Analysis of the Relationships between Magnesium, Zinc, Calcium, Potassium, Cholesterol and Creatine Kinase Concentrations According to the Severity of Ischemia

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Zusammenfassung

Es wird eine statistische Auswertung der Plasma (Pl) und/oder Erythrozytenkonzentrationen (Erc) von Magnesium (Pl-Mg; Erc-Mg), Zink (Pl-Zn; Erc-Zn), Calcium (Pl-Ca), Kalium (Erc-K), Gesamtcholesterin, HDL sowie der Aktivität von Gesamtkreatininkinase (CK) und ihrem Isoenzym MB (CK-MB) bei 26 Männern mit instabilem Präinfarkt-Syndrom (PIS) und 34 Männern mit akutem Myorkardinfarkt (MI) dargelegt. Die unterscheidende Analyse ermöglichte einen globalen Vergleich der beiden Gruppen und die Bestimmung der statistisch signifikantesten Variablen CK und Pl-Zn. Die nichthierarchische Einstufung der IM-Männer führte zur Aufstellung dreier homogener Untergruppen mit signifikant unterschiedlichen CK-, CK-MB- und Pl-Zn-Werten. Die Baumdiagramme der PIS- und MI-Männer wurden durch hierarchische Einstufung erhalten. Auf diese Weise wurden Korrelationen zwischen den Variablen und Variablengruppen nachgewiesen. Bei den PIS-Männern war Pl-Zn mit Pl-Ca korreliert, während bei den MI-Männern Pl-Zn mit Pl-Mg korreliert war. Die schrittweise Regression zeigte, daß Pl-Zn bei den PIS-Männer und bei den MI-Männern die signifikanteste CK oder CK-MB Variable war.
Diese statistischen Auswertungen zeigen alle einerseits Unterschiede zwischen den Beziehungen der untersuchten Variablen je nach dem Ischämiegrad und andererseits die besondere Bedeutung von Pl-Zn für die Diagnose und wahrscheinlich auch Prognose. Nach MI vermittelt möglicherweise die Absonderung von Interleukin-1 die beobachtete Hypozinkämie durch die Bildung eines kardialen Zn-Metallthionein-Komplexes.

Summary

A statistical interpretation of plasma (Pl) and/or erythrocyte (Erc) concentrations of magnesium (Pl-Mg; Erc-Mg), zinc (Pl-Zn; Erc-Zn), calcium (Pl-Ca), potassium (Erc-K) and total and HDL-cholesterols, as well as of the activity of total creatine kinase (CK) and CK isoenzyme-MB (CK-MB), is presented in 26 men with preinfarction syndrome (PIS) and 34 men with acute myocardial infarction (MI).
Discriminant analysis allowed overall comparison of both groups and determination of the most significant variables: CK and Pl-Zn. Nonhierarchical cluster analysis was used to define 3 homogeneous subgroups among MI men in which CK, CK-MB and Pl-Zn differed significantly between the groups. Tree diagrams of PIS and MI men were obtained by hierarchical cluster analysis displaying the correlations among variables and groups of variables; in PIS men, Pl-Zn was correlated with Pl-Ca, whereas in MI men Pl-Zn was correlated with Pl-Mg. Stepwise regression indicated that Pl-Zn was the most significant regressor of CK in PIS men and of CK-MB in MI men.
All these statistical interpretations indicate both the differences among the relationships of the variables studied according to the degree of ischemia and the quite special role of Pl-Zn in diagnosis and perhaps prognosis. After MI, interleukin-1 release may possibly mediate observed hypozincemia via a heart Zn-metallothionein.

Résumé

Une interprétation statistique des concentrations plasmiques (Pl) et/ou érythrocytaires (Erc) de magnésium (Pl-Mg; Erc-Mg), zinc (Pl-Zn; Erc-Zn), calcium (Pl-Ca), potassium (Erc-K), des cholestérols total et HDL ainsi que de l'activité de la créatine kinase totale (CK) et de son isoenzyme MB (CK-MB) a été rapportée chez 26 hommes souffrant d'angor instable (AI) et 34 hommes présentant un infarctus aigu du myocarde (IM). L'analyse discriminante a permis la comparaison globale des deux groupes et la détermination des variables les plus significatives: CK et Pl-Zn. La classification non hiérarchique des hommes IM a conduit à définir trois sous-groupes homogènes pour lesquels CK, CK-MB et Pl-Zn différaient significativement entre les groupes. Les dendrogrammes des hommes AI et IM ont été obtenus par classification hiérarchique mettant en évidence les corrélations entre les variables et les groupes de variables; chez les hommes AI, Pl-Zn était corrélé avec Pl-Ca tandis que, chez les hommes IM, Pl-Zn était corrélé avec Pl-Mg. Les équations de régression pas à pas ont montré que Pl-Zn était la variable la plus significative de CK chez les hommes AI et de CK-MB chez les hommes IM.
Toutes ces interprétations statistiques indiquent d'une part des différences entre les relations des variables étudiées selon le degré d'ischémie, d'autre part le rôle spécial de Pl-Zn dans le diagnostic et vraisemblablement le pronostic. Après IM, la sécrétion d'interleukine 1 serait susceptible d'expliquer l'hypozincémie observée par la formation d'une Zn-métallothionéine cardiaque.

