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Review Article

Apoptosis in Human Acute Myocardial Infarction: The Rationale for Clinical Trials of Apoptosis Inhibition in Acute Myocardial Infarction

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The objective of the present review was to examine apoptosis in patients with acute myocardial infarction (MI) and to address (i) the prevalence of apoptosis in acute MI, (ii) techniques to determine apoptosis, (iii) time period from the onset of acute MI to the detection of apoptosis, (iv) criticisms about apoptosis in acute MI. A systematic literature search identified over 20 publications comprising over 400 patients. The prevalence of apoptosis varied from over 90% in nuclear imaging studies using annexin binding to 0.25% in an autopsy study using monoclonal antibody to single-stranded DNA. Apoptosis was present in 50–60% of infarcted hearts within 24 hours of MI (detected by Bax and activated caspase-3), 26% of myocytes in patients who died within 11 days of MI (pooled mean from 5 studies using only TUNEL staining), and 12% of the myocytes of patients who died, on average, 20 days after onset of MI (pooled mean from eight studies using dual staining with caspase-3 plus TUNEL). Criticisms of the TUNEL assay appear unjustified as TUNEL is at least 85% specific using caspase-3 activation as a marker of apoptosis. Taken together, DNA fragmentation on agarose gel electrophoresis, TUNEL staining of nuclei, caspase-3 activation, bcl-2 and Bax expression, and annexin V binding overwhelming support apoptotic cell death as an important component of MI. The amount of cardiac apoptosis correlates with the presence of heart failure and fatal arrhythmias. Heart failure as a complication of MI carries a high mortality and indicates the amount of myocardium lost during the infarct. Taken together, these findings suggest the need for clinical trials in acute MI to confirm whether inhibition of apoptosis can reduce patient morbidity and mortality.

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1. Introduction

Apoptosis, one of the main forms of cell death, is a highly regulated process which leads to the destruction of a cell utilizing a unique set of biochemical processes and leading to a characteristic set of morphologic changes [1]. Oncosis, the other major form of cell death, has been traditionally considered to be the dominant form of cell death in acute myocardial infarction (MI) [2]. There was a long delay from the initial description of apoptosis [1, 3] until the recognition of its occurrence in the heart [4]. Experimental models of ischemia followed by reperfusion, or hypoxia followed by reoxygenation induce apoptosis in the heart of different species including mice, rabbit, rat, dog, and pig [4, 5]. There is abundant experimental evidence that isolated cardiomyocytes undergo apoptotic cell death from activation of the extrinsic pathway through the activation of cell surface receptors or through the intrinsic pathway mainly involving mitochondria. Stimuli such as high concentrations of the saturated fatty acids, nitric oxide, some catecholamines, or cytokines induce apoptosis in isolated cardiomyocytes [6–9]. Despite the experimental evidence, there is recurrent concern that apoptosis does not occur in the human heart because it has not been demonstrated to occur in human heart to any meaningful extent. It is universally accepted, however, that the death of cardiomyocytes is the fundamental basis of myocardial infarction and its subsequent complications. Therefore, it is of paramount importance to clearly understand the extent to which apoptosis maybe the primary mode of cell death in patients with acute MI. The purpose of this paper is to critically review the evidence supporting the hypothesis that apoptosis occurs in patients with acute MI and to evaluate whether apoptosis, in the setting of acute MI, is a worthwhile therapeutic target.

2. Methods

The Medline database was searched from its inception to August 2008. The search terms were "apoptosis" and "myocardial infarction." The search was restricted to humans and further restricted to studies that examined the hearts of humans after the onset of an acute myocardial infarction.

This review was performed to specifically address the following issues: (i) prevalence of apoptosis in acute myocardial infarction, (ii) techniques that were used to determine whether apoptosis was present, (iii) time period from the onset of the acute myocardial infarction to the detection of apoptosis and its potential for producing variations in the prevalence of apoptosis in acute myocardial infarction, and (iv) criticisms in the literature about cardiac apoptosis in acute MI.

3. Results

A review of the human pathologic studies and their methodology for the detection of apoptosis shows that there were 20 autopsy studies over a ten-year period from 1995 to 2007 (Table 1). One study examined the myocardium in a patient with an infarcted intraventricular septum that was partially resected at surgery [10]. Studies excluded were those contained cases with profound ischemia without documented myocardial infarction and those that were explanted at the time of a cardiac transplant with ischemia but without a recent infarction or examined only the right ventricle. The present review, therefore, encompasses 437 acute MI patients that were included in studies that evaluated cases with documented myocardial infarction. As some of the authors published several of these papers and since the respective methodology sections did not specifically indicate which cases, if any, were included in other publications, it is assumed that no overlap exists in the cases reported across these studies.

