

# Utility of C4d Immunohistochemistry in Acute Myocardial Infarction

C Jenkins, D Cardona and S Normann

Pathology and Laboratory Medicine, University of Florida, Gainesville, FL, United States

**Background:** The pathologic presence of acute myocardial infarction (AMI) is determined by gross and histological examination. These findings are generally not seen early in the course of an AMI (<9-12 hours). The earliest indication of cell death is contraction band necrosis, which can be seen within minutes of reperfusion. However, it is often absent in typical infarction and is considered an artifact in endomyocardial biopsies. C4d (and C3d) is an end-product of the classical complement cascade which covalently binds the target tissue. The utility of C4d immunohistochemistry (IHC) is well described in the evaluation of antibody-mediated rejection of cardiac allografts. Full activation and involvement of the complement pathway following AMI has been shown. The aim of our study is to determine the specificity and potential timeline in C4d detection on necrotic myocytes following an AMI. **Design:** Sixteen autopsy cases with a total of 26 areas of infarcted myocardium were reviewed. The clinical record and microscopic examination of H&E slides showed that the areas of infarction ranged from a few hours to months in age. IHC was performed on paraffin sections of formalin-fixed tissue using the ABC/peroxidase method with C4d. Staining patterns and intensity were recorded. Five cases without evidence of infarction were used as controls and histologically normal myocardium functioned as an internal control. **Result:** C4d antibody strongly and diffusely stained necrotic myocytes in all cases of 8 to 48 hours in age (10/10). Adjacent histologically normal myocytes were nonreactive, resulting in clear delineation of damaged myocardium by C4d. Four cases with only scattered contraction band necrosis showed focal to no C4d reactivity, indicating that contraction bands may not be specific for necrotic myocytes. Three cases with 3-7 day old infarcts showed variable staining. Areas of fragmented myocytes showed focal weak staining. Infarctions of >1-2 months and negative controls were nonreactive. The use of C4d provided new diagnoses in two cases, including evidence of reinfarction and a newly diagnosed AMI. **Conclusion:** C4d is specific for necrotic myocytes as areas of histologically apparent necrosis stained strongly for C4d and normal appearing myocytes did not. C4d staining of necrotic myocytes is apparent prior to the influx of inflammatory cells making this a useful diagnostic tool in the event of uncertainty. The contrast achieved by IHC impressively creates a clear delineation between viable and necrotic myocytes, making C4d an efficient diagnostic tool.

Activation of the complement cascade following myocardial ischemic events has been well documented. Complement 4d (C4d) is one of the end products of this process, and it covalently binds to the target tissue. Therefore, following an acute ischemic insult C4d should covalently bind to necrotic myocytes. Areas of myocardial tissue that are not affected by a lack of perfusion should appear histologically normal and should not bind C4d.

Guidelines for assessing the age of an acute myocardial infarction (AMI) are well established by using gross and microscopic examination. There is, however, ambiguity in diagnosing infarction prior to the influx of inflammatory cells (<9-12 hours duration). The utility of immunohistochemical reactivity of C4d has been well described in evaluating antibody-mediated rejection of renal and cardiac allografts, while complement binding to necrotic myocytes has been shown in previous studies. However, no sensitivity or specificity related to C4d immunoreactivity has been determined. Studies have shown that complement plays a role in myocyte necrosis in acute myocardial ischemia as well as acting as a chemotactic factor for inflammatory cells. Determining the specific timeline at which C4d reactivity is present may establish C4d as a helpful tool in confirming the presence of AMI at time of autopsy, especially prior to the influx of inflammatory cells.

Through this study we hope to provide better assurance of the existence of an AMI by showing immunoreactivity to C4d early in the course of an AMI. Given the patient's clinical history, gross and histologic findings, and immunoreactivity for C4d, a lower limit of detection for myocardial infarction age will be determined.

All autopsy cases with myocardial ischemia from the University of Florida, between January 1, 2005 to August 1, 2007, were retrospectively collected. Thirty-one autopsy cases with a total of fifty areas of injury were reviewed with the clinical history, gross and histologic findings, and final diagnoses recorded. Histologic age of injury was determined by standard criteria (Table 1). Immunohistochemistry with commercially available C4d antibody was performed on paraffin sections of formalin-fixed tissue. Samples were considered immunoreactive for C4d when >10 adjacent cells stained positive. Extent of immunoreactivity was graded by comparing the overall area of perceived injury on H&E histologic sections to the area highlighted by C4d immunoreactivity. Each sample was determined to have a greater extent of injury, equivalent extent of injury, or lesser extent of injury by C4d immunoreactivity. Five cases without evidence of injury were used as controls and histologically normal myocardium functioned as an internal control.

