Necropsy study of association between sudden death and cardiac enzymes

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Abstract

Aims: To determine if cardiac enzymes measured at necropsy could be used to predict early myocardial infarction.

Methods: Cardiac enzyme activities were measured in body fluids at necropsy. Coroners' necropsies were grouped by gross and microscopic findings into cases of definite myocardial infarction, cases with occlusive coronary artery atheroma but no identifiable myocardial infarction, and non-cardiac cases. Pericardial fluid, peripheral venous blood, and blood from the right atrium were collected. Total creatine phosphokinase, creatine phosphokinase isoenzymes, aspartate aminotransferase and hydroxybutyrate dehydrogenase activities were measured and the results analysed by logistic regression.

Results: The values of creatine phosphokinase and its isoenzymes were raised in those who had died of cardiac disease and were most discriminatory. Cases of early myocardial infarction without evidence of infarction on routine histological examination could be identified from enzyme activities.

Conclusions: Measurement of cardiac enzymes in blood and pericardial fluid at

necropsy can provide valuable additional information in cases of sudden death as a result of myocardial ischaemia which have occurred before macroscopic or microscopic evidence of myocardial infarction.

Sudden death from cardiac disease is the most common cause of death identified in necropsies performed for the Coroner. Identifiable myocardial infarction is not uncommon, but in most post mortem examinations severe occlusive coronary artery atheroma, without macroscopic or microscopic evidence of myocardial infarction, is the most common finding.¹ Various techniques have been used to try to identify infarction microscopically earlier than can be seen on haematoxylin and eosin staining but these are not regularly used because of various difficulties including ease of use and variability of results.²⁻¹³

Methods

Necropsies performed for the Coroner were grouped into non-cardiac deaths, deaths from occlusive coronary artery atheroma without evidence of infarction, and deaths from definite myocardial infarction.

Multiple blocks of paraffin wax embedded myocardium, stained with haematoxylin and eosin, were examined to identify or exclude infarction. Blood from the right atrium (into which the coronary sinus drains), blood from a peripheral vein, and pericardial fluid were collected, and cardiac enzyme activities measured in each. The following enzymes were measured: aspartate aminotransferase (AST); hydroxybutyrate dehydrogenase (HBD); total creatine phosphokinase (CPK); creatine phosphokinase isoenzymes CPK-MM, CPK-MB, and CPK-BB.

The creatine phosphokinase isoenzymes were measured by quantitative electrophoresis (Ciba-Corning, Palo-Alto, California, USA). Enzyme activity was measured by a standard procedure using a centrifugal analyser (Multistat IL, Warrington, England). Total CPK on each sample was measured, then a second sample with activity up to 1000 IU/l applied for electrophoresis. Samples with activity greater than 1000 IU/l were diluted using isotonic saline accordingly.

A total of 72 cases were collected in which full data were included, and in 66 of these cases the interval between death and necropsy was also known (the post mortem examination interval).

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Table 1 Non-cardiac deaths (n = 29)

Site and enzyme	Range (IU/l)	Median	Interquartile range
<i>Right atrial blood:</i>			
Total CPK	418-74500	18300	9000-38680
BB	0-782	0	0-9
MB	0-16811	2220	1217-6439
MM	348-68316	13542	7147-29712
BB %	0-7	0	0-0.75
MB %	0-38.7	16.7	10.75-24.45
MM %	61.3-100	83.2	75.6-89.3
HBD	320-43600	7410	2338-17275
AST	170-10125	1700	650-4363
<i>Peripheral venous blood:</i>			
Total CPK	220-257000	17850	7815-32575
BB	0-2325	0	0-450.5
MB	0-8940	664	200-1477
MM	177-248776	17750	7248-30686
BB %	0-10.9	0	0-3.55
MB %	0-11.7	5.4	3.65-7.4
MM %	80.5-100	93.7	89.3-96.4
HBD	210-30000	1400	625-4525
AST	38-6250	500	250-1450
<i>Pericardial fluid:</i>			
Total CPK	122-100750	18120	6630-46260
BB	0-1680	0	0-151.5
MB	0-27513	2443	658-4344
MM	77-97728	17178	5814-38370
BB %	0-13.1	0	0-1.7
MB %	0-31.3	14.1	6.6-19.1
MM %	63.3-100	85.8	78.1-93.4
HBD	175-23300	2985	1318-7435
AST	19-7700	550	238-1425

Results

From the results of the necropsy combined with the histological evidence, 29 of the 72 cases were classified as non-cardiac deaths, 15 as definite myocardial infarction, and 28 as

Table 2 Possible infarct (*n* = 28)