Across these respective studies, there was found to be a wide range of allowable time intervals for patient inclusion from the initial onset of acute MI symptoms to eventual patient death. Some studies only focused on cases where death had recently occurred within 2 hours while other studies included cases 80 days after the acute MI. The time from the onset of symptoms to death is a determinant of the presence and thus the prevalence of apoptosis [14] as well as the assessment of comparability between studies. Two studies were subdivided as reported by the authors into different time periods from the onset of symptoms. Another issue is that 5 of 22 of the published studies did not report the time from the onset of symptoms to death. Most autopsies were performed within 24 to 30 hours of patient death because excessively long time periods would have limited the accurate detection of apoptosis [21]. Unfortunately, in half of the studies, the time from the death to autopsy is uncertain.

Different methodologies were utilized to assess the heart for the presence of apoptosis (Table 2). These methods encompassed direct detection of the specific DNA fragmentation characteristic of apoptosis seen on agarose gel electrophoresis, as well as immunohistochemical changes of DNA fragmentation or immunohistochemical evidence of activation of apoptotic pathways. Not all of these techniques can provide quantitative information. The results should be considered for their consistency between studies using the same methodology.

Apoptosis was originally defined based on distinct morphological features due to internucleosomal DNA degradation resulting from activation of specific endonucleases or DNAses [1]. The morphologic features of apoptosis include chromatic margination, nuclear condensation and fragmentation, as well as condensation of the cell with preservation of organelles [1]. The cellular enzymes active in the process of apoptosis also produce fragmentation of the cell into membrane-bound apoptotic bodies, which undergo phagocytosis by nearby cells without associated inflammation [3, 35].

3.1. Histologic Evidence of DNA Damage Consistent with Apoptosis. Histologic staining of the myocardium to show nuclei with specific DNA fragmentation has been accomplished by staining for in situ end-labeling (ISEL), TUNEL, or ssDNA. The early studies used ISEL or TUNEL and reported either the presence or absence of apoptosis with the conclusion that apoptosis was present in most cases of acute MI [10, 14, 16, 21] (Table 3). These studies, therefore, demonstrated that widespread apoptosis occurs in acute MI since apoptosis was evident after only a few hours after the onset of MI and prior to the appearance of necrosis [14]. In addition, apoptosis was not detected in normal myocardium [14]. In older infarcts, the incidence of apoptosis declined in myocytes, but increased in invading inflammatory cells [14].

Quantitation of the number of myocytes showing evidence of ISEL- or TUNEL-stained nuclei, as a proportion of the total myocytes nuclei visualized microscopically has been referred to as the "apoptotic index." The earliest study found that 12% of myocytes in the border zone of the myocardial infarct showed DNA strand breaks, whereas 1% of cells were undergoing apoptosis in the remote myocardium; indicating a significant increase in apoptosis in acute MI [12]. Subsequent studies reported apoptotic indexes using this detection methodology range from 0.8 to 43.6% (Table 3). Although the 0.8% value is the outlier, it is from a study in which the occluded artery had been reopened, potentially, minimizing the amount of cell death. This value is lower than the level of apoptosis in a similar study [30]. Some of the studies report data as the mean and others as median. If one assumes that the two indexes of central tendency are similar and calculate a weighted average, then 25% of myocyte nuclei show evidence of apoptosis in acute MI (Table 3). Apoptosis index varies dramatically across studies, in part because some studies did not indicate from which region of the infarct the apoptotic index was derived [18, 19]. This is unfortunate because there is little or no TUNEL staining in the core of the infarct where cellular nuclei are either absent or severely disintegrated [23], considerable TUNEL-stained nuclei in the border zone and much less at a site remote form the infarct. The increase in apoptotic cells in the border zone around the infarct area was consistently many fold greater than the area of myocardium remote from the infarct [12, 34].

Table 1: The number of studies that examined apoptosis in patients with acute MI, the number of cases, the time from the onset of symptoms to death, and the time from death to autopsy. (Studies present the times as either mean or median.)