Age of Injury	Gross Findings	Histologic Findings
0-8 hours	None	Contraction bands (Cb) or no apparent histologic alteration
8-16 hours	Occasional dark mottling	Cb, hemorrhage, myocyte hyper eosinophilia, nuclear pyknosis, coagulation necrosis, <b>no inflammatory infiltrate</b>
9-24 hours	Dark mottling	Cb, hemorrhage, myocyte hyper eosinophilia, nuclear pyknosis, coagulation necrosis, & <b>early polymorphonuclear (PMN) cell infiltrate</b>
1-2 days	Mottling with yellow-tan center	Cb, hemorrhage, myocyte hyper eosinophilia, nuclear pyknosis, coagulation necrosis, & <b>interstitial PMN cell infiltrate</b>
3-7 days	Hyperemic border, central yellow-tan softening	Hemorrhage, myocyte hyper eosinophilia, nuclear pyknosis, coagulation necrosis, interstitial PMN cell infiltrate, & <b>myocyte phagocytosis by macrophages at borders</b>
7-10 days	Maximally yellow-tan and soft, with depressed red-tan margins	Myocyte hyper eosinophilia, nuclear pyknosis, coagulation necrosis, <b>myocyte phagocytosis by macrophages, &amp; early granulation tissue</b>
10 days to 4 weeks	Red-tan depressed borders with progressive gray-white scar	Granulation tissue with early collagen fibrosis
>1-2 months	Scar	Dense collagen fibrosis

Table 1. Histologic Criteria for Determining Age of Injury

The suspected areas of myocardial injury were reviewed and are summarized as follows: 12 samples showed injury of 0-8 hours, 13 samples showed injury of 8-16 hours, 5 samples showed injury of 9-24 hours, 1 sample showed injury of 1-2 days, 3 samples showed injury of 3-7 days, 6 samples showed injury of 7-10 days, no samples showed injury of 10 days to 4 weeks, and 10 samples showed injury of >1-2 months in age. C4d antibody strongly and diffusely reacted with the necrotic myocytes in all samples of 8 hours to 2 days in age (19/19), (Figures 1 and 2, Table 2, Chart 1). Adjacent histologically normal myocytes were nonreactive, resulting in clear delineation of damaged myocardium by C4d (Figure 1). Twelve samples with only scattered contraction bands showed no C4d reactivity (0/12), (Figure 4). Overall, the level of reactivity declined significantly after three days duration. Two samples with 3-7 day old injuries showed no reactivity to C4d (2/3) and one 3-7 day old injury showed minimal reactivity (1/3) when compared to the histologically apparent area of injury. Areas of 7-10 day old injury showed no reactivity in two cases (2/6), minimal reactivity in three cases (3/6), and equivalent reactivity in one case (1/6). Injuries of >1-2 months and negative controls were nonreactive (Figure 3). Upon review of the final diagnoses at time of autopsy, the use of C4d provided new diagnoses in two cases, including evidence of reinfarction and a newly diagnosed AMI.

Age of Injury	Samples	C4d Immunoreactivity		Extent of Injury Determined by C4d vs Histology		
		Non-reactive	Reactive	Greater than	Equal	Less than
0-8 hours	12	12 (100%)	0 (0%)	0	0	0
8-16 hours	13	0 (0%)	13 (100%)	6	7	0
9-24 hours	5	0 (0%)	5 (100%)	1	4	0
1-2 days	1	0 (0%)	1 (100%)	1	0	0
3-7 days	3	2 (67%)	1 (33%)	0	0	1
7-10 days	6	2 (33%)	4 (67%)	0	1	3
>1-2 months	10	10 (100%)	0 (0%)	0	0	0