Site and enzyme	Range (IU/l)	Median	Interquartile range
Right atrial blood:			
Total CPK	1550–75700	16825	9125–29950
BB	0–2102	0	0–160
MB	281–13626	3081	1247–5159
MM	1202–62074	13263	7924–25445
BB %	0–4.8	0	0–1.5
MB %	6.4–29.9	16.85	6.4–29.9
MM %	68.2–93.6	83.15	76.2–87.2
HBD	1050–24400	4900	2680–10720
AST	300–5600	1950	875–3788
Peripheral venous blood:			
Total CPK	469–150750	16575	3688–54345
BB	0–5427	0	0–136.5
MB	0–7326	556	145–2391
MM	411–138087	15062	3536–50788
BB %	0–6.1	0	0–3.3
MB %	0–17.5	4.75	2.76–7.0
MM %	80.5–100	93.1	89.9–97.4
HBD	230–16900	1350	825–3038
AST	93–5125	450	250–1050
Pericardial fluid:			
Total CPK	6300–135000	24320	13700–55400
BB	0–983	0	0–0
MB	0–25342	1955	941–3290
MM	5218–13500	21114	12791–49135
BB %	0–1.4	0	0–0
MB %	0–36.1	10.5	5.2–14.5
MM %	62.4–100	89.6	85.5–94.8
HBD	1300–28700	4075	2047–6700
AST	250–7850	950	650–2350

Table 3 Definite infarction (*n* = 15)

Site and enzyme	Range (IU/l)	Median	Interquartile range
Right atrial blood:			
Total CPK	6250–112600	39120	9200–76200
BB	0–641	0	0–148.8
MB	635–13878	4765	1394–7672
MM	4867–106069	32154	7288–53085
BB %	0–7	0	0–2.1
MB %	3.7–23.1	15.2	11.7–17.9
MM %	75.0–96.3	83.4	79.9–88.3
HBD	1650–28550	9713	4863–16013
AST	200–9275	2263	1344–5130
Peripheral venous blood:			
Total CPK	2160–203800	13350	4838–40470
BB	0–2252	109	0–260
MB	0–4170	467	212–1206
MM	2029–203800	12401	4340–39072
BB %	0–5.6	2.1	0–4.7
MB %	0–6.4	4.8	3.8–5.8
MM %	88.2–100	93.4	90.0–96.2
HBD	335–31400	2800	850–7800
AST	100–8275	775	250–3500
Pericardial fluid:			
Total CPK	1390–215400	16400	6920–58500
BB	0–571	0	0–0
MB	97–38183	1288	607–4344
MM	1293–184813	15793	6076–53293
BB %	0–10.8	0	0–0
MB %	3.7–28.2	10.3	7.9–12.7
MM %	71.8–96.3	89.1	85.8–92.1
HBD	189–40100	3700	2050–13050
AST	50–7800	650	425–2575

Table 4 Model using total CPK enzymes and isoenzyme percentages

Logit (<i>p</i>) = 1.120 + 1.438 log (total right atrial CPK)		
– 0.040 (% isoenzyme MB in peripheral venous blood)		
– 0.130 (% isoenzyme MB in pericardial fluid)		
– 0.072 (% isoenzyme MM in pericardial fluid)		
Classification	Predicted non-infarct	Predicted infarct
Non-cardiac	25	4
Infarct	9	6
Possible infarct	24	4

possible myocardial infarction (occlusive coronary artery atheroma only).

The enzyme activities varied widely (tables 1–3) and there was a steady rise in enzyme activities the longer the interval before post mortem examination. The percentage of isoenzymes, however, remained fairly constant. The post mortem examination interval varied from two to 99 hours (mean 31.8 hours, median 21 hours).

The results were statistically analysed using logistic regression and discriminant analysis.^{14,15} Both logistic regression and discriminant analysis gave similar results. Logistic regression is the preferred technique, because it does not assume multivariate normality of the predictor variables and leads to a direct estimate of probability of death from cardiac cause without resorting to Bayes' theorem.

Using logistic regression, $L = \text{logit}(p) = \ln(p/[1-p])$, whence $p = \exp(L)/1 + \exp(L)$. *p* is the estimated probability of the death being due to myocardial infarction, and "predicted positive" means $p > 0.5$ (which is the same as $L > 0$); two models were found to be the best predictors.

Using a combination of total CPK in the right atrial blood together with percentage isoenzyme MB in peripheral venous blood, percentage isoenzyme MB in pericardial fluid, and percentage isoenzyme MM in pericardial fluid, the results in table 4 were obtained. When the post mortem examination interval was known and included in the analysis, a better rate of classification was obtained (table 5). In this model, about 75% of cases of myocardial infarction and non-cardiac deaths would be correctly classified according to enzyme results. Ten of the 27 cases of possible myocardial infarction (the occlusive coronary artery atheroma group) would be reclassified as myocardial infarction. Hydroxybutyric dehydrogenase and aspartate aminotransferase did not provide any additional independent information.

Activities of CPK-BB up to 5% were found in pericardial fluid in several cases with a variety of causes of death which included occlusive coronary artery atheroma (3), bronchopneumonia (6), lobar pneumonia (1) cerebral haemorrhage (1), asphyxia following choking on food (1), hypothermia (1), pancreatitis (1), congestive cardiac failure (1) and gastrointestinal haemorrhage (1).

Higher CPK-BB values in pericardial fluid were seen in cerebral infarction (39.8%), cerebral damage caused by lithium toxicity (13.1%), and epilepsy (6.4%).

Discussion

Reliable markers of early myocardial infarction still elude pathologists. Early histological changes of myocardial infarction seen on haematoxylin and eosin stained sections include oedema, eosinophilia, and granularity of fibres. These changes can also be produced by autolysis and variation in section thickness

Table 5 Model using post-mortem examination interval, total CPK, and percentage isoenzymes

Logit (p) = 5.529 - 0.653 log (PMI in hours) + 1.003 log (total right atrial CPK) - 0.187 (% isoenzyme MB in peripheral venous blood) - 0.162 (% isoenzyme MB in pericardial fluid) - 0.078 (% isoenzyme MM in pericardial fluid)		
Classification	Predicted non-infarct	Predicted infarct
Non-cardiac	24	2
Infarct	8	5
Possible infarct	17	10

and staining, all of which make accurate determination of early changes difficult. Similarly, waviness of fibres, another early change, may be seen in the absence of infarction.

The factors which make early recognition of myocardial infarction unreliable have prompted searches for a more reliable method of detection of early myocardial infarction. Such techniques have included staining of paraffin wax sections with haematoxylin-basic fuschin-picric acid, acridine orange (and subsequent examination under ultraviolet light) and enzyme histochemistry using frozen sections. These methods have either proved unreliable or difficult to perform.²⁻¹³

Measurement of cardiac enzymes in pericardial fluid at necropsy, but not in blood, have been undertaken by Luna and colleagues,^{16,17} and by Stewart and his colleagues.¹⁸ In their first paper Luna *et al* measured total LDH and its isoenzymes and total CPK in a variety of cases at necropsy. They found raised total CPK values in people dying from myocardial infarction, violent asphyxia, and multiple trauma compared with deaths from pulmonary embolism, infectious bronchopulmonary disease, haemorrhage and trauma of the central nervous system and a group labelled miscellaneous deaths. Total LDH values were not discriminatory, but the LDH₁:LDH₂ isoenzyme ratio was increased in those who had died from myocardial infarction. In a second study Luna *et al* found increased CPK-MB values in those who had died from myocardial infarction compared with deaths from other causes. They did not find any significant difference between values in myocardial infarction and cases of multiple trauma. In neither study did they differentiate between cases of definite myocardial infarction and occlusive coronary artery atheroma.

Stewart *et al* measured creatine phosphokinase, lactate dehydrogenase and their isoenzyme activities in pericardial fluid at necropsy. They found raised cardiac enzyme activities in cases of cardiac death compared with non-cardiac deaths. Enzyme activities were not statistically different between those cardiac cases with histological evidence of infarction and those without (occlusive coronary artery atheroma only). Values were raised in people where resuscitation had been attempted before death, and like Luna *et al*, they found raised activities in those who had died a violent death, defined by Stewart *et al* as a death in which there was or should have been severe physical or emotional distress. The exact cause of death in these cases of violent death was not defined but, interestin-

gly, they had the highest values of any group, with no evidence of myocardial necrosis. This was attributed to the action of catecholamines ("stress" cardiomyopathy).

Luna *et al* did not find CPK-BB in pericardial fluid in any of their cases, but Stewart *et al* did in two cases with central nervous system pathology. We found high CPK-BB activities in pericardial fluid in cases with intracranial disease, as well as low values in cases with various causes of death.

Our study has shown that death caused by early myocardial infarction can be predicted from cardiac enzyme activities at necropsy. Most non-cardiac deaths were correctly predicted (24 out of 26) using the model in table 5. Five of the 13 cases in which there was histological evidence of infarction were predicted as cardiac deaths using this model. The fact that eight cases were not is not entirely surprising as the rise in enzymes may have occurred a few days before death and then returned to normal. In the cases of possible myocardial infarction 10 cases were classified as infarction, based on the enzyme activities. In these cases the rise in enzymes suggests that infarction was occurring but that death happened before histological evidence of infarction appeared. In the other 17 cases there was no histological or biochemical evidence of infarction. This is not surprising. In many of these cases there was probably a cardiac arrhythmia and very rapid death without any infarction. Very early infarction, with no rise in enzymes, or an insignificant rise, however, cannot be excluded.

The results of this study show that early myocardial infarction can be identified at necropsy using cardiac enzyme measurement of body fluids. The accuracy is increased if blood from the right atrium and peripheral venous blood is used in addition to pericardial fluid. If the post mortem examination interval is included in the analysis the accuracy is increased still further. The failure to identify a rise in enzymes in all cases of the definite infarction group does not, of course, present any practical problem, as infarction will be identified by gross or routine histological examination. Although not all cases of early myocardial infarction can be predicted using our model, any additional information when only occlusive coronary artery atheroma is present may be useful in controversial cases. We recommend, therefore, in cases of sudden unexpected death, when post mortem examination indicates myocardial ischaemia as the likely cause of death, but where doubt still exists, that pathologists make use of this valuable additional information by taking samples of blood and pericardial fluid for enzyme analysis.

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