Z Rechtsmed (1989) 103:111–119

Zeitschrift für
Rechtsmedizin

Usefulness of different myocardial sampling zones for the postmortem diagnosis of myocardial infarction

Emilia Lachica[1], **Aurelio Luna**[2], **and Enrique Villanueva**[1]

[1]Cátedra de Medicina Legal, University of Granada, Facultad de Medicina, Avda. de Madrid s/n, 18071 Granada, Spain
[2]Cátedra de Medicina Legal, University of Murcia, Murcia, Spain

Summary. The diagnosis of myocardial infarction requires the use of a group of tests that are very efficient, quick and inexpensive. Another important consideration is the choice of myocardial sampling zones, especially in cases of differential diagnosis between a cardiac injury secondary to a trauma or violent asphyxia and others, secondary to myocardial infarction. The aim of this work was to choose, through discriminant analysis, the most useful zones of cardiac tissue for the quantification of free fatty acids and free carnitine and for the performance of the K/Na quotient, as biochemical parameters for the postmortem diagnosis of myocardial infarction. According to the discriminant analysis performed, seven zones of cardiac tissue are necessary to achieve a differential diagnosis among "myocardial infarction," "other natural deaths," and "violent deaths" with a 71.9% efficacy. Greater diagnostic efficacy was found (78.1%) for differentiating between "natural deaths" and "violent deaths."

Key words: Thanatochemistry, myocardial infarction – Myocardial infarction, biochemical analysis

Zusammenfassung. Für die postmortale Herzinfarkt-Diagnose braucht man heutzutage eine einfache und billige, aber aussagekräftige Gruppe von Labortests. Das erste wichtige Problem ist die Auswahl von geeigneten Herzgebieten für die biochemischen Analysen, vor allem wenn eine Differentialdiagnose zwischen Trauma, Erstickung oder Herzinfarkt vorgenommen werden muß. In dieser Arbeit haben wir die geeigneten Herzgebiete für eine postmortale Herzinfarkt-Diagnose mit verschiedenen Tests (Quotient K/Na, freie Fettsäuren und freies Karnitin) untersucht.

Bei Anwendung der Diskriminanzanalyse sind acht verschiedene Herzgebiete erforderlich, um für die Differentialdiagnose zwischen „Herzinfarkt-Tod", „gewaltsamen Tod" und „natürlichen Tod" mit 71.9% Genauigkeit machen zu können.

Offprint requests to: E. Lachica

Eine sehr hohe diagnostische Sicherheit (78.1) haben die Verf. für die Differentialdiagnose zwischen „natürlichem Tod“ und „gewaltsamen Tod“ gefunden.

Schlüsselwörter: Thanatochemie, Herzinfarkt – Herzinfarkt, biochemische Analyse

Introduction

Nowadays, the high frequency of coronary artery alterations in the normal population can lead to false-positive diagnoses of myocardial infarction in cases of violent death. This fact can lead to error, for example, in cases of accidental death suspected to have been provoked by a heart attack. Demonstrating a myocardial infarction when the patient has died within the first few hours after onset of the disease (that is, when most cardiac deaths occur) is still an unsolved problem in forensic medical autopsies, and numerous attempts have been made to find more sensitive diagnostic methods. Discrimination between patients with and without myocardial infarction can, however, be improved by appropriate selection of laboratory investigations.

The purpose of this work (which restricts itself to the biochemical assessment of myocardial infarction) is to answer the following two questions:

- Which are the best myocardial sampling zones for identifying sudden deaths of cardiac origin?
- Does the combination of two or several biochemical tests improve the diagnostic performance of the tests taken separately?

In this paper we have tested the efficiency of three biochemical parameters for the postmortem diagnosis of myocardial infarction: free fatty acids, free carnitine, and the K/Na ratio in seven different regions of the human heart.

