increase in the intensity of staining [9].

Brinkmann et al. studied various immunohistochemical markers in a human sample of cardiac deaths with or without coronary thrombosis, which were subdivided on the basis of histological evidences of infarction or not. The studied markers were: desmin, myoglobin, fibrinogen and C5b-9 complement complex. Particularly, the authors highlighted that desmin and myoglobin reacted very much in parallel, even if the myoglobin indicated a higher degree of sensitivity. The immunohistochemical staining in the ischaemic fibres showed the depletion of desmin, the negative reaction for

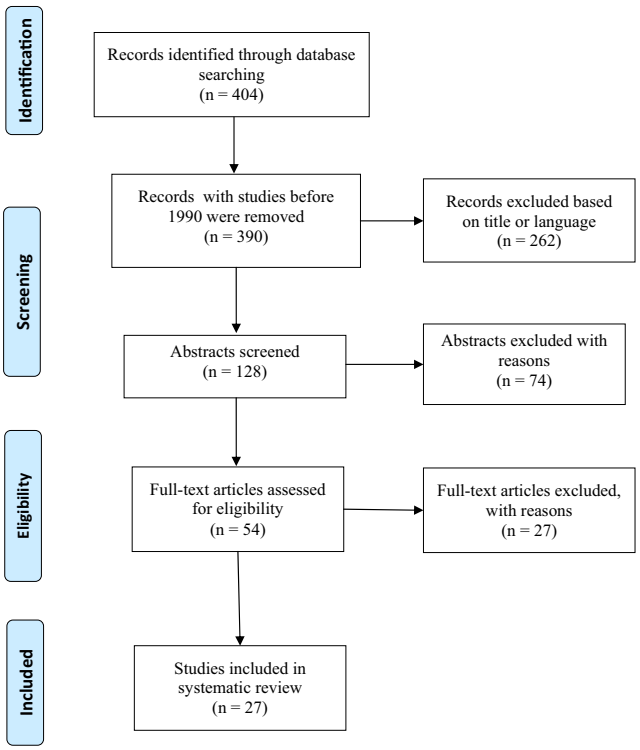

Fig. 1 Flowchart of the results of literature search

Table 1 Studies evaluating the immunohistochemical markers for early myocardial infarction diagnosis and timing

Authors	Year	Sample	Marker	Range of expression	Manner of expression
Casscells et al. [9]	1990	Animals	Fibronectin	≥4 h	+
Brinkmann et al. [10]	1993	Human	C5b-9	NHS	+
			Desmin		−
			Myoglobin		−
			Fibrinogen		+
Thomsen et al. [11]	1994	Human	C5b-9	0–170 h	+
Väkevä et al. [12]	1994	Animals	C1, C3, C8, C9	≥3 h	+
			CD59	≥6 h	−
Zhang et al. [13]	1996	Human	Desmin α-Actinin	≥1 h	−
			Vinculin		
Hu et al. [14]	1996	Human	Fibronectin	NHS	+
Hansen et al. [15]	1999	Human	Troponin I	NHS	−
Ortmann et al. [16]	2000	Human	FABP	NHS	−
			Troponin CT		−
			Desmin–myoglobin		−
			Fibrinogen–fibronectin		+
			C5b-9		+
Robert-Offerman et al. [17]	2000	Human	C9	NHS	+
Piercecchi-Marti et al. [18]	2001	Human	C9	NHS	+
Xiaohong et al. [19]	2002	Animals	Fibrinogen	>30 min	+
			Myoglobin		−
Fishbein et al. [20]	2003	Animals	CT-T, CT-I	>30 min	−
Panpìn et al. [21]	2006	Human	HIF-1α	≈2 h	+
Meng et al. [22]	2006	Animals Human	H-FABP	15 min–4 h NSH	−
Willam et al. [23]	2006	Animals	HIF-1	6–24 h	+
			PHD2, PHD3		−
Jasra et al. [24]	2012	Human	CT-I	<6 h	−
			C9		+
Hashmi et al. [25]	2013	Animals	Dystrophin	≥20 min	+
Kakimoto et al. [26]	2013	Human	SORBS2	<7 h	−
Bi et al. [27]	2013	Animals Human	S100A1	≥15 min <6 h-NHS	−
Kawamoto et al. [28]	2014	Human	npCX-43	NHS	+
			CX-43		−
Mayer et al. [29]	2014	Human	Dityrosine	≥4	+
Al-Salam et al. [30]	2014	Animals	GAL-1	20 > min < 60	+
Turillazzi et al. [31]	2014	Human	IL-15, MCP-1	≤4–6 h	+
Hashmi et al. [32]	2015	Animals	GAL-3	≥30 min	+
Jia et al. [33]	2015	Animals	CTnI	0.50–1 h	+
				>1 h	−
		Human		HS	−
Shabaiek et al. [34]	2016	Human	H-FABP	NHS	−