Table 2. Immunohistochemical Results: C4d Immunoreactivity vs. H&E

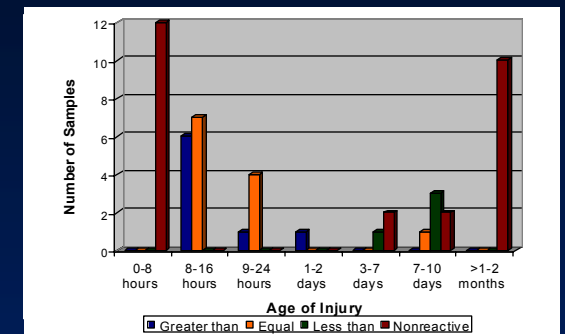


Chart 1. C4d Immunoreactivity by Age of Injury

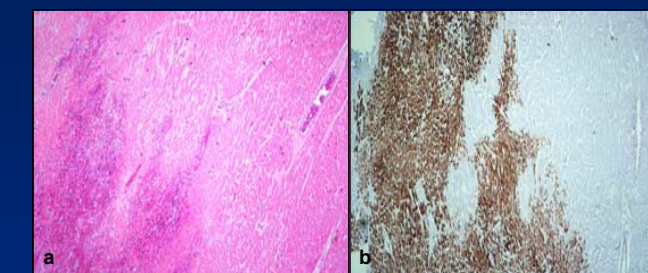


Figure 1. a) Hypereosinophilic myocytes and hemorrhage consistent with injury of 8-16 hours. b) Same infarcted myocardium shows strong reactivity to C4d creating a clear delineation between necrotic and viable myocytes.

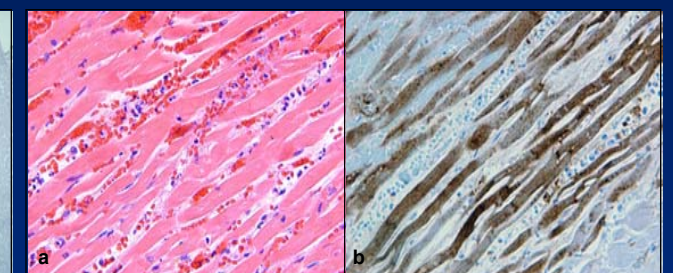


Figure 2. a) Hypereosinophilic myocytes with loss of nuclei and an interstitial polymorphonuclear cell infiltrate consistent with injury of 1-2 days. b) The same infarcted myocardium shows strong reactivity to C4d.

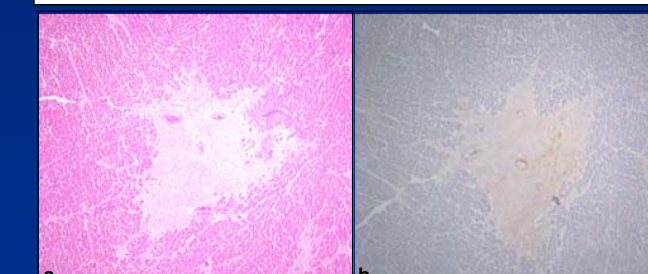


Figure 3. a) Dense collagenous scar consistent with an infarction >1-2 months in age. b) The same scar is nonreactive for C4d with only mild background staining.

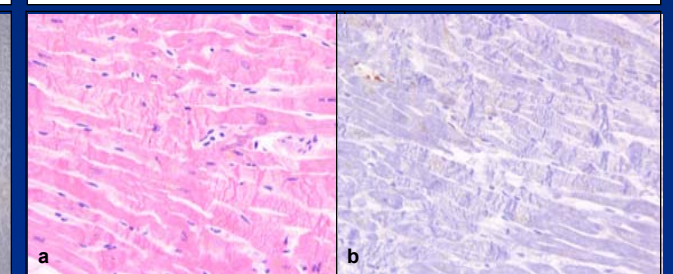


Figure 4. a) Contraction bands consistent with an infarction of 0-8 hours in age. b) The same sample is nonreactive for C4d with only mild background staining.

- Immunoreactivity for C4d is specific for necrotic myocytes and can label the areas of injury prior to the influx of inflammatory cells, making this a useful diagnostic tool in the event of uncertainty.
- The utility of C4d antibody appears greatest when used to diagnose a myocardial infarction of eight hours to two days in age, as the immunoreactivity is most intense within this timeframe.
- The lower limit of detection for myocardial infarction by use of C4d IHC is eight hours, or possibly less as reliable data is not available on documented infarcts of less than eight hours. All samples which showed only contraction bands by histologic examination were nonreactive for C4d.
- The contrast achieved by IHC creates clear and impressive delineation between viable and necrotic myocytes.
- The lack of immunoreactivity in areas of contraction bands suggests that either this change may not represent true necrosis or that it occurs prior to or possibly without the activation of the complement cascade.