Author	Year	Number of MI	Time after onset MI	Time death to autopsy
Itoh et al. [11]	1995	19	"12 hours to several days"	Uncertain
Olivetti et al. [12]	1996	20	~4 (<10) days	<24 hours
Bardales et al. [13]	1996	32	Uncertain	Uncertain
Veinot et al. [14]	1997	8	Uncertain	<24 hours
Saraste [15]	1997	8	38 (6 to 120) hours	17.3 (4 to 48) hours
Toyoda et al. [10]	1998	1	7 days	0*a*
James [16]	1999	12	Uncertain	Uncertain
Ottaviani et al. [17]	1999	10	<2.5	<24 hours
Piro et al. [18]	2000	16	0.4 days (<14 hours)	Uncertain
Rodríguez-Calvo et al. [19]	2001	14	2.9 (0.5 to 11) days	Uncertain
Abbate et al. [20]	2002	24	~27 (15 to 40) days	Uncertain
Nakatome et al. [21]	2002	6	<2 hours	<24 hours
Nakatome et al. [21]	2002	6	0.5 to 5 hours	24 to 63 hours
Baldi et al. [22]	2002	16	23 (12–62) days	<30 hours
Edston et al. [23]	2002	10	Uncertain (Sudden deaths)	3.6 days
Bussani et al. [24]	2003	12	~20 (14 to 44) days	Uncertain
Abbate et al. [25]	2003	30	23 (14 to 40) days	Uncertain
Abbate et al. [26]	2003	14	~16 (12 to 34) days	Uncertain
Biondi-Zoccai et al. [27]	2004	21	~25 (11–80) days	<30 hours
Biondi-Zoccai et al. [28, 29]	2005	21	~22 (12–50) days	Uncertain
Abbate et al. [30]	2005	30	17 (9 to 60) days	<30
Akasaka et al. [31]	2005	43	Uncertain	<6 hours
Zidar et al. [32]	2006	30	<24 hours	Uncertain
Zidar et al. [32]	2006	20	>24 hours	Uncertain
Sinagra et al. [33]	2007	14	18.8 (10 to 62) days	Uncertain

The presence of TUNEL staining in nuclei of myocardial cells is a function of the time from death to autopsy because postmortem autolysis damages the DNA and limits detection of the characteristic apoptosis. Nakatome et al. reported that all 6 cases with autopsies between 5 to 30 hours after death showed TUNEL-positive cells in the infarct while none of 6 cases who had autopsies 24 to 63 after death showed TUNEL-positive cells [21].

Monoclonal antibody to single-stranded DNA (ssDNA) is more specific and sensitive cellular marker of apoptosis than TUNEL. Akasaka et al. studied 43 persons with acute MI at autopsy and related the detection of ssDNA to histologic estimation of the stage of MI from earliest, to coagulation necrosis, macrophage infiltration, granulation tissue, and scar tissue [31]. This study is difficult to relate to the others because it reported more stages of MI than hearts examined. The maximum prevalence of apoptosis was only 0.25% [31]. As the time from death to autopsy is uncertain in that study, it is possible that this variable influenced their results. It is difficult to reconcile this assessment of apoptosis in MI with the other studies of apoptosis in the heart (Table 3).

3.2. DNA Fragmentation on Agarose Gel Electrophoresis. DNA fragmentation into fragments of 180 to 200 base pairs detected on agarose gel electrophoresis is the hallmark of apoptosis because of the activation of specific endonucleases during on the apoptosis process [1]. Itoh et al. were the first to report DNA fragmentation in the infarcted myocardium but not in normal myocardium [11]. It has been examined in seven studies and the characteristic DNA fragmentation has been consistently demonstrated (Table 2) in the myocardium of patents following MI [11, 12, 14, 18, 19, 21, 34]. In contrast, there is no DNA fragmentation in hearts from individuals who did not die of MI or in normal myocardium remote from the MI [12, 14, 19]. The DNA ladder pattern is found in cases of MI less than 72 hours old and more often with high prevalence of apoptotic DNA [19]. DNA fragmentation was seen in the early stages of AMI (12 to 24 hours) and was not seen in older infarcts [14]. With the caveat that most studies evaluated only a few cases from their study population, the consistency of the findings across all studies in which it was examined is noteworthy.

3.3. Caspase-3. Caspases are a family of proteases that cleave cellular polypeptides with the subsequent production of the majority of cellular and morphological events that occur during apoptotic cell death [3]. Caspase activation is unique to apoptosis as it does not occur in other forms of cell death and provides strong evidence for the presence of apoptosis [35]. Caspase-3 activation has been found in each of the 9

Table 2: Methodologies used to determine the presence of apoptosis in the heart.