Table 1 (continued)

Authors	Year	Sample	Marker	Range of expression	Manner of expression
Sabatasso et al. [35]	2016	Animals	Troponin CT	≤1 h	−
			Myoglobin		−
			Fibronectin		+
			C5b-9	≤2 h	+
			npCX-43	≤30 min	+
			Jun B		+

+: positive staining; −: loss of staining

NHS: without histological signs (assumed time, ≤6 h); *HS*: with histological signs (assumed time, >6 h)

the myoglobin, the extensive positive reaction for fibrinogen and a slight positive reaction for C5b-9 [10].

Thomsen et al. conducted a study on C5b-9 complex in cardiac autopsy material. This study showed intense and specific immunohistochemical staining in the cases with limited possibility of histological diagnosis of myocardial ischaemia, particularly in subjects with an average survival time of 7–10 h post-infarction [11].

On the basis of the knowledge about the complement activation following the occurrence of ischaemia, Väkevä et al. studied the expression of C1, C3, C8 and C9 in a sample of rats killed at different time points after left coronary ligation. Immunostaining at 2 h showed foci of C3 deposition in the myocardial areas supplied by the ligated vessels, whilst the positivity of the other complement components was found at 3 h, where haematoxylin and eosin staining pointed out the earliest signs of myocardial infarction [12].

Zhang et al. analysed the expression of three cytoskeletal proteins—desmin, α-actinin and vinculin—in a sample of autopsy cases with sudden death. The immunohistochemistry showed the myocardial extensive loss of the proteins in the subject who died after 1 h from the onset of the symptoms; moreover, larger cellular depletion was observed for α-actinin and vinculin [13].

Hu et al. conducted a study on fibronectin expression in human autopsy material of subjects who died from definite or suspected myocardial infarction. In the suspected myocardial infarction group without microscopic evidences of ischaemic injuries, immuno-analysis showed positive staining for fibronectin in the cytoplasm of myocytes which was distributed in individual myocytes or group of myocytes, or in confluent areas, or segmentally in ischaemic myocytes [14].

Hansen et al. carried out a study on autopsy heart about the expression of cardiac troponin I. Significant reduction or total loss of staining was observed in the cases with definite infarcted areas, as well as in the groups of possible macroscopic signs of acute infarction with or without microscopic findings [15].

Ortmann et al. conducted a comparative study on the analysis of the depletion of the cardiac antigens FABP, troponin C and T, desmin and myoglobin, loss of CD59 and deposition of the plasma antigens fibrinogen, fibronectin and the complement complex C5b-9. These markers were tested in autopsy samples of subjects who died from acute myocardial infarction and acute cardiac death. The study confirmed the usefulness of C5b-9 as a marker of early myocardial ischaemia and highlighted that the depletion of cardiac antigens started earlier than the deposition of plasma antigens [16].

Another study regarding the complement C9 component was conducted by Robert-Offerman et al. in a human sample. The authors pointed out the positive staining only in some cases without definite histological signs of myocardial infarction and concluded that C9 immunostaining clearly pointed towards myocardial infarction in 47 % of cases, where routine histological evaluation was not conclusive [17].