Introduction

In previous papers, we studied the concentrations of various trace elements and biological variables at day 1 in two groups of men: one with preinfarction syndrome (PIS) and the other with acute myocardial infarction (MI) [1, 2]. We now present a statistical study using cluster analysis and stepwise regression to show possible relationships among the variables investigated and changes according to the severity of ischemic heart diseases. The following plasma (Pl) and erythrocyte (Erc) variables were studied: magnesium (Pl-Mg; Erc-Mg), zinc (Pl-Zn; Erc-Zn), calcium (Pl-Ca), potassium (Erc-K) and total and HDL-cholesterols, as well as the activity of total creatine kinase (CK; EC 2.7.3.2.) and CK isoenzyme-MB (CK-MB).

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Populations and Methods

Populations

Samples were obtained from 60 white men (26 patients with preinfarction syndrome, 46 to 82 yr of age, and 34 patients with acute myocardial infarction, 32 to 82 yr of age), all residents of the Nantes area in France. The 60 patients were admitted to an intensive care unit less than 10 hours after the first clinical signs. The therapy of these patients consisted of administration of six tablets of isosorbide dinitrate per day and of calcium heparinate injections three times per day. The distinction between the two groups was made in subsequent days according to biological and ECG signs. Subjects with prior therapy or who had not fasted for several hours were excluded from the study.

Assay techniques

Blood specimens (5 ml) were drawn into Terumo Venoject Tubes (Ref. T 206 LH, Code VT 050 HL 1) containing lithium heparin (Terumo France, 78181 Saint-Quentin-en-Yvelines Cedex, France) and then immediately centrifuged at 3500 x g for 8 min at 10 °C.

Magnesium, Ca and Zn concentrations were measured by flame atomic absorption spectrometry using a Hitachi 180–80 model with Zeeman effect (Skalar Analytique, 75015 Paris, France). Potassium was measured by emission spectrometry using the same apparatus. Procedural details have been described previously [3]. HDL-cholesterol (Boehringer Precipitant 400 971: phosphotungstic acid-Mg^{2+}) and total cholesterol were determined by an enzymatic colorimetric cholesterol C-system "CHOD-PAP" method; Boehringer, Mannheim, FRG). CK activity and CK isoenzyme-MB were measured using the "R-CK NAC-activated Merckotest" (ref. 14317 and 14333, respectively; E. Merck, 6100 Darmstadt, FRG) at 25 °C and 334 nm.

Statistical analysis

Individual comparison of the variables was performed using Student's t-test; there were about 30 subjects in each group.

A discriminant analysis allowing overall comparison of both groups revealed the most significant variables.

Non-hierarchical cluster analysis was used to define homogeneous subgroups among MI men. Finally, tree diagrams of PIS an MI men were obtained by hierarchical cluster analysis [4]. For the last two analyses, data were transformed into ranks, as was also done for stepwise regression [5].

All these statistical procedures were implemented from Systat software (Systat, Inc. Evanston, IL, USA).

Results

The results are given in Tables 1 to 3 and in Figure 1 (A, B).

In Table 1, the different variables of PIS and MI men are compared; CK and Pl-Zn differed significantly between the two groups.

As might be expected, the variables allowing the best discrimination between PIS an MI men presenting cardiac ischemia at two different stages of severity were, in terms of decreasing importance, CK followed by Pl-Zn.