Material and methods

Hearts were obtained from 67 corpses during autopsy, according to normal autopsy procedures, and dissected in seven different regions:

- Anterosuperior zone from the left ventricle (LV_{a-s})
- Anteroinferior zone from the left ventricle (LV_{a-i})
- Posterosuperior zone from the left ventricle (LV_{p-s})
- Posteroinferior zone from the left ventricle (LV_{p-i})
- Superior zone from the interventricular septum (S_s)
- Inferior zone from the interventricular septum (S_i)
- Superior zone from the right ventricle (RV_s)

Samples were classified in relation to the different causes of death according to their physiopathological similarity:

- Group I, myocardial infarction ($n = 21$)
- Group II, other natural deaths ($n = 10$)
- Group III, violent deaths ($n = 36$); this group included 11 cases of “hanging,” 11 cases of “deaths due to cerebral injury,” 8 cases of “multiple trauma,” 2 cases of “poisoning,” and 4 cases of “other violent asphyxy”

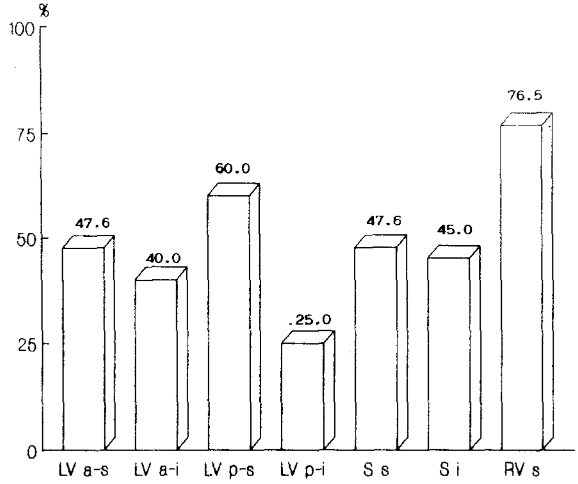

Fig. 1. Topographical distribution of ischemic areas in the group "myocardial infarction" ($n = 21$)

The diagnosis of myocardial infarction was carried out by using two kinds of histological techniques: (a) the routine technique (hematoxylin-eosin stain) and (b) the fluorescent method (acridine orange). The topographical distribution of ischemic areas in the group "myocardial infarction" is shown in Fig. 1.

Free fatty acis concentrations were determined according to the method of Lowry and Tinsley (1976) after previous extraction of total lipids according to the Bligh and Dyer method (1959). Free carnitine determinations were carried out using the Pearson et al. technique (1974). Potassium and sodium concentrations were assayed by flame emission photometry after homogenizing the cardiac tissue with distilled water (1:20, w:v).

The average postmortem interval was 17.75 ± 7.34 h, and its range was 5–40 h. The age distribution of the cases was: myocardial infarction, 61.9 ± 14.6 years; other natural deaths, 66.3 ± 20.1 years; violent deaths, 45.9 ± 20.3 years.

We studied 21 variables: free fatty acids and free carnitine concentrations and K/Na ratios in the seven myocardial regions mentioned above.

Statistical analysis was carried out using BMDP discriminant analysis. The general problem in statistical discriminant analysis is to allocate an item of unknown type to one, two, or more types, using measurements from one or more so-called classification variables on that item. In this paper the item is "cause of death" and types are:

I. First discriminant
   Group A, myocardial infarction
   Group B, other natural deaths
   Group C, violent deaths
II. Second Discriminant
   Group A, natural deaths
   Group B, violent deaths

Results and discussion

In our previous work we studied the behavior of the biochemical parameters, free fatty acids (FFA) and free carnitine (FC), in cardiac tissue in relation to different causes of death (Lachica et al. 1988a) and the efficiency of three techniques (formazan test, histological methods, and potassium/sodium ratio) for the postmortem diagnosis of myocardial infarction (Lachica et al. 1988b). The

Table 1. Selected variables for discriminant analysis in three groups of causes of deaths

Variable	Classification functions		
	Myocardial infarction	Other natural deaths	Other violent deaths
FFA-LV_{a-i}	−0.00171	−0.07040	0.03393
FFA-LV_{p-i}	−0.00928	0.04404	−0.01481
FFA-RV_s	0.02763	0.08245	−0.02521
FC-S_s	0.02482	0.00710	0.03252
K/Na-LV_{a-s}	0.02724	0.01343	0.04852
K/Na/LV_{a-i}	0.02353	0.03402	−0.01598
K/Na-LV_{p-s}	0.00328	0.02768	0.02545
K/Na-S_i	0.02692	0.00585	0.06504
Constant classification matrix	−8.25353	−12.80206	−13.64757