Xiaohong et al. carried out an experimental study on the expression of fibrinogen and myoglobin in a sample of rats killed at different time points after left coronary ligation. Immunostaining showed local and incomplete depletion of myoglobin and light fibrinogen positivity in the 30-min group. These patterns gradually intensified with the prolongation of ischaemia (at 1, 2 and 3 h), and the staining extended from the subendocardium cells to the middle myocardium and subepicardial cells [18].

The same marker was studied by Piercecchi-Marti et al., who tested C9 in a human cardiac sample with histological signs of myocardial infarction and without or not obvious histological signs. The cases with non-obvious signs presented a well-documented history of myocardial ischaemia of less than 10 h old and severe or moderate atherosclerotic damage; the cases without histological signs presented severe atherosclerotic coronary disease and a survival estimated range of 1–3 h. The immunohistochemical study revealed intense cytoplasmic and sometimes membranous immunostaining in the group with myocardial ischaemia of less than 10 h, but no sensitive and no specific immunostaining in the subject where the death occurred within 1 h from the onset of the symptoms [19].

Fishbein et al. analysed the loss of cardiac troponins T and I in an experimental study conducted on animals that underwent permanent coronary occlusion for 0.5–6 h, or occlusion of 0.75–6 h followed by reperfusion, and with or without histological signs of myocardial necrosis. The study revealed that loss of both cardiac troponins T and I could be detected in the necrotic myocardium of animals with permanent occlusion. In these cases, cardiac troponin loss was greater at the periphery than at the centre of the lesions; moreover, loss of cardiac troponin T appeared earlier and greater than loss of cardiac troponin I. The same results are observed in the animals with occlusion followed by reperfusion and histologic evidences of myocardial necrosis. The authors highlighted that immunohistochemical staining for cardiac troponins T and I may be more sensitive than routine haematoxylin–eosin staining for the recognition of myocardial ischaemia over 30 min [20].

Pampìn et al. evaluated the immunohistochemical expression of hypoxia-inducible factor (HIF-1α) in cases of sudden cardiac death with suggestive circumstance of antemortem myocardial ischaemia, but neither microscopic evidence. The study showed positivity to antibody in about 79 % and particularly revealed myocyte nuclear staining followed by mixed nuclear and cytoplasmic staining. The authors observed nuclear positivity in the subpopulation with duration of previous symptoms ≤2 h and mixed staining in the subpopulation with duration of previous symptoms >2 h [21].

Meng et al. studied the heart-type fatty acid binding protein (H-FABP), performing an immunohistochemical study on an animal sample and forensic autopsy sample. The animal sample was represented by rats, with acute myocardial infarction produced by left anterior descending coronary ligation and killed at various ischaemia intervals. This study showed full depletion of H-FABP involving almost all myocardium in the group of rats killed after 4 h from coronary ligation and patchy depletion in the 2- and 1-h groups. In the autopsy sample, the authors showed extensive or focal loss of H-FABP in the group of subjects who died from suspected early myocardial infarction, where death occurred within 6 h from the onset of the symptoms [22].

Willam et al. analysed the expression of HIF-1α in relation to the expression of prolyl hydroxylase PHD2 and PHD3 that regulate the HIF activity. The study was conducted on a sample of rats subjected to ligation of the left anterior descending coronary artery and then killed after different postoperative intervals. The authors showed marked positivity of HIF close to the infarcted area and for PHD evaluated a minor patchy peri-infarct staining for PHD2 and a lesser staining for PHD3, at the 6-h and 1-day time points [23].

Jasra et al. tested the expression of cardiac troponin I (CT-I) and complement C9 component in a sample of autopsy cases with no histological signs of infarction (<6 h) and with microscopically visible signs with traditional H&E staining. The immunohistochemical analysis of all the cases <6 h highlighted negative to weakly positive staining for CT-I, demonstrating loss of cardiac antigen; more than 85 % of these cases showed a weakly positive staining or a few isolated cells staining for C9 [24].

Another study conducted by Hashmi et al. was on the expression of dystrophin in a sample of mice killed after induction of myocardial infarction at different time points. The immunohistochemistry of the 20- to 30-min post-infarction samples showed well-demarcated foci of complete sarcolemmal loss of dystrophin staining in the region supplied by ligated artery and areas of partial loss of membranous staining in vicinity to those [25].