Author	Year	Number MI	DNA gel electrophoresis	ISEL or TUNEL	Caspase-3	ssDNA	Bax
Itoh et al. [11]	1995	19	Yes	Yes	No		
Olivetti et al. [12]	1996	20	Yes	Yes	No		
Bardales et al. [13]	1996	32	No	Yes	No		
Veinot et al. [14]	1997	8	Yes	Yes	No		
Saraste et al. [34]	1997	8	Yes	Yes	No		
Toyoda et al. [10]	1998	1	No	Yes	No		
James [16]	1998	12	No	Yes	No		
Ottaviani et al. [17]	1999	10	No	Yes	No		
Piro et al. [18]	2000	16	Yes	Yes	No		
Rodríguez-Calvo et al. [19]	2001	14	Yes	Yes	No		
Abbate et al. [20]	2002	24	No	Yes	Yes		
Nakatome et al. [21]	2002	12	Yes	Yes	No		
Baldi et al. [22]	2002	16	No	Yes	Yes		Yes
Edston et al. [23]	2002	10	No	Yes	No		
Abbate et al. [25]	2003	30	No	Yes	Yes		
Bussani et al. [24]	2003	12	No	Yes	Yes		
Abbate et al. [26]	2003	14	No	Yes	Yes		
Biondi-Zoccai et al. [27]	2004	21	No	Yes	Yes		Yes
Biondi-Zoccai et al. [28, 29]	2005	21	No	Yes	Yes		Yes
Abbate et al. [30]	2005	30	No	Yes	Yes		
Akasaka et al. [31]	2005	43	No	No	No	Yes	
Zidar et al. [32]	2006	50	No	No	Yes		
Sinagra et al. [33]	2007	14	No	Yes	Yes		

studies of acute MI in which it was examined [20, 22, 24–27, 30, 32, 33]. Immunohistochemical examination of MI demonstrated caspase-3 activation in myocardial cells in 60% of patients who died within 24 hours of the onset of MI, 30% of those dying 1 to 7 days after the infarct, and in none of the cases dying 1 to 4 weeks after the onset of infarction [32].

Because caspase activation is a relatively specific marker of apoptosis, investigators have used double staining of cardiomyocytes with TUNEL plus caspase-3 as the criteria to indicate apoptosis. Using this set of criteria, the apoptosis index varies from 5.9 to 25.4%. On average, 13.2% of cardiomyocytes in the heart of patients dying of acute MI manifested apoptosis (Table 3). The dual labeling (TUNEL plus caspase-3) approach has been utilized mainly by a common group of investigators. Unfortunately, most of the studies using the more specific TUNEL plus caspase-3 criteria examined heart of patients who had died 20 days after the onset of MI (Table 1). Considering that caspase-3 activation is maximum within 24 hours after acute MI and declines thereafter [32], the percentage of apoptotic cells using this criteria is dependent of the time after acute MI that the heart was examined.

Caspase-3 activation was significantly greater in patients who received reperfusion treatment compared to those that did not receive this form of therapy [32]. The site of the infarct that expresses the greatest amount of caspase-3 activation, that is, center or border zone is unresolved between studies [22, 32].

3.4. Bax and Bcl-2 Expression. The Bcl-2 family of proteins regulate the various protein-protein and protein-membrane interactions affecting the permeability of the outer mitochondrial membrane which maybe viewed as the point of no return in apoptotic cell deaths [36]. Two important Bcl-2 signaling molecules in apoptosis related to the intrinsic or mitochondrial pathway in apoptosis are Bax and bcl-2 [36] both of which regulate caspase activation.

Myocytes with positive bcl-2 immunoreactivity were seen in 60% of hearts with acute MI while there were no myocytes with positive bcl-2 immunoreactivity in the controls or in hearts with old MI [37]. There is a predominance of bcl-2 localized to the border areas surrounding the infarcted tissues [37]. Piro et al. did not find immunoreactivity for bcl-2 but attributed this failure to the autolysis process as most of their specimens for analysis of bcl-2 were taken 24 hours after death [18]. Bax expression is increased in acute MI. Bax immuno-reactivity is evident in 52% of myocytes at the site of the infarction which is significantly greater than in areas remote from the MI [22]; Bax protein expression colocalizes with TUNEL [22]. Myocardial Bax expression was increased in patients with multivessel disease [27].

3.5. Demonstration of Apoptosis in Patients with MI by Cardiac Imaging. The studies reviewed above have been mainly autopsy studies with the attendant concerns about post-mortem changes in tissue and technical issues surrounding TUNEL or caspase-3 immunohistochemistry. Annexin V is

Table 3: The percentage of confirmed apoptosis using different assays. (Data are presented in two ways: the data in parenthesis is the percentage of infarcted hearts with apoptosis; otherwise it is the percentage of cardiomyocytes that show evidence of apoptosis.)