A procedure for assigning individuals to groups was defined by a computer program. A random sample drawn from both groups showed that the percentage of correctly ranked subjects was about 100 % for PIS and 50 % for MI. These results indicated that the PIS group was very homogeneous and the MI group very heterogeneous, which led us to attempt to define subgroups of homogeneous subjects in the MI group by means of non-hierarchical cluster analysis (K-means procedure) [6]. Classification of MI in 4 subgroups produced one subgroup containing a single, apparently atypical subject (young: 44 yr; CK quite elevated: 976 U/1; CK-MB: 58.9 U/1; and Pl-Zn very low:

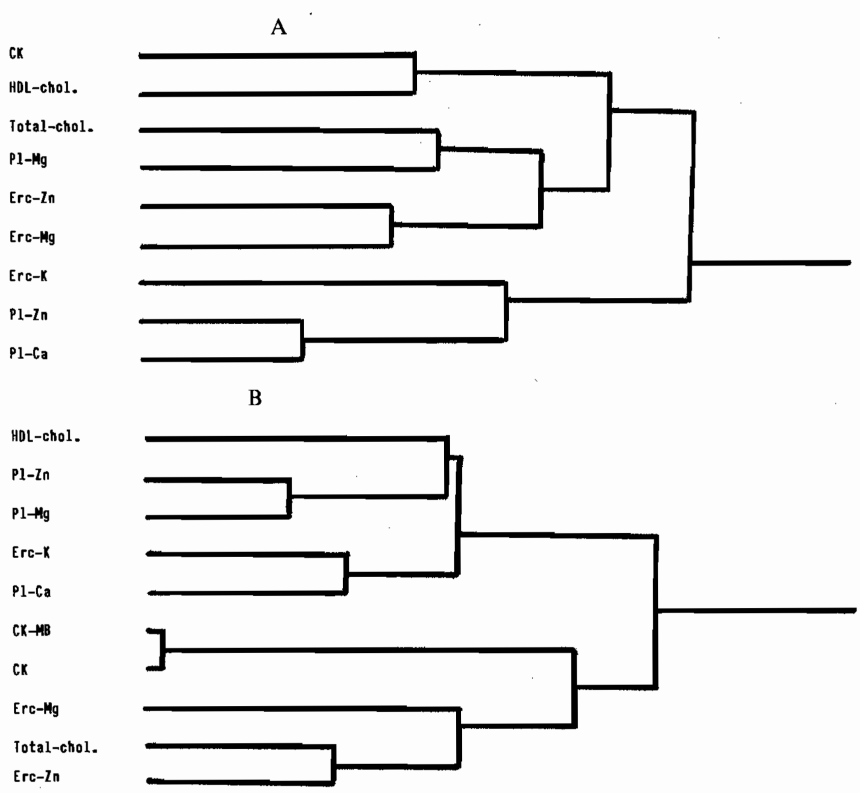

Fig. 1: Tree diagrams of (A) PIS patients, (B) MI patients.

Tab. 1:

Results (means and SD) for populations of 26 preinfarction syndrome (PIS) men and 34 acute myocardial infarction (MI) men at day 1 after admission to hospital

Age years	Plasma magnesium mmol/l	Erythrocyte magnesium mmol/l	Plasma zinc µmol/l	Erythrocyte zinc µmol/l	Plasma calcium mmol/l	Erythrocyte potassium mmol/l	Total-cholesterol mmol/l	HDL-cholesterol mmol/l	CK activity U/l	CK-MB activity U/l
Preinfarction syndrome men (n = 26)										
64.4 (10.5)	0.77 (0.09)	2.31 (0.27)	9.12 (1.89)	166 (27.7)	2.10 (0.13)	88.6 (5.59)	5.37 (1.10)	1.14 (0.46)[a]	40.9 (33.6)[a]	
Myocardial infarcted men (day 1) (n = 34)										
61.1 (10.9)	0.76 (0.09)	2.19 (0.25)	8.07 (1.67)*	162 (20.6)	2.08 (0.13)	86.3 (5.96)	5.43 (1.07)	1.07 (0.31)	364 (260)**	38.4 (29.3)[a]

[a] Nonnormal distribution.
*$p<0.05$, **$p<0.001$: Student's t-test between PIS men and MI men.

4.40 µmol/l). This subject was excluded from the study. By non-hierarchical cluster analysis, 3 subgroups were obtained (Table 2). The results show that the size of the necrotic area increased from Group A to Group C [7]. The 3 variables involved differed significantly (**p** < 0.05) between the 3 groups.

In Table 3, the non-parametric stepwise regression equations are shown. The equation obtained for MI subjects at day 2, given for information only, serves to confirm the role of Zn. Pl-Zn appears to be the variable most correlated (**p** < 0.05) with CK or CK-MB.