Kakimoto et al. analysed the expression of sorbin and SH3 domain-containing protein 2 (SORBS2), an adapter protein that functions in cytoskeletal organization, cell adhesion and signalling pathways. The sample was represented by autopsy cardiac material of patients who died within 7 h from the onset of the symptoms in which there were histological signs of contraction band or wavy fibres or irregular patterns such as fragmented myocytes. The study showed different patterns in relation to the histological evidences: in contraction bands was observed a blurry immunostaining; in wavy fibres was observed the negative staining of some myocytes and the weakly staining of the adjacent myocardial cytoplasm; in irregular fibres, the immunostaining signals clearly disappeared from the Z lines. Thus, the immunohistochemistry showed the early loss of SORBS2 in infarcted heart tissues [26].

Bi et al. performed a study on the downregulation of S100A1, a dimeric Ca^{2+}-binding protein, in a sample of rats with induced myocardial ischaemia and in a sample of human myocardial tissue obtained from autopsies. In the animal sample, the authors highlighted the depletion of S100A1 in a few cardiomyocytes as low as 15 min after artery occlusion; after 4 h, the immunohistochemical staining showed well-demarcated areas of complete depletion of cytoplasmic staining. In the human sample without microscopic evidences of myocardial infarction, there were cases that showed massive depletion of S100A1 expression and cases with patchy depletion or sparse loss in a few myocytes [27].

Kawamoto et al. evaluated the expression of connexin43 (Cx43), non-phosphorylated Cx43 (np-Cx43) and zonula occludens-1 (ZO-1). Cx43 is an important gap junction protein in human cardiomyocytes localized at the intercalated discs in which stimuli such as ischaemia induce non-phosphorylation (np-Cx43) and redistribution to the cytoplasm and/or lateral cell border of myocytes; ZO-1 is a tight junction protein playing a role in anchoring Cx43 to the cytoskeleton. On the basis of this knowledge, the immunohistochemical study was conducted on a sample of human hearts obtained from autopsies. Particularly, the analysis showed positive distribution of Cx43 and np-Cx43 in intercalated discs, cytoplasm and lateral cell border; the positive distribution of ZO-1 was in the cytoplasm. The most important

findings resulted from np-Cx43 that was detected more frequently and intensely in the cytoplasm of the cardiac tissues of subjects who died from sudden cardiac death without apparent myocardial necrosis [28].

Mayer et al. performed a study to evaluate the expression of dityrosine as a protein product of oxidative stress to use in the diagnosis of early myocardial infarction. The study was conducted on a sample of autopsy heart of a human subject who died following myocardial infarction, then subdivided on the basis of the histological age. The authors showed intracellular space positivity in some cases with estimated survival times of 0–4 h and in most cases belonged to the group with survival times of 4–24 h. Positive staining was evaluated also in some cases of the control group in which the subjects died after a presumably shorter agony. Moreover, the authors compared these results with fibronectin and C5b-9 and assumed that dityrosine appears also quite early after an ischaemic event [29].

Al-Salam et al. tested the expression of galectin-1 (GAL-1) as hypoxia-induced protein of early myocardial infarction. The authors carried out a study on a mice sample with induced myocardial ischaemia at different time points, between 20 min and 24 h. The increased expression of GAL-1 was very well demarcated in all tested time points; moreover, there was evidence of an increase of demarcated areas of no or low expression in relation to the increase in ischaemia time, which means that as time proceeds, dying cells stop the expression of GAL-1 [30].

Turillazzi et al. performed an immunohistochemical study on some inflammatory mediators: CD15, IL-1β, IL-6, TNF-α, IL-15, IL-8, MCP-1, CD18 and tryptase. The study evaluated the expression of these mediators in an autopsy sample of subjects who died following myocardial infarction with survival times ranging from 4–6 h to more than 12 h from the onset of the symptoms. The most important results were observed for MCP-1 (monocyte chemotactic protein 1) and IL-15; in fact, immunostaining showed intense positivity of both markers in the very early phase between 0 and 4 h and their gradual reduction after 6 h [31].