		TUNEL	TUNEL + Caspase	TUNEL ± Caspase	Casp-3				
	N	MI (%)	MI (%)	Remote (%)	% Hearts	Control (%)	*N*	Control	Remote
Olivetti et al. [12]	20	12 B		0.74					$P < .05$
Bardales et al. [13]	32	(All)				8	13		
Saraste et al. [34]	8	0.8 B		0.005		0.007	6		
Veinot et al. [14]	8	(All)				0	3		
James [16]	12	(All)							
Ottaviani et al. [17]	10	A				A	3	$P < .05$	
Piro et al. [18]	16	43.6		38.1		0	0	Not done	ns
Rodríguez-Calvo et al. [19]	14	36				0	5		
Nakatome et al. [21]	6	(All)				0			
Abbate et al. [20]	24		19.5	0.5					$P < .001$
Baldi et al. [22]	16		25.4	0.7					$P < .001$
Edston et al. [23]	10	26				0	8		
Abbate et al. [25]	30		11	4					
Abbate et al. [26]	14		19						
Bussani et al. [24]	12		16.7						
Biondi-Zoccai et al. [27]	21			0.71		0.01	4		
Biondi-Zoccai et al. [28, 29]	21		8.1	0.41+		0.01	4		
Abbate et al. [30]	30		6.9	0.9		0.01	5	$P < .01$	$P < .01$
Zidar et al. [32]	30				(60)	0	5		
Zidar et al. [32]	20				(15)	0	5		
Sinagra et al. [33]	14		5.9						

A: data expressed as arcosine
All: present in all hearts and there is no quantitative data
B: border zone
+: the data are extracted from the graph.

a naturally occurring human protein that binds avidly to membrane-associated phosphatidylserine which is actively transported to the outer layer as an early event in apoptosis, and indicated the presence of apoptosis in isolated cardiomyocytes [38]. In six of seven patients, increased uptake of Tc-^{99}m-labelled annexin-V was seen in the infarct area of the heart on nuclear (SPECT) imaging [39]. No increased uptake was seen in the heart outside the infarct area. All patients with increased Tc-^{99}m-labelled annexin-V uptake in the infarct area showed a matching perfusion defect [39]. In a control individual, no increased uptake in the heart was seen [39]. In another series of 9 patients, all patients showed accumulation of Tc-^{99}m-labelled annexin A5 at the infarct site [40]. These data suggest that 94% of patients with acute MI manifest apoptosis [39, 40]. The major critique of this methodology is the inability to distinguish between apoptosis in cardiomyocytes from apoptosis in the other cell types in the heart, and annexin V labeling of platelets in the coronary thrombus producing the infarct [41, 42]. Although labeling of platelets may not be that relevant of an issue [43], the proportion of the total labeling that can be confirmed to be only due to myocytes remains a challenge. This technique, however, has validity [44] and provides supporting evidence for the existence of apoptosis in the process of acute MI.

3.6. Relationship of Apoptosis to Reperfusion Injury or Heart Failure. While experimental data support a relationship between apoptosis and reperfusion injury, it is difficult to obtain this kind of data in patients after acute MI in large part because autopsy studies do not come from randomized trials comparing reperfusion and control therapy. However, apoptosis was associated with histologic features of myocardium that is reperfused after brief ischemia whereas necrosis occurs more often in areas of prolonged ischemia without reperfusion [14]. DNA fragmentation (ISEL) was visible in recent infarcts primarily in myocytes containing contraction bands, which occur predominantly in regions of reperfused myocardium [14]. The myocardium supplied by an occluded infarct-related artery (IRA) was associated with significantly higher amount of apoptosis compared to the myocardium supplied by a patent IRA [30, 45]. The investigators suggested that the benefits observed with a patent IRA may in part be due to reduced myocardial apoptosis [30, 45].

The amount of apoptosis present in the heart at autopsy is a significant indicator of the presence of heart failure. Considering that heart failure was a complication of MI that carried a high mortality and acts as a major indicator of the amount of myocardium lost during the infarct, individuals who experienced early occurrence of heart failure had a

four-fold increase in the prevalence of apoptosis compared to individuals without heart failure [26]. Multivariate analysis including variables such as the amount of apoptosis at autopsy, the use of fibrinolytic agents, as well as the history of a previous remote MI showed that only heart failure remained significantly associated with increased apoptosis [26]. Individuals with poor prognosis at the onset of acute MI, assessed by the Norris coronary prognostic index (age, presence of pulmonary congestion, heart size, and history of previous MI), had a higher prevalence or greater proportion of apoptosis both in the infarct as well as the area remote from the infarct compared to low-risk individuals [25]. Patients with biventricular enlargement had extremely high cardiomyocyte apoptosis at necropsy compared to individuals who had no cardiac dilatation after MI [24]. Patients with severe postinfarction LV diastolic dysfunction had significantly higher rates of apoptosis, determined by colocalization of TUNEL and caspase-3 [33]. Thus, the amount of apoptosis is an important determinant of the amount of left ventricular dysfunction which in turn is a major determinant of an unfavorable outcome.

