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# Susceptibility of C5b-9$_{(m)}$ to postmortem changes

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**Summary.** C5b-9$_{(m)}$ is a specific and sensitive marker for myocardial cell necrosis. The diagnostic value of this marker would be considerably limited in forensic practice if its immuno-histochemical demonstration were hampered by putrefaction or autolysis. We could demonstrate C5b-9$_{(m)}$ immunohistochemically in necrotic myocardium due to infarction up to the 11th day of experimentally induced putrefaction and autolysis, when reliable demonstration of myocardial infarction with hematoxylin-eosin was no longer possible. Under the experimental conditions of this study, no false positive immunohistochemical staining occurred.

**Key words:** C5b-9$_{(m)}$ – Immunohistochemistry – Myocardial infarction – Putrefaction – Autolysis

**Zusammenfassung.** C5b-9$_{(m)}$ ist ein spezifischer und sensitiver Marker für Myokardzellnekrosen. Sein diagnostischer Wert in der forensischen Praxis wäre aber stark eingeschränkt, wenn die immunhistologische Darstellung des Komplexes empfindlich gegen Autolyse und Fäulnis wäre. Wir konnten auch nach elftägiger experimentell forcierter Autolyse und Fäulnis C5b-9$_{(m)}$ immunhistochemisch in infarziertem Myokard sicher nachweisen. Zu diesem Zeitpunkt wäre die Diagnose eines Myokardinfarktes in der Hämatoxylin-Eosin-Färbung nicht mehr möglich gewesen. Im Untersuchungszeitraum waren falsch positive immunhistologische Färbungen nicht zu beobachten.

**Schlüsselwörter:** C5b-9$_{(m)}$ – Immunhistochemie – Myokardinfarkt – Fäulnis – Autolyse

## Introduction

The literature contains few reports on the demonstration of myocardial infarction in decomposed myocardium. The acridine orange method appeared to be reliable for 2 or 3 days after death in warm weather [1], the microenzyme staining of malate dehydrogenase could show early infarction in refrigerated cadavers 4–5 days postmortem [2]. The haematoxylin basic fuchsin picric acid stain of Lie et al. [3] is hampered by its susceptibility to autolysis [4]. Zollinger used chromotrope aniline blue staining, but found that false negative results due to autolysis could not be excluded [5]. The fluorescence of haematoxylin and eosin stained sections is a useful way to demonstrate myocardial infarction in mild to moderately decomposed bodies [6].

Immunohistochemical detection of the terminal complement complex C5b-9$_{(m)}$ has been demonstrated in infarcted myocardium [7–9], even in cases of "sudden death" due to infarction and in cases with disseminated single cell necrosis [10].

It is unclear how long C5b-9$_{(m)}$ can be detected postmortem in advanced putrefactive and autolytic myocardium and whether these conditions produce unspecific cross-reactions that can result in false positive staining. We investigated the reliability of immunohistochemical detection of C5b-9$_{(m)}$ versus conventional hematoxylin-eosin staining in systematically induced autolysis and putrefaction of human myocardium.

## Materials and methods

Four hearts were taken at autopsy 1–2 days post mortem from cases of myocardial infarction, that were clinically diagnosed, confirmed macroscopically and microscopically. An average survival time of 7–10 hours post infarction was assumed, since hematoxylin-eosin staining merely exhibited general eosinophilia of the muscle fibers and depletion of the cardiac muscle nuclei with very rare leucocytic migration into the peripheral zone of the infarction. The hearts were stored in toto in a moist chamber at an ambient temperature of 18–22°C (hour "0" in our study). Thin specimens were taken at intervals over a period of 16 days (in one case 20 days) from marginal zones of infarcted myocardium in the left ventricle and embedded in paraffin. Each specimen included both infarcted and non-infarcted myocardial tissue. Two sections were cut from each block, one section was stained immunohistochemically with polyclonal antibodies against C5b-9 and the alkaline phosphatase anti-alkaline phosphatase (APAAP)-method, the other by conventional hematoxylin-eosin. Immunohistochemistry was performed as previously decribed in detail [9]. Myocardium from a different case of fresh myocardial infarction with disseminated single cell necroses and necroses of fiber bundles was used

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as a positive control. Non-infarcted myocardium from the 4 hearts in our study was used as a negative control.