Hashmi et al. studied the expression of galectin-3 (GAL-3), involved in mitosis, proliferation of cells and anti-apoptotic mechanism. The study was conducted on a sample of mice where the myocardial infarction was induced by left anterior descending coronary artery ligation; then, the animals were killed at different time points. The immunohistochemistry highlighted both cardiac myocyte and endothelial cell staining at 30–60 min and 4 h. A very characteristic pattern was evaluated in the 24-h post-myocardial infarction group; in fact, there was a high expression of GAL-3 in the area supplied by ligated artery, surrounding an area of very low or no expression of GAL-3. The area showing no expression of GAL-3 was the area of the infarct and consisting of dead myocytes, whilst the surviving cardiomyocytes and endothelial cells surrounding the infarct were the ones showing very high staining [32].

Jia et al. tested the expression of cardiac troponin inhibitor (CTnI), the inhibitor subunit that forms part of the regulatory troponin complex. The expression of CTnI was evaluated in samples of rabbits and human myocardium. In the animal study, the authors found increased cytoplasmic immunostaining for CTnI in 0.5 h of ligation, and after 1 h, the well-defined corresponding infarcted areas began to show a clear reduction in CTnI expression; so, as the ligation time increased, the immunoreactivity was reduced, and complete depletion was observed after 8 h of ligation. The immunohistochemical marker was also tested in the myocardial tissues of subjects who died from acute myocardial infarction demonstrating the partial or diffuse depletion of CTnI expression in the infarcted areas [33].

Shabaiek et al. [34] tested again H-FABP in autopsy hearts. The immunohistochemical study showed cytoplasmic staining by various degrees, ranging from minimal depletion and up to near-total depletion, in the group of hearts with probable signs of ischaemia and coronary stenosis.

Sabatasso et al. carried out an experimental study on the expression of troponins I and T, myoglobin, fibronectin, C5b-9, non-phosphorylated Cx43 (np-Cx43), JunB and cytochrome C. The study was performed on a sample of rats killed after induction of myocardial ischaemia at different time points. The authors reported the most important immunohistochemical results on the earliest expressions of np-Cx43 and JunB, in which immunopositivity was observed in the interval 15–30 min after coronary ligation. np-Cx43 positivity was in the gap junction, whilst JunB showed a nuclear positivity. A larger and more intense staining was observed, proportionally, with increasing durations of ischaemia. The other markers' expressions were observed in the interval 1–2 h [35].

Discussion

Acute myocardial infarction (AMI) is a common cause of sudden unexpected death and often follows severe coronary artery occlusion [36]. The clinical diagnosis of AMI can be easy using electrocardiography, biochemical markers and imaging [37].

Postmortem diagnosis is generally based on macroscopic evidences of myocardial necrosis and on routine histology findings. Haematoxylin and eosin staining is a useful tool in cases where polymorphonuclear leucocyte infiltration and cell death can be demonstrated. These findings begin to be detectable after 6–8 h from the onset of ischaemic injury, so the early diagnosis of myocardial infarction is a challenge for the forensic pathologist when it occurs before this period. This is

because several hours elapse for cells death, over the first 6 to 24 h [38, 39]. The earliest (before 6 h) histological signs described are mild myofiber eosinophilia, elongation of sarcomeres and nuclei, wavy fibres, interstitial oedema and contraction band, but these cannot be considered pathognomonic of myocardial infarction [40–44].

Many authors have suggested the use of immunohistochemistry to fill the gaps in the histological diagnosis of early myocardial infarction. In view of this, the current knowledge about the chronology of the myocardial tissue responses to the occurrence of ischaemia has an important role [45–47]. Fundamental are the knowledge about the innate immune response, the consequent inflammatory reaction, and the cellular and extracellular alterations. Thus, the immunohistochemical detection of immune-inflammatory and cellular phenomena accompanying the cardiac alterations during the early inflammatory phase of myocardial ischaemia could be an excellent diagnostic tool.

In this regard, the presented review summarises and analyses the studies about the immunohistochemical markers tested between 1990 and September 2016.

The most studied marker for the forensic diagnosis of early myocardial infarction is the membrane attack complex C5b-9. The C5b-9 complex is the terminal product of the complement system activation. MAC interacts and damages the plasma membrane, causing direct cell lysis [48]. The first studies about the C5b-9 complex demonstrated the immunohistochemical detection of the marker in the myocardial infarction areas, so it was judged to be specific for necrosis [49, 50]. Then, this antigen was detected in myocardial ischaemic tissues in the cases that do not show histological signs and/or after 30–40 min from the beginning of the ischaemic injuries [9, 10, 14]. On the basis of the experimental results, pathologists agree to consider it useful to visualize early ischaemic myocardial damage.

The literature review highlights that the other analysed markers are complement components and others are inflammatory mediators, cardiac cell proteins and plasma proteins.

The role of the activation of the complement system has been documented in both experimental and clinical studies of myocardial infarction [51–53]. Complement activation can occur through three pathways that begin with the fragmentation of the C3 component, to which follows the activation of C5 and the formation of the membrane attach complex C5b-9. The immunohistochemical studies were conducted for the diagnosis of early myocardial infarction on the complement components with regard to C1, C3, C8 and C9. All markers are tested in animal samples demonstrating an early immune positivity already 3 h after the occlusion of the coronary [12]. C9 components were also studied in human samples showing clearly positive immunostaining in half of the myocardial infarction cases within approximately 6 h [17]. Moreover, another study assesses the specificity and sensitivity of C9 expression as 85 % (62.11–96.79 % confidence interval) in the cases without histological evidences of myocardial infarction and survival times between 3 and 10 h [18].

Knowledge of the chronology of the responses of the myocardial tissue to the ischaemic injury, and particularly about the early inflammatory phase, has suggested the possibility to test some inflammatory mediators [54, 55]. The usefulness of these mediators is documented by one study that reports the immunohistochemical evaluation of CD15, IL-1β, IL-6, TNF-α, IL-15, IL-8, MCP-1, CD18 and tryptase. Whilst CD15, IL-1β, IL-6, TNF-α, IL-8, CD18 and tryptase show a larger immune reaction gradation over 6 h, IL-15 and MCP-1 reveal intense immunostaining very early, earlier than 4 h [31].

Several studies on the cardiac cell proteins concern: myoglobin, cardiac troponins I and T (CT-I and CT-T, respectively), heart-type fatty acid binding protein (H-FABP) and cytoskeletal proteins.

About myoglobin and cardiac troponins, the early loss of proteins detectable within 6 h from the onset of the symptoms is clearly documented in all immunohistochemical studies on human samples [10, 15, 16, 24]. These evidences are also associated with the results obtained from animal studies in which the loss of cardiac troponins and myoglobin appeared earlier [19, 35]; moreover, it was observed that the loss of troponin T appeared earlier and is greater than the loss of cardiac troponin I [20]. Moreover, there is a study on the expression of cardiac troponin inhibitor (CTnI), the inhibitor subunit that forms part of the regulatory troponin complex, which shows a clear reduction in CTnI expression from 1 h after the induced myocardial ischaemia [33].

H-FABP is an abundant protein in the cytoplasm of myocardial cells that set in the uptake and oxygenation of long-chain fatty acids. It has been demonstrated that H-FABP is more sensitive and specific than myoglobin for clinical detection of myocardial infarction within 12 h after the onset of the symptoms by quantifying its plasma concentration [56, 57]. The sarcolemmal damage due to ischaemia determines the cellular leaks of H-FABP and its passage in blood vessels. Thus, the immunohistochemical studies about H-FABP expression in myocardial infarcted tissues highlight the partial depletion of the marker after 15 min and the full loss after 4 h in experimental animal models [22]. The usefulness of the immunohistochemical detection of H-FABP is also demonstrated in human samples where the myocardial infarction was suspected and no or nonspecific histological signs were present [16, 22, 34].