In Figure 1 (A, B), the two tree diagrams after hierarchical cluster analysis display the results in homogeneous groups so as to allow any relationship between the groups to be revealed [4]. Interpretation is straightforward: the more to the left the grouping of two variables is, the more highly these variables are correlated [8].

Tab. 2: Non-hierarchical cluster analysis of 33 MI patients (means and SD).

	Group A (n = 15) age = 59.5 (12.2)	Group B (n = 8) age = 65.0 (5.81)	Group C (n = 10) age = 62.1 (9.73)
CK (U/l)	134 (62.1)	351 (66.5)	659 (98.6)
CK-MB (U/l)	15.3 (7.13)	35.5 (7.07)	73.4 (25.3)
Pl-Zn (µmol/l)	8.81 (1.16)	7.94 (1.67)	7.43 (1.55)

Tab. 3: Non-parametric stepwise regression equations.

26 PIS men:

$$CK = 11.347 - 0.536\ Pl\text{-}Zn + 0.318\ Erc\text{-}Mg + 0.378\ HDL\text{-}cholesterol\ (p < 0.01)$$

34 MI men:

day 1 $CK\text{-}MB^{a} = 23.449 - 0.340\ Pl\text{-}Zn\ (p < 0.05)$

day 2 $CK\text{-}MB^{a} = 22.704 - 0.408\ Pl\text{-}Zn + 0.429\ total\text{-}cholesterol + 0.293\ Erc\text{-}Mg - 0.304\ HDL\text{-}cholesterol - 0.308\ Pl\text{-}Ca\ (p < 0.001)$

[a] After elimination of CK activity.

Discussion

Many recent papers have sought to present significant information about the biochemical and pathobiochemical pathways of trace elements and electrolytes in humans [9–11].

In our study, comparison of means between PIS and MI men showed a single significant difference in Pl-Zn (**p** < 0.05) in addition to the difference concerning CK (normal CK values for PIS men). The importance of Pl-Zn was also found in non-hierarchical cluster analysis and stepwise regression. In very recent papers, the quite important role of creatine kinase data in detecting myocardial infarct extension and serum-Zn in the acute-phase response to stress has been pointed out [7, 12]. A high correlation between CK-MB-estimated infarct size and anatomic infarct size has been reported, and early peaking of CK-MB activity has been found to be associated with MI extension [7]. Zinc, an essential trace element required for DNA and RNA synthesis and the functioning of over 200 metalloenzymes, is involved in numerous metabolic pathways of clinical importance. Certain cytokines such as interleukin-1 are known to cause a decrease in serum-Zn concentration as part of the acute phase response to stress and/or inflammation. Interleukin-1 stimulates production of metallothionein, a tissue zinc-binding protein [12]. After MI, a significant decrease in Pl-Zn has been consistently observed, and several hypotheses have been advanced to account for this drop [2, 13]. Our own results relative to men and women who died after MI of the left ventricle indicate that only the Zn level of the necrotic area was decreased (**p** < 0.01) as compared to that of the left ventricle of reference subjects [14]. In the right ventricle and the non-necrotic left ventricle, Zn concentrations were even slightly higher than those of control groups. Accordingly, in view of the drop in Pl-Zn and the negative significant correlation between Pl-Zn and CK or CK-MB on the first days after MI [15], the hypothesis would seem feasible that circulating Zn is taken up by non-necrosed myocardial tissue, in proportion to the extent of the necrotic area, as part of the reparative process [6, 13]. Interleukin-1 release might mediate this observed hypozincemia via a heart Zn-metallothionein.

The two tree diagrams of Figure 1 (A, B) show the well-known relationship discussed in our previous works [15, 16] between Pl-Zn and CK or CK-MB as well as other correlations, particularly, in PIS men, between Pl-Zn and Pl-Ca; and between Pl-Zn and Pl-Mg in MI. In PIS men, the correlation between Pl-Zn and Pl-Ca is similar to that found previously by us in 58 control men [3]. Serum-Zn concentration, like that of Ca, is maintained within a narrow range in healthy humans [17, 18]. Even though the metabolism of Ca and Zn differs substantially, Ca-regulating hormones are known to affect Zn metabolism [17]. In MI men, the relationships are modified, and Pl-Zn is correlated with Pl-Mg. All these statistical interpretations indicate both the differences among the relationships of the variables studied according to the severity of ischemia and the quite special role of Pl-Zn in diagnosis and perhaps prognosis. Our current investigations are continuing along these lines.

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