## Results

*Hematoxylin-eosin staining*

*0–30 hours.* Infarcted myocardium was markedly eosinophilic and contained only a few indistinct nuclear remnants. Centrally located areas of degeneration, with segmental thickening and infrequent rupturing were seen. Transverse striation was effaced. A few granulocytes were present. Diagnosis of myocardial infarction was possible.

*30–100 hours.* Infarcted myocardial cells were increasingly fragmented. The fragments were dilated and dissociated, with occasional longitudinal cleavage. Nuclei could not be detected. Transverse striation was effaced. Leucocytes were markedly basophilic, pyknotic, and could not be differentiated. Diagnosis of myocardial infarction was possible.

*100–170 hours.* Infarcted areas could not be clearly distinguished. Zones that were clearly infarcted in previous preparations now possessed maculate, eosinophilic cell fragments. Diagnosis of myocardial infarction was only possible within limits.

*170–240 hours.* Abundant peripheral mycelia and ubiquitous bacterial clusters were now present. Wide clefts produced dissociated, homogeneously red-staining myocardial fragments. Eosinophiles were totally absent. Blurry, basophilic remnants could be seen; cell margins were indistinct. Connective tissue fibers were shrunken and widely-spaced; only a few pyknotic nuclei were evident. Diagnosis of myocardial infarction was not possible (Fig. 1).

*240–350 hours (up to 480).* Increasing formation of gas bubbles, with septum-like cleavage of homogeneously pale red-staining myocardium. The structure was almost totally effaced.

*Immunohistochemistry*

*0–170 hours.* Intense, specific staining of infarcted myocardial cells; no staining of non-infarcted areas. C5b-9$_{(m)}$ was detected chiefly in the inner layers of arterial walls. Diagnosis of myocardial infarction possible.

*170–340 hours.* Less intense, but specific staining of necrotic myocardium. Increased weak, pale-red background staining of non-infarcted areas; no false positive staining. Persistent intense staining of C5b-9$_{(m)}$ in some inner layers of arterial walls. Diagnosis of myocardial infarction possible (Fig. 2).

*Up to 390 hours.* Non-specific staining of myocardium, uniformly weak background staining, no false positivity. C5b-9$_{(m)}$ could be demonstrated in a few arterial walls. Diagnosis of myocardial infarction was not possible.

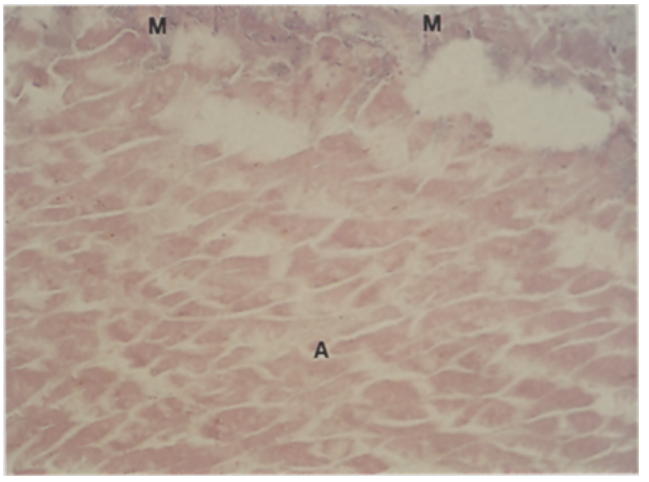


**Fig. 1.** Infarcted myocardium exhibiting experimentally induced putrefaction and autolysis (at 280 hours) with homogeneous pale red staining of myocytes. Diagnosis of myocardial infarction is no longer possible. *(A)* Artery; *(M)* mycelium; Hematoxylin-eosin staining, × 280)