3.7. Potential Relevance of Apoptosis in the Border Zone of Acute MI. Several studies have shown that apoptosis is greater in the border zone of MI or the area at the border of the infarct compared to normal myocardium [31, 34]. The number of apoptotic nuclei is consistently greater in the peri-infarcted region than in that away from infarction [12]. Potentially fatal arrhythmias such as ventricular tachycardia can originate in pathways in the heart at the border zone of an acute MI [46, 47] perhaps due to the mixture of living and death cells at the border zone [12, 31]. Thus, apoptosis may be an important component in the generation of potentially fatal cardiac arrhythmias.

4. Discussion

The major contribution of this paper is the synthesis of data involving over 400 MI patients whose myocardium was examined for the presence of apoptosis. The demonstration of DNA fragmentation, caspase-3 activation, Bax expression, and annexin V binding provides overwhelming support for the contention that apoptotic cell death is a component of myocardial infarction in acute MI patients. The major question is whether the extent of apoptosis in acute MI was evaluated. The differences in the technique that is used to quantitate apoptosis makes it difficult to compare the studies but it is estimated that the amount of apoptosis in the initial (24 hours) of acute MI may be approximately 60% of cases based on myocytes that demonstrate the presence of Bax, Bcl-2, or caspase-3 activation [22, 32, 37]. After 20 days, 12% of myocytes show evidence of DNA fragmentation (TUNEL) and caspase-3 activation in the absence of signs of DNA repair [20, 22, 24–27, 30, 32, 33].

Most of the studies have relied, all or in part, on TUNEL staining of nuclei to conclude that apoptosis was evident in MI. A number of the earlier studies presented dichotomous results namely that TUNEL-positive cells were either present or not present. The existence of apoptotic nuclei suggested that apoptosis was present in all cells in acute MI and clashed with prevailing thinking of the dominance of oncosis as the main form of cell death in acute MI [2]. Criticism of the TUNEL methodology, upon which opinions of the prevalence of apoptosis in MI were based, was powerful. Some investigators contend that there is no direct evidence of apoptosis in the heart and rather "all of the evidence for apoptosis in cardiomyocytes is indirect, based on detection of DNA fragmentation and/or so-called apoptosis-related factors" [48]. This contention is based on the following arguments: (i) electron microscopy is the gold standard for the diagnosis of apoptosis and no human studies have utilized this methodology to establish apoptosis in acute MI [48], (ii) TUNEL methodology does not have a high specificity for apoptosis because it detects both apoptotic as well as oncotic cell death [49], (iii) TUNEL can also indicate DNA activity associated with DNA repair or increased proteins synthesis and not only apoptosis [50, 51], (iv) DNA fragmentation in the nucleus is not necessarily related to apoptotic morphology in the nucleus or other indicators of apoptosis such as caspase activation [49, 52], (iv) DNA ladders, as performed, are nonspecific for cardiomyocytes as they are from extracts of heart that may also contain the nonmyocytes elements of the heart [48]. Another criticism of the occurrence of apoptosis in the heart questions whether cells initially undergoing apoptosis eventually die by oncotic mechanisms so that apoptosis is not a major concern for the heart. Stated in another way, the triggers for both kinds of cell death can be one and the same, and the cell may switch from one type of cell death (apoptosis) to the other (oncosis) mode of cell death [53]. Secondly, DNA fragmentation as indicated by TUNEL or caspase activation does not inevitably mean cell death [54]. These criticisms can be examined by applying the patient data examined in this study.

4.1. Analysis and Rebuttal to the Critique of Apoptosis in Acute Myocardial Infarction.

TUNEL methodology does not have a high specificity for apoptosis because it detects both apoptotic as well as oncotic cell death. This criticism was initially advanced by Ohno et al. based on the poor correlation of electron microscopic (EM) evaluation for signs of apoptosis and TUNEL staining of cardiomyocytes in rabbit heart subjected to 30 minutes of ischemia followed by reperfusion [49]. There are two camps on this issue. Takemura and Fujiwara contend that apoptosis can only be conclusively identified by EM which does not identify enough of the characteristic morphologic features to substantiate the existence of apoptosis in cardiomyocytes [48]. In contrast, the other position has been well formulated by Anversa who contend that EM evaluates too small a sample of the myocardium or cardiac nucleus to be able to identify cardiac apoptosis so that immunohistochemical techniques are preferred [55]. Nevertheless, there are EM data to substantiate that apoptosis does exist in human heart [56]. Studies in human heart with classic indicator of apoptosis, namely, caspase-3 activation found a high correlation of TUNEL-positive cells with caspase-3, as 85% of TUNEL-positive cells also manifest caspase-3 activation