The sarcolemmal damage following myocardial ischaemia is expressed by the cytoskeletal damage, and this could lead to cellular loss of the cytoskeletal proteins. The immunohistochemical studies reviewed the cytoskeletal alterations following early myocardial infarction with regard to desmin, vinculin and α-actinin [10, 13, 16]. These studies confirm the

depletion of cellular staining of all markers particularly detectable after 1 h from the onset of the symptoms. Also, comparing the three markers, desmin decrements later than vinculin and α-actinin. Moreover, a study about dystrophin, a cytoskeletal protein that forms a complex with glycoproteins involved in contractile force transmission and stabilization of the plasma membrane, has been reviewed [58, 59]. As well as for other proteins, the immunohistochemical study on a mice sample shows loss of dystrophin staining in ischaemic myocytes [25]. The dystrophin loss is detectable from 20 min after induced ischaemia, and it appears as areas of complete sarcolemmal loss and areas of partial sarcolemmal loss.

In the group of myocardial cell proteins tested as immunohistochemical markers of early myocardial infarction, there are positive findings about sorbin and SH3 domain-containing protein 2 (SORBS2) and non-phosphorylated connexin 43 (np-Cx43). The adapter protein SORB2 has been proven to be an early marker with different patterns of distribution in relation to the histological evidences, showing quantitative patterns of expression ranging from blurred loss to total loss according to findings of contraction bands, wavy fibres or irregular fibres [26]. About np-Cx43, the study conducted on human hearts shows intense staining of the cytoplasm in the cases without signs of myocardial infarction, confirming that ischaemic injury induces non-phosphorylation of Cx43, normally localized in intercalated discs [28, 60, 61]. The expression of this marker in rat samples shows very early staining (15–30 min), but a different distribution pattern (nuclear) [35].

The most important evidences of the literature review about plasma proteins concern fibronectin. The plasma extravasation following the cardiac matrix network degradation was highlighted. The protein contributes in the formation of a fibrin-based provisional matrix that serves as a scaffold for migration and proliferation of infiltrating inflammatory cells, endothelial cells and fibroblast [62]. The usefulness of fibronectin in the forensic diagnosis of early myocardial infarction is documented by experimental studies that show the overexpression of the antigen in various areas of the ischaemic tissue and an increased staining in relation to the increase of the interval between the coronary ligation and death of the animals [9, 63]. The positive staining in animal models was highlighted after 4 h from the induced ischaemia, and similar results were obtained in human myocardium [14, 16, 35].

The review showed that some markers were studied because the association with heart failure was highlighted. S100A1 is abundant in cardiomyocytes and appears rapidly in the serum after the clinical onset of myocardial ischaemia, demonstrating the cellular depletion of the protein [64, 65].

Table 2 Summary of the results for the main evaluated immunohistochemical markers

Marker	Staining	Time after onset		
		Animals	Human	
Fibronectin	Positive	≤1 h to ≥4 h	Without histological signs	
Troponins	Loss	>30 min		<6 h (CT-I)
Myoglobin	Loss	>30 min	Without histological signs	
Fibrinogen	Positive	>30 min	Without histological signs	
C5b-9	Positive	≤2 h	Without histological signs	
C9	Positive	≥3 h		<6 h
Desmin	Loss			≥1 h
α-Actinin	Loss			≥1 h
Vinculin	Loss			≥1 h
H-FABP	Loss	15 min–4 h	Without histological signs	
HIF-1α	Positive			≈2 h
Dystrophin	Loss	≥20 min		
SORBS2	Loss			<7 h
S100A1	Loss	≥15 min		<6 h
npCX-43	Positive		Without histological signs	
CX-43	Loss		Without histological signs	
Dityrosine	Positive			≥4 h
GAL-1	Positive	20 > min < 60		
GAL-3	Positive	≥30 min		
IL-15, MCP-1	Positive	≤4–6 h		
JunB	Positive	≤30 min		

Moreover, other studies show that in cardiovascular diseases, downregulation of S100A1 contributes to the progressive contractile dysfunction of the heart and increases cardiac-related death [66, 67]. On the basis of this knowledge, the protein was tested by an immunohistochemical study showing in an experimental animal model, in which ischaemia was induced, the very early myocyte depletion of S100A1. Also, the depletion was evaluated in human tissue without microscopic findings of myocardial infarction [27]. The same clinical evidences about galectin-3 were demonstrated [68–70], so this protein was tested for the diagnosis of early myocardial infarction, demonstrating positive results as very early loss of GAL-3 (between 30 and 60 min) from cardiomyocytes and endothelial cells in an experimental animal model [32].