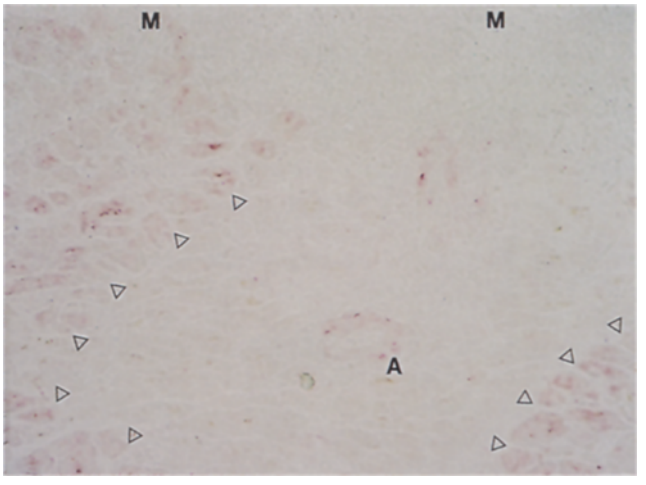


**Fig. 2.** Immunohistochemical demonstration of C5b-9$_{(m)}$ in infarcted myocardium with advanced autolysis and putrefaction (taken from the same specimen as in Fig. 1). Necrotic cells *(arrow heads)* and deep arterial wall layers *(A)* show weak but specific staining. Note the absence of staining in the non-infarcted area surrounding the artery. *(A)* Artery; *(M)* mycelium; APAAP, × 240)

*Up to 480 hours.* No false positive staining of C5b-9$_{(m)}$.

The changes caused by experimentally induced putrefaction and autolysis were relatively uniform and varied little in their chronological sequence. The immunohistochemical findings for C5b-9$_{(m)}$ are shown in Fig. 3.

## Discussion

Immunohistochemical demonstration of C5b-9$_{(m)}$ can aid the diagnosis of myocardial necrosis, even in very early ischemic injury [6–10]. C5b-9$_{(m)}$ has also been detected in the inner layers of arterial walls [11–13]. In the present study, C5b-9$_{(m)}$ was found to be a surprisingly reliable

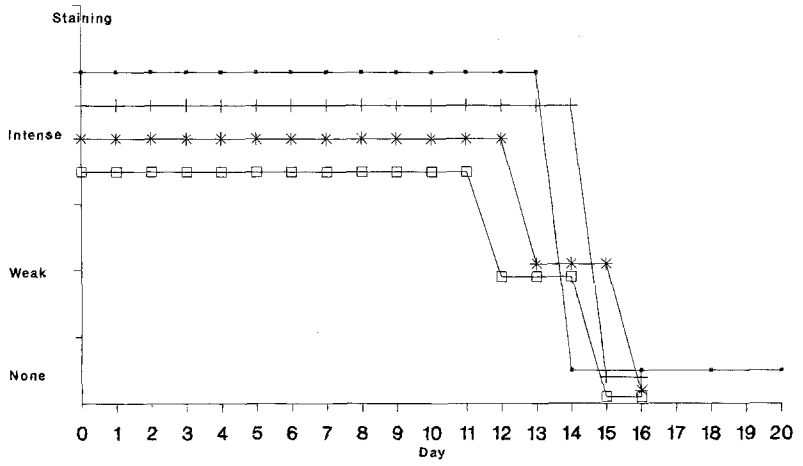


**Fig. 3.** Immunohistochemical detection of C5b-9$_{(m)}$ in experimentally induced putrefaction and autolysis of infarcted myocardium. (—⊡—) heart I; (—+—) heart II; (—✳—) heart III; (—⊟—) heart IV

marker of necrosis in myocardium with advanced putrefaction and autolysis up to the 11th day postmortem, in one instance even longer. Diagnosis of myocardial infarction using C5b-9$_{(m)}$ was possible after a longer postmortem interval than with conventional hematoxylin-eosin. False positive staining did not occur despite an increase in background staining with time.

However, due to the extreme experimental conditions employed (closed humid chamber, ambient temperatures between 18° and 22°C), the present findings do not allow a strict time limit to be placed on the duration of reliable C5b-9$_{(m)}$ staining. Diagnosis must take into account the degree of putrefactive and autolytic changes. By the 9th day postmortem the hearts were covered by fungal growth, the myocardial tissue had become saponaceous, gas bubbles had formed, and there was extensive liquefaction of subepicardial fat tissue.

Based on these results, immunohistochemical detection of C5b-9$_{(m)}$ can assist in the diagnosis of suspected infarction, even in myocardial tissue with advanced putrefaction and autolysis.

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