[22]. Considering that the processes of DNA fragmentation and caspase-3 activation should not necessarily be expected to occur simultaneously as the process of apoptosis takes time to complete, a 100% correlation should not be anticipated. Furthermore, the classic marker of apoptosis, DNA ladder pattern in cases of MI, correlated strongly with a high prevalence of TUNEL-stained nuclei in cardiac myocytes [19]. The high correlation between TUNEL and caspase-3 activation and DNA laddering assures that TUNEL is at least 85% specific for the presence of apoptosis in the heart and supports the contention that TUNEL-positive cells are primarily apoptotic cells.

TUNEL can also indicate DNA activity associated with DNA repair or increased protein synthesis and not only apoptosis. False-positive TUNEL staining may be more of a problem in heart failure or cardiomyopathy [51]. In patients with MI, however, TUNEL-positive-stained cardiomyocytes are overwhelmingly negative for immunohistochemical evidence of DNA repair. Specifically, none of the myocytes (0%) which coexpressed TUNEL and caspase-3 were positive for PCNA or SC35 which, respectively, indicates an increased DNA and RNA synthesis [22]. Thus, during the early part of the time frame and sequence of cellular events that occur during an acute MI, TUNEL-positive cells do not represent cells undergoing DNA repair or increased protein synthesis.

DNA fragmentation on agarose gel electrophoresis is not specific for cardiomyocytes. Although DNA fragmentation of agarose gel electrophoresis is the classic hallmark of apoptosis, criticism of this data centers on the uncertainty of how much cardiomyocytes rather than the other elements of the heart contributed to the generation of the DNA fragmentation and the inability of this technology to provide data on what percentage of cardiomyocytes underwent apoptosis. However, DNA fragmentation on agarose gel was observed mainly in the areas of the myocardial infarction that had the majority of apoptotic cells identified by intense ISEL- or TUNEL-stained nuclei of cardiomyocytes [34]. Similarly, there was no relationship between areas of the myocardium that manifested DNA fragmentation and the distribution of ISEL-positive nonmyocyte or inflammatory cells [34].

TUNEL-positive cells may include apoptosis in other elements of the heart and in inflammatory cells. Elements of the heart other than cardiomyocytes undergo apoptosis after acute MI. Inflammatory cells that invade the infarct also undergo apoptosis. This event is a later stage in acute MI while the greatest amount of apoptosis occurs before the inflammatory response [14]. While these non-cardiomyocytes can stain TUNEL, autopsy studies comment specifically on their evaluation only of the myocyte element of the heart either directly or using special stains for myocytes [14, 22, 25, 27, 45]. Thus, there is only a low probability that TUNEL-positive cells represent apoptosis in non-cardiomyocyte elements of the heart.

Cardiomyocytes undergoing apoptosis eventually die by oncotic mechanisms so that apoptosis is not a major concern for the heart. Currently available data shows a strong relationship between evidence for apoptosis and the severity of the myocardial infarction thus providing a compelling argument that apoptosis is an important process in the heart during acute MI. The amount of apoptosis present in the heart at autopsy is a significant predictor of the presence of heart failure—a complication of myocardial infarction that carried a high mortality. Individuals who experienced early occurrence of clinical manifestations of heart failure had a four-fold greater prevalence of apoptosis compared to individuals who did not have heart failure [25, 26]. Patients with biventricular enlargement had extremely high rates of cardiomyocyte apoptosis, compared to individuals who had no cardiac dilatation after MI [24]. Patients who died after an acute MI with severe postinfarction left ventricular (LV) diastolic dysfunction had significantly higher rates of apoptosis than those without LV dysfunction [33]. Thus, the amount of apoptosis is an important determinant of the amount of LV dysfunction which in turn is a major determinant of an unfavorable outcome pertaining to patient morbidity and mortality.