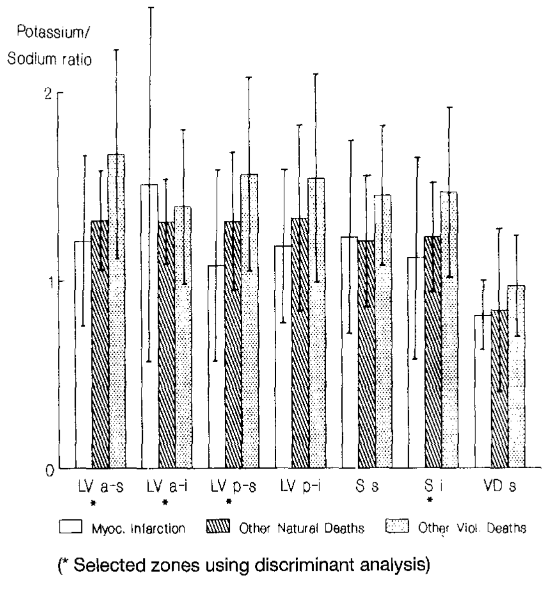

Fig. 2. Potassium/sodium ratio behaviors in myocardial tissue in relation to the various causes of deaths

aim of this work was to determine the best biochemical tests and sampling zones to use to diagnose deaths of ischemic cardiac origin.

Our results confirm the difficulty in establishing postmortem diagnosis of early myocardial infarction. In the discriminant analysis performed, eight variables were necessary to achieve a 71.9% rate of correct diagnosis according to Jacknifed's classification (Table 1).

Discriminant analysis for the K/Na ratios shows significant results in the following cardiac zones (Fig. 2): anterosuperior portion of the left ventricle, anteroinferior zone of the left ventricle, posterosuperior zone of the left ventricle, and inferior portion of the septum interventricular.

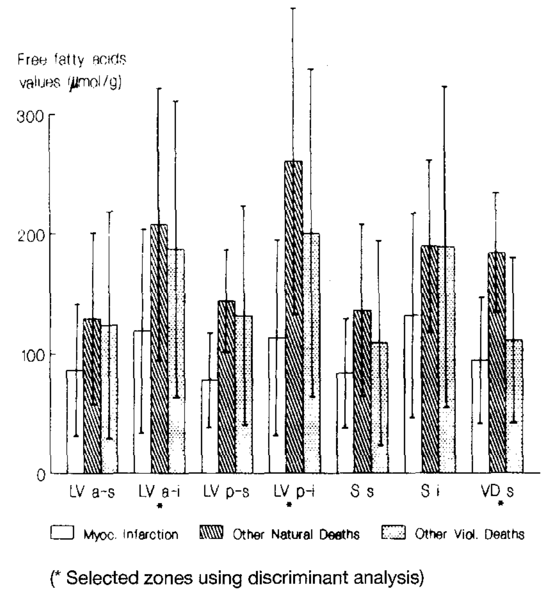

Fig. 3. Free fatty acids values in myocardial tissue in relation to the various causes of death

In addition to the possible interferences due to autolysis and the possibility of passive diffusion processes as a consequence of the increase of tissue permeability, another problem in relation to the potassium and sodium determinations in the cardium is the choice of sampling zones. Several such zones have been proposed, including the anterior and lateral walls of both the superior and inferior portions of the left ventricle (Pedersen 1980), the endocardial portion of the anterior and posterior walls of the left ventricle (Rammer and Jansson 1976), and the anterosuperior and posterosuperior walls of the left ventricle (Navarrete 1976). Obviously, the probability of false-negative diagnosis of myocardial infarction increases as the number of sampling zones decreases.

On the basis of our results, it would appear necessary, at least, to study the four zones mentioned above (that is, LV_{a-s}, LV_{a-i}, LV_{p-s}, and S_i), although they do not correspond to the most frequent sites of cardiac ischemia reported in clinical studies. On the other hand, discriminant analysis searches out the greatest differences between zones. Therefore, an altered K/Na ratio can be found in cardiac emergency situations such as asphyxia, where blood is used for irrigation of regions with higher metabolic requirements, as well as in cardiac injuries after multiple trauma (Polkin et al. 1982), cardiac reanimation (Tonkin et al. 1975), and traffic accidents (Lindsey et al. 1978; Hamilton et al. 1984) where the myocardial damage has been reported as a serum CK-MB enzyme increase.

Discriminant analysis for free fatty acid concentrations shows significant results in the following cardiac zones (Fig. 3): anteroinferior and posteroinferior zones from the left venctricle and superior zone from the right ventricle. In relation to free carnitine, the region selected by discriminant analysis has been the superior zone of the septum interventricular (Fig. 4).

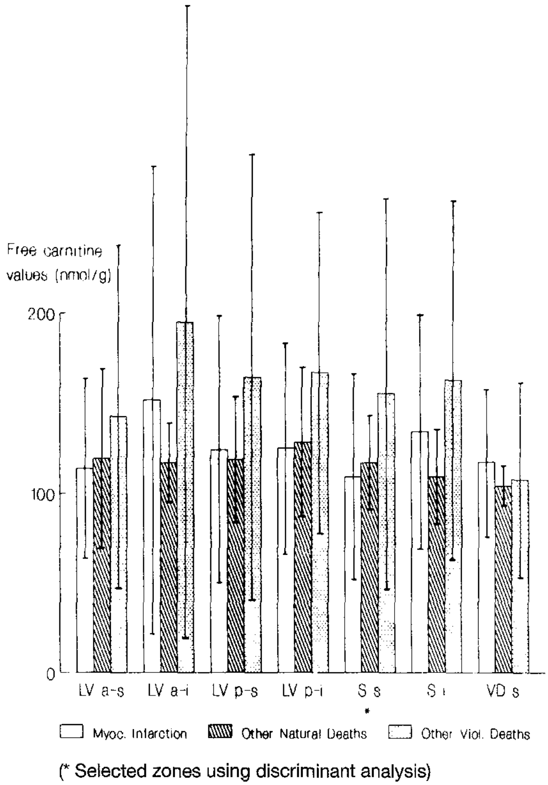

Fig. 4. Free carnitine values in myocardial tissue in relation to the various causes of death

Table 2. Statistically significant values for the canonical correlation

	Canonical correlations	
	0.79790	0.59287
Variable	Coefficients for canonical variables	
FFA LV_{a-i}	−0.02532	0.01685
FFA LV_{p-i}	0.01129	−0.01778
FFA RV_s	0.02943	−0.00826
FC S_s	−0.00597	0.00466
K/Na LV_{a-s}	−0.01045	0.00037
K/Na LV_{a-i}	0.01674	0.00467
K/Na LV_{p-s}	−0.00417	−0.01339
K/Na S_i	−0.01804	−0.00061
Constant	1.74772	1.86602
Canonical variables evaluated at group means		
Myocardial infarction	0.94885	0.81194
Other natural deaths	2.19175	−1.39729
Other violent deaths	−1.12967	−0.19660

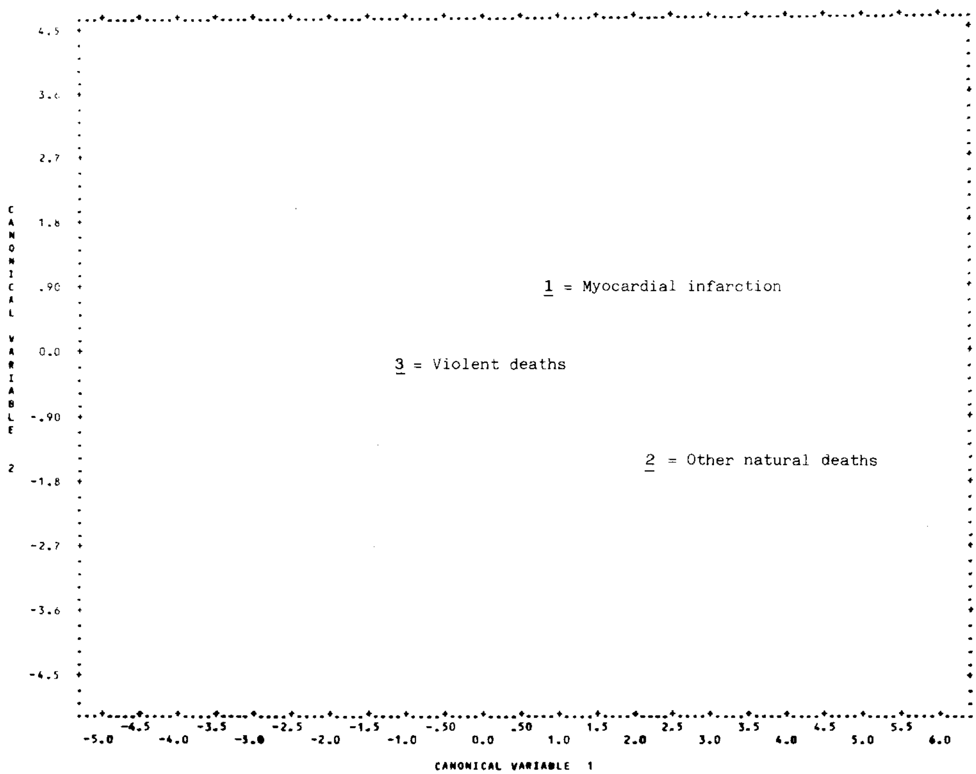

Fig. 5. Graphic representation of the three groups of causes of death in relation to the canonical variables

The results of the canonical correlation for the first discriminant analysis are shown in Table 2, and Fig. 5 shows the different spatial localization of the three group of causes of death in relation to the canonical variables.

According to experimental studies, during myocardial ischemia there is a depletion in carnitine tissue levels, with the resultant intracellular accumulation of free fatty acids (Katz and Messineo 1981; Feuvray and Plouët 1981; Liedtke et al. 1984; Silverman et al. 1985). There is no agreement between our results and those from experimental works. Nevertheless, there is an explanation regarding the length and intensity of the agonal proccess.

During agony there is a mobilization of catecholamines and other biogenic amines (Laves and Berg 1965; Luna et al. 1983) that cause activation of lipases with an increase in serum free fatty acids levels. In processes with very intense agonal suffering (for example, asphyxia), we found a serum free fatty acids rise that, in the initial phase, occurs in tissues with the highest metabolic demand (brain, heart) for their latter metabolization. In violent deaths, with enough survival time, we found the highest free fatty acids values, while in myocardial infarction cases, with slight survival time, these values were the lowest – because death occurs suddenly and, although there is an initial rise of serum free

Table 3. Selected variables for discriminant analysis in the groups "natural deaths" and "violent deaths"

	Classification functions	
Variable	Natural deaths	Violent deaths
FFA-LV_{a-i}	0.00176	0.02568
FFA-S_s	−0.01222	−0.04054
FFA-S_i	−0.00314	0.02882
FFA-RV_s	0.03953	−0.02688
K/Na-LV_{a-s}	0.02627	0.05474
K/Na-LV_{a-i}	0.02681	−0.02807
K/Na-LV_{p-s}	0.01045	0.04652
K/Na-S_i	0.01133	0.04714
Constant classification matrix	−7.17312	−11.84553

Table 4. Summary table

Variable	*F* value	Variables included (*n*)	*U* statistic	Approximate *F* statistic	Degrees of freedom	
K/Na LV_{a-s}	3.1135	1	0.8232	3.113	2	29
FFA RV_s	2.4751	2	0.6996	2.738	4	56
FFA LV_{a-i}	3.8008	3	0.5459	3.181	6	54
FFA LV_{p-i}	2.1873	4	0.4673	3.009	8	52
K/Na LV_{a-i}	2.3363	5	0.3937	2.969	10	50
K/Na S_i	3.0854	6	0.2989	3.317	12	48
FC S_s	1.7348	7	0.2597	3.162	14	46
K/Na LV_{p-s}	1.1241	8	0.2356	2.915	16	44

fatty acids, there is not enough time for the increase of these compounds in myocardial tissue.

The discriminant analysis performed, using as variables the groupings "natural deaths" and "violent deaths" (Table 3), shows similar results, but it includes a higher number of zones to study free fatty acids concentrations. The percentage of correct diagnosis was 78.1% according to Jacknifed's classification. Table 4 summarizes the statistical values for the variables chosen by the discriminant analysis, as well as their significant differences.

In conclusion, we think that the main contribution of our work is to suggest a group of techniques useful in differentiating false-positive diagnoses of cardiac ischemia, where a previous myocardial infarction is suspected in cases of violent death. The zones and parameters studied showed different behaviors in relation to the mechanism and etiology of death, which means that they had some ability to classify. We are aware of the low number of cases studied here (only 67), and therefore have to be cautious in establishing diagnostic conclusions. Nevertheless, we think our results have a definite orientation value.

References

1. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
2. Feuvray D, Plouët J (1981) Relationship between structure and fatty acid metabolism in mitochondria isolated from ischemic rat hearts. Circ Res 48:740–747
3. Hamilton JRL, Dearden C, Rutherford WH (1984) Myocardial contusion associated with fracture of sternum: important features of the belt syndrome. Injury 16:155–156
4. Katz AM, Messineo FC (1981) Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. Circ Res 48:1–16
5. Lachica E, Villanueva E, Luna A (1988a) Regional study of free fatty acids and free carnitine behavior in cardiac tissue in relation to different causes of death. J Forensic Sci 33: 1483–1490
6. Lachica E, Villanueva E, Luna A (1988b) Comparison of different techniques for the postmortem diagnosis of myocardial infarction. Forensic Sci Int 38:21–26
7. Laves WC, Berg S (1965) Agonie. Physiologisch-chemische Untersuchungen bei gewaltsamen Todesarten. Schmidt-Römhild, Lübeck
8. Liedke AJ, Nellis SH, Whitesel LF (1984) Effects of carnitine isomers on fatty acids metabolism in ischemic swine hearts. Circ Res 54:859–866
9. Lindsey D, Navin TR, Finley PR (1978) Transient elevation of serum activity of MB isoenzyme of creatine phosphokinase in drivers involved in automobile accidents. Chest 74:15–18
10. Lowry RR, Tinsley IJ (1976) Rapid colorimetric determination of free fatty acids. JAOCS 53:470–472
11. Luna A, Villanueva E, Hernandez-Cueto C, Morales I (1983) Verhalten der 5-hydroxytriptamin und der 5-hydroxy-indol-essigsäure in Zusammenhang mit der Agonie (I). Z Rechtsmed 89:216–226
12. Navarrete M (1976) Demostración postmortem del infarto de miocardio reciente. Publicaciones de la Universidad de Sevilla, Sevilla
13. Pearson DJ, Tubbs PK, Chase JFA (1974) Carnitine and acylcarnitines. In: Bergmeyer HU (ed) Methods of enzymatic analysis, 2nd edn, vol 4. Academic Press, New York, pp 1758–1771
14. Pedersen PK (1980) Determination of potassium/sodium ratio in heart tissue. Evaluation of its use as an index of myocardial ischemic damage. Comparison with the Nitro-BT test. Forensic Sci Int 16:271–280
15. Rammer L, Jansson O (1976) Determination of electrolytes in the myocardium as a tool for the postmortal diagnosis of recent infarction. Forensic Sci 8:127–130
16. Polkin RT, Warner A, Troeaugh O (1982) Evaluation of noninvasive tests of cardiac damage in suspected cardiac contusions. Circulation 66:627–631
17. Silverman NA, Schmitt G, Wishwanath M, Feinberg H, Levitky S (1985) Effect of carnitine on myocardial function and metabolism following global ischemia. Ann Thorac Surg 40:20–24
18. Tonkin AM, Lester RM, Guthrow CE (1975) Persistence of MB isoenzyme of creatine phosphokinase in serum after minor iatrogenic cardiac trauma. Circulation 51:627–631

Received November 10, 1988