Some reviewed studies examine the expression of stress- or hypoxia-induced factors expressed during ischaemia as a result of oxygen unavailability. These studies concern HIF-1, a factor studied in animal models that appears rapidly accumulated in the nuclei after exposure to hypoxic conditions [71, 72]. The possible use of HIF-1 as an immunohistochemical marker of early myocardial infarction is supported by evidences of nuclear or mixed (nuclear and cytoplasmic) immunostaining in the myocardial tissues of subjects who died both before and after 2 h from the onset of the symptoms [21]. In relation to the overexpression of HIF-1, there is also an immunohistochemical study about prolyl hydroxylase PHD2 and PHD3, which are involved in the HIF-alpha chain hydroxylation on which depends the inhibition of HIF function [73]. Comparing with the early increase of HIF, the analysis shows the low expressions of PHD2 and PHD3 [23].

Another hypoxia-induced protein tested is galectin-1 (GAL-1), which has been found in brain and lung hypoxic conditions [74, 75]. The immunohistochemical study shows GAL-1 expression in myocardial ischaemic tissue and an early positive staining which allows defining the ischaemic area within 24 h from the induction of ischaemia [30].

With regard to the protein products of oxidative stress, many authors describe the reactive species produced in ischaemic damage. These reactive species react with the proteins, causing the formation of amino acid radicals [76]. Knowledge about the oxidative stress factors suggests that these could be markers for the detection of myocardial infarction. Thus, one of the factors studied is dityrosine, which results from the interaction of the tyrosyl radicals with each other [77]. The most important results highlight the intracellular distribution of the dityrosine and positive immunostaining more documented in heart tissues from subjects with survival times of 4–24 h; also described is the dityrosine non-specificity for myocardial infarction [29].

The last evaluated marker is JunB, a transcription factor belonging to the activator protein 1 family, which regulates gene expression in response to a variety of stimuli including apoptosis [78]. The study shows very early nuclear expression, as soon as 30 min after rats' coronary ligation in the subendocardial region, and a gradient of expression towards the epicardium with increasing duration of ischaemia [35].

The most important results about the time and pattern of expression of the evaluated immunohistochemical markers are summarised in Table 2.

The reviewed studies show many limitations regarding the influence on the usefulness of immunohistochemical markers of some factors as autolysis or cardiopulmonary resuscitation. Only three studies refer to performing cardiopulmonary resuscitation and cardioversion (chemical or electrical) and merely highlight their influence on myocardial integrity [16, 26, 29]. For this reason, the immunohistochemical evaluation can be misrepresented, especially that related to the expression of myocyte proteins [15, 28]. The influence of postmortem phenomena is well studied on the C5b-9 complex, which results as a reliable marker even in myocardial tissue with advanced putrefaction and autolysis [11]. Moreover, a C9 study shows intense and specific staining up to 10 days on the infarcted areas after the experimental autolysis had begun [18]. There are no other evidences related to the reliability of other markers in cases with autolysis or putrefaction, but it is plausible to consider not useful myocyte proteins in light of the breakdown of the cells through decomposition.

In conclusion, all studies demonstrate that knowledge on the pathophysiological mechanisms involved in the response to myocardial infarction is a very important tool for the forensic investigation of early myocardial ischaemia.

The studies reviewed allow us to consider the important application of the immunohistochemical analysis of some biomarkers. In fact, immunohistochemistry has been shown to provide a substantial contribution on the issue of determining myocardial infarct age, particularly when the routine histological staining results are not specific. Moreover, the reviewed studies have focused on molecules with different tissue distributions that could be used in combination to demonstrate cellular and extracellular damage and to study the possible differences in the evolution of damage between these compartments.

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