DNA fragmentation as indicated by TUNEL or caspase activation does not inevitably mean cell death. Clinical trial data on the effect of interruption of apoptosis on clinical outcomes in patients after acute MI would address this issue but are absent. This necessitates the reliance on experimental data. These studies can be grouped into two kinds. Those studies using genetic overexpression or knockout models have been uniformly supportive of a role for apoptosis in acute MI while studies with synthetic caspase inhibitors have been controversial. Caspase-3 transgenic mice that had cardiac tissue-specific overexpression of the proapoptotic gene caspase-3 manifested increased infarct size and a pronounced susceptibility to die after experimentally induced MI [57]. Adenoviral gene transfer of the caspase inhibitor p35 leads to a significant reduction of myocardial infarct size and improved cardiac function after induction of acute MI in the rat [58]. Caspase-1-knockout (KO) mice, lacking caspase-1 exhibited both improved peri-infarct survival and a decreased rate of ventricular dilatation, possibly due in part to a reduction in the rate of apoptosis after experimental MI [59]. Reduction in bax levels in the bax knock out mouse was associated with reduced infarct size and improved long-term function after MI [60].

Synthetic caspase inhibitors have the risk of inherent drug toxicity as well as their potential beneficial effect from caspase inhibition. Several caspase inhibitors protected the heart against lethal reperfusion injury [61–64] while others do not [65–68]. This is more difficult to extrapolate from data on caspase inhibitors because the details of their systemic adverse effects may vary between the different caspase inhibitors.

Interestingly, some drug therapy that improves prognosis after myocardial infarction such as ACE inhibitors, beta-adrenergic receptor antagonists, and aldosternone antagonists have antiapoptotic properties [69–71]. Some novel therapies such as the interleukin-1 receptor antagonist anakinra, which inhibits caspase-1 and -9 activities, limits cardiomyocyte apoptosis and ameliorates the remodeling

after experimentally induced acute MI [72]. These comments are not meant to provide a detailed review of strategies to prevent cardiac apoptosis. Rather data on caspase inhibition in acute MI suggest that if the apoptosis process is not antagonized, then cell death is more likely to occur or that accentuation of apoptosis leads to enhanced cardiac cell death. These findings refute the criticism that caspase activation is of no consequence in myocardial infarction.

4.2. Summary. The strength of the overwhelming evidence supports the contention that apoptosis occurs in cardiomyocytes in patients following acute MI. This is based on immunohistochemical staining of myocytes showing DNA fragmentation, caspase-3 activation, as well as bcl-2 and Bax overexpression: the presence of the characteristic DNA fragmentation on agarose gel electrophoresis; and is nuclear imaging using radio-labeled annexin V. Some authors contend that TUNEL methodology is not accurate or specific for the recognition of cardiac apoptosis. Review of this contention suggests that TUNEL may have been excessively criticized and its role in detection of apoptosis has been too readily dismissed. While each methodology has its flaws, they are all consistent in their demonstration of the presence of apoptosis, and taken together, the data offer a strong and compelling argument in favor of this hypothesis.

The principle question that exists is the extent of apoptosis in acute MI. The studies that were reviewed do not provide a clear answer for several reasons, the predominant reason is that present study designs and methodology do not allow for serial follow-up, in the human heart, starting with the activation of apoptotic pathways in individual cardiomyocytes as well as in the determination of whether their eventual death is exclusively due to apoptosis. Nuclear imaging studies in patients with an antibody specific for caspase activation or annexin V, coupled with a probe to ensure the radiolabel is targeted to cardiomyocytes, may prove to be the best method to identify apoptosis in patients to obviate the need to obtain myocardial tissue. Cleary, autopsy studies and sectioning of the heart at the time of death provide a picture at a single time point, and the numbers of myocytes that have already died due to apoptosis are not included in the evaluation. The prevalence of apoptosis may be as high as 60% in the first day after acute MI and approximately 12% three weeks later. The above is a disturbingly high number because the dynamic nature of acute MI means the maximum cell death occurs in the early stages of MI and the major mode of cell death may actually be apoptosis.

Thus, this potentially high prevalence of apoptotic cell death appearing to be unreasonably high has been has been discussed by Elsässer et al. [73]. In 2000, Elsässer et al. drew attention to the unresolved issues in the role of apoptosis in myocardial ischemia including some that are clinically relevant today, namely, the need to determine the reason cells, under ischemic conditions, die by either apoptosis or oncosis and the need to determine the reversibility of apoptosis [73]. The association of high amounts of apoptosis in myocytes in the heart in heart failure or in areas of the heart that can generate potentially fatal arrhythmias underscores the importance of apoptosis in MI.

Clinical trials are needed in patients with acute MI to determine the extent to which apoptosis is reversible or preventable in patients with acute MI. The latter may lead to considerable changes to treatment strategies that are considered best practices for MI. Considering that our present treatment strategies still allow the occurrence of a high morbidity and mortality from acute MI; these clinical trials are long overdue.

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