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Non-survivor patients with malignant middle cerebral artery infarction showed persistently high serum malondialdehyde levels

Leonardo Lorente[1*], María M. Martín[2], Pedro Abreu-González[3], Rafael Sabatel[4], Luis Ramos[5], Mónica Argueso[6], Jordi Solé-Violán[7], Juan J. Cáceres[8], Alejandro Jiménez[9] and Victor García-Marín[10]

Abstract

Objective: Previously there have been found higher circulating malondialdehyde levels during the first week of ischemic stroke in patients with worst neurological functional outcome, and at moment of ischemic stroke in non-survivor patients. Thus, the aim of our study was to determine the potential role of serum malondialdehyde levels during the first week of a severe cerebral infarction to mortality prediction.

Methods: This study was observational, prospective, and multicenter. We included patients with a severe malignant middle cerebral artery infarction (MMCAI) defined as patients with computed tomography showing acute infarction in more than of 50% of the territory and Glasgow Coma Scale (GCS) lower than 9. We determined serum concentrations of malondialdehyde on days 1, 4 and 8 of MMCAI.

Results: Serum malondialdehyde concentrations at days 1 ($p < 0.001$), 4 ($p < 0.001$), and 8 ($p = 0.001$) of MMCAI in non-survivor patients ($n = 34$) were higher than in survivor patients ($n = 34$). ROC curve analyses showed that serum malondialdehyde concentrations at days 1, 4, and 8 of MMCAI had an AUC (95% CI) to predict 30-day mortality of 0.77 (0.65–0.86; $p < 0.001$), 0.82 (0.69–0.91; $p < 0.001$) and 0.84 (0.70–0.93; $p < 0.001$) respectively.

Conclusions: The new findings of our study were that serum malondialdehyde levels during the first week of MMCAI could be used as biomarkers to mortality prediction.

Keywords: Malondialdehyde, Ischemic stroke, Patients, Mortality, Prognosis

Introduction

A large quantity of disabilities, deaths and resources consumption are generated by ischemic stroke [1]. In ischemic stroke, in addition to cell death produced by brain vasculature obstruction that causes a reduction of blood containing oxygen and substrates to neurons, could appears a secondary brain injury mediated by oxidative stress [2–6]. Different end-products could appear during lipid peroxidation such as malondialdehyde, which is formed during cellular membrane phospholipids degradation [3, 4]. Afterwards malondialdehyde could be released to extracellular space and appears in the blood; and circulating malondialdehyde levels have been used as lipid oxidation biomarker [7, 8].

Previously have been found higher circulating malondialdehyde levels during the first week of ischemic stroke in patients with worst neurological functional outcome [9–12], and at moment of ischemic stroke in non-survivor patients [13, 14]. Thus, the aim of our study was to determine the potential role of serum malondialdehyde levels during the first week of a severe cerebral infarction to mortality prediction.

* Correspondence: lorentemartin@msn.com
[1]Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n. La Laguna –, 38320 Santa Cruz de Tenerife, Spain
Full list of author information is available at the end of the article

Methods

Design and subjects

This study was observational and prospective. This multicentre study was performed with the Institutional Review Board approval of the six participating hospitals and with the written informed consent of patient legal guardians. This study was carried out in the Intensive Care Units of the following hospitals: H. General de La Palma from Breña Alta, H. Universitario Dr. Negrín from Las Palmas de Gran Canaria, H. Insular from Las Palmas de Gran Canaria, H. Universitario Nuestra Señora de Candelaria from Santa Cruz de Tenerife, H. Clínico Universitario de Valencia from Valencia, and H. Universitario de Canarias from La Laguna.

We included patients with a severe malignant middle cerebral artery infarction (MMCAI), defined as computed tomography showing acute infarction in more than of 50% of the territory and Glasgow Coma Scale (GCS) [15] lower than 9; and there were excluded patients with brain hemorrhage, less than 18 years of age, inflammatory or malignant disease, or pregnancy.

Previously, our team determined serum malondialdehyde concentrations in some of those patients in the day of a severe MMCAI [14]. In this current work, we determine serum malondialdehyde concentrations at days 1, and also at days 4 and 8.

Clinical and demographic variables

We recorded the following variables from the patients: age, arterial hypertension, sex, chronic obstructive pulmonary disease (COPD), diabetes mellitus, heart failure, chronic renal failure, temperature, CGS, lactic acid, sodium, bilirubin, creatinine, glycaemia, pressure of arterial oxygen (PaO_2), fraction inspired oxygen ($FI0_2$), PaO_2/FIO_2 ratio, platelets, leukocytes, haemoglobin, fibrinogen, international normalized ratio (INR), activated partial thromboplastin time (aPTT), Acute Physiology and Chronic Health Evaluation II (APACHE II) score [16], thrombolysis, volumen infarction, haemorrhagic transformation, midline shift, and descompresive craniectomy. Thirty-day mortality was considered as the end-point study.

Meassure of serum malondialdehyde concentrations

We obtained serum blood samples on days 1, 4 and 8 of MMCAI and were frozen at − 80 °C until the determination of serum malondialdehyde concentrations. All assays for the meassure of malondialdehyde concentrations were carried out in the Physiology Department of Medicine Faculty of La Laguna University (Tenerife, Spain). The meassure of malondialdehyde concentrations was performed according to thiobarbituric acid-reactive substance (TBARS) method by Kikugawa et al. [17]. We mixed serum (200 μL), thiobarbituric acid (2.5 mL at 0.8%), sodium dodecyl sulfate (200 μL at 8.1%), trichloroacetic acid (1.5 mL with pH 3.5) and butylated hydroxytoluene (50 μL at 0.8%). We kept the mixture during 1 h at 5 °C and later it was heated during 1 h at 100 °C. Afterwards, n-butanol was extracted. Finally, the sample was placed doubly in a 96-well plate and read at 535 nm with a spectrophotometer reader (Benchmark Plus, Bio-Rad, Hercules, CA, USA). The assay detection limit, intra-assay coefficient variation, and inter-assay coefficient variation were of 0.08 nmol/ml, 1.82, and 4.01% respectively.

Statistical methods

Medians (and interquartile ranges) were used to describe continuous variables, and frequencies (and percentages) to describe categorical variables. Wilcoxon-Mann-Whitney test was used to compare continuous variables between survivor and non-survivor patient groups, and chi-square test to compare categorical variables between patient groups. Receiver operating characteristic (ROC) analyses were used to determine the capacity for 30-day mortality prediction by serum malondialdehyde levels at day 1, 4 and 8 of MMCAI. Area under curve (AUC), and sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predicted value and negative predicted value of serum malondialdehyde levels cut-offs for mortality prediction are showed with its 95% confidence intervals (CI). Optimal cut-off values at days 1, 4 and 8 were selected according to Youden J index. Multiple logistic regression was carried out to determine whether exists an association between serum malondialdehyde levels and 30-day mortality after to control for platelet count, lactic acid and GCS. *P*-values< 0.05 was the point considered to determine as statistically significant. SPSS 17.0 (SPSS Inc., Chicago, IL, USA), LogXact 4.1 (Cytel Co., Cambridge, MA), and NCSS 2000 (Kaysville, Utah) were the programs used for statistical analyses.

Results

In Table 1 is possible to see that significant differences were not found between patients groups, non-survivors ($n = 34$) and non-survivors (n = 34), in age, arterial hypertension, sex, COPD, diabetes mellitus, heart failure, chronic renal failure, temperature, lactic acid, sodium, bilirubin, creatinine, glycaemia, PaO_2, PaO_2/FIO_2 ratio, leukocytes, haemoglobin, fibrinogen, INR, aPTT, APACHE-II, thrombolysis, volumen infarction, haemorrhagic transformation, midline shift, and descompresive craniectomy. However, lower GCS and platelets were found in non-survivor than in survivor patients.

Serum MDA concentrations at days 1 ($p < 0.001$), 4 ($p < 0.001$), and 8 ($p = 0.001$) of MMCAI in non-survivor patients were higher than in survivor patients (Table 2 and Fig. 1).

Table 1 Clinical and biochemical characteristics of 30-day survivor and non-survivor MMCAI patients

	Non-survivors ($n = 34$)	Survivors ($n = 34$)	*P*-value
Age (years) - median (p 25–75)	63 (53–70)	59 (47–68)	0.36
Arterial hypertension - n (%)	16 (47.1)	19 (55.9)	0.63
Gender female - n (%)	13 (38.2)	14 (41.2)	0.99
COPD - n (%)	1 (2.9)	1 (2.9)	0.99
Diabetes mellitus - n (%)	9 (26.5)	4 (11.8)	0.22
Heart failure - n (%)	1 (2.9)	1 (2.9)	0.99
Chronic renal failure - n (%)	2 (5.9)	2 (5.9)	0.99
Temperature (°C) - median (p 25–75)	36.9 (36.0–37.3)	36.4 (36.0–37.0)	0.15
GCS score - median (p 25–75)	6 (3–7)	7 (6–8)	0.01
Lactic acid (mmol/L)-median (p 25–75)	1.55 (1.00–2.70)	1.20 (0.90–1.70)	0.05
Sodium (mEq/L)- median (p 25–75)	140 (139–145)	139 (136–145)	0.38
Bilirubin (mg/dl) - median (p 25–75)	0.60 (0.33–1.10)	0.60 (0.40–0.83)	0.95
Creatinine (mg/dl) - median (p 25–75)	1.00 (0.70–1.25)	0.80 (0.60–1.13)	0.19
Glycemia (g/dL) - median (p 25–75)	136 (118–162)	127 (100–170)	0.40
PaO2 (mmHg) - median (p 25–75)	115 (94–267)	156 (105–293)	0.26
$PaO2/FI0_2$ ratio - median (p 25–75)	254 (192–325)	300 (198–369)	0.24
Platelets - median*$10^3/mm^3$ (p 25–75)	175 (136–216)	202 (171–265)	0.02
Leukocytes-median*$10^3/mm^3$ (p 25–75)	13.9 (9.7–20.1)	12.4 (9.6–16.9)	0.32
Hemoglobin (g/dL) - median (p 25–75)	12.5 (11.0–14.8)	12.1 (11.4–14.0)	0.81
Fibrinogen (mg/dl) - median (p 25–75)	419 (337–631)	443 (416–489)	0.90
INR - median (p 25–75)	1.20 (1.01–1.31)	1.06 (1.00–1.20)	0.07
aPTT (seconds) - median (p 25–75)	27 (26–32)	28 (25–30)	0.91
APACHE-II score - median (p 25–75)	22 (19–27)	20 (16–25)	0.06
Thrombolysis - n (%)	10 (29.4)	11 (32.4)	0.99
Volumen infarction (ml) - median (p25–75)	180 (60–277)	173 (100–231)	0.64
Haemorrhagic transformation - n (%)	6 (17.6)	7 (20.6)	0.99
Midline shift (mm) - median (p 25–75)	9.0 (3.5–15.0)	6.0 (2.5–11.5)	0.43
Decompressive craniectomy – n (%)	7 (20.6)	9 (26.5)	0.78
Malondialdehyde (nmol/mL)-median (p 25–75)	2.96 (2.01–4.44)	1.84 (1.33–2.28)	< 0.001

P 25–75 percentile 25th–75th, *COPD* chronic obstructive pulmonary disease, *GCS* Glasgow Coma Scale, *PaO_2* pressure of arterial oxygen/fraction inspired oxygen, *FIO_2* fraction inspired oxygen, *INR* international normalized ratio, *aPTT* activated partial thromboplastin time, *APACHE II* Acute Physiology and Chronic Health Evaluation

Table 2 Receiver operation characteristic analysis using serum malondialdehyde levels at day 1, 4 and 8 of severe malignant middle cerebral artery infarction as predictor of mortality at 30 days

	Day 1	Day 4	Day 8
Cut-off of malondialdehyde (mmol/mL)	> 2.87	> 2.12	> 2.01
Sensitivity and 95% CI	58.8 (40.7–75.4)	83.3 (58.6–96.4)	83.3 (51.6–97.9)
Specificity and 95% CI	94.1 (80.3–99.3)	91.2 (76.3–98.1)	94.1 (80.3–99.3)
Positive likelihood ratio and 95% CI	10.0 (2.5–39.5)	9.4 (3.1–28.4)	14.2 (3.6–55.6)
Negative likelihood ratio and 95% CI	0.4 (0.3–0.7)	0.2 (0.1–0.5)	0.2 (0.1–0.6)
Positive predicted value and 95% CI	90.9 (71.7–97.5)	83.3 (62.5–93.8)	83.3 (56.0–95.2)
Negative predicted value and 95% CI	69.6 (60.3–77.5)	91.2 (78.5–96.7)	94.1 (81.8–98.3)

CI confidence intervals

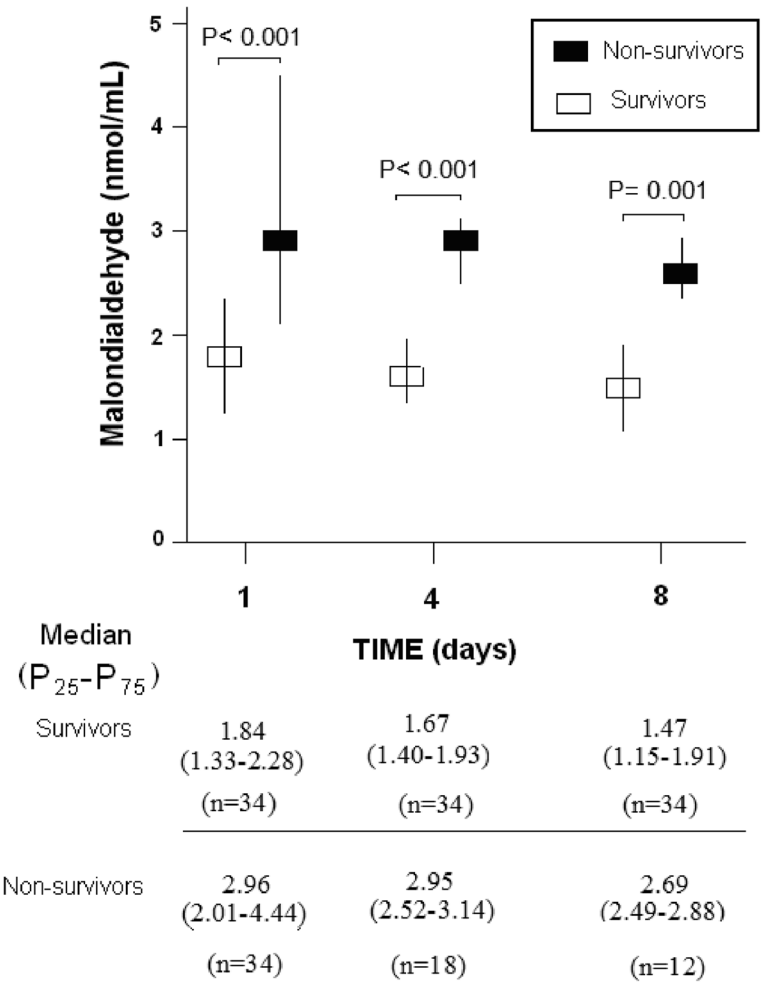

Fig. 1 Serum malondialdehyde levels at day 1, 4 and 8 of severe malignant middle cerebral artery infarction in 30-day survivor and non-survivor patients

ROC curve analyses showed that serum malondialdehyde concentrations at days 1, 4, and 8 of MMCAI had an AUC (95% CI) to predict 30-day mortality of 0.77 (0.65–0.86; $p < 0.001$), 0.82 (0.69–0.91; $p < 0.001$) and 0.84 (0.70–0.93; $p < 0.001$) respectively. Table 2 showed sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predicted value and negative predicted value of serum malondialdehyde levels cut-offs at days 1, 4, and 8 of MMCAI for mortality prediction.

Multiple logistic regression an association between serum malondialdehyde levels> 2.87 mmol/mL and 30-day mortality (OR = 6.509; 95% CI = 2.095–20.222; $p = 0.001$) after to control for platelet count, lactic acid and GCS (Table 3).

Discussion

The new findings of our study were that serum malondialdehyde levels during the first week of MMCAI could be used as biomarkers to mortality prediction.

Previously have been found higher circulating malondialdehyde levels during the first week of ischemic stroke in patients with worst neurological functional outcome

Table 3 Multiple logistic regression analysis to predict 30-day mortality

Variable	Odds Ratio	95% Confidence Interval	*P*-value
Platelet count (each 1000/mm^3)	0.995	0.985–1.006	0.39
Lactic acid (mmol/L)	0.967	0.486–1.925	0.92
Glasgow Coma Scale (points)	0.528	0.336–0.829	0.01
Serum malondialdehyde > 2.87 mmol/mL	6.509	2.095–20.222	0.001

[9–12], and at moment of ischemic stroke in non-survivor patients [13, 14]. Thus, the higher serum MDA levels during the first week of MMCAI observed in non-survivor patients in respect to survivor patients, and that those levels could be used as mortality prediction are two novel findings of our study.

We believed that those higher concentrations of serum malondialdehyde during the first week of MMCAI in non-survivor patients reflects a higher ROS production and lipid peroxidation in comparison to survivor patients, and the use of antioxidant agents could be a new therapeutic to explore in MMCAI patients. The administration of melatonin in animal models of ischemic stroke has been associated with a reduction of oxidation [18–22], specifically a reduction of malondialdehyde levels [21, 22], and even an increase of survival. In patients with ischemic stroke, the oral administration of different antioxidant vitamins (B2, B6, B12, C, E) during the first 14 days of stroke has been associated with lower plasma levels of malondialdehyde [23–25]. Thus, although we recognize that our study has the limitation that other oxidant state compounds were not reported, we think that all those findings could open the interest for study in patients with ischemic strike the oxidative stress and the potential role of antioxidant agents in your treatment.

Conclusions

The new findings of our study were that serum malondialdehyde levels during the first week of MMCAI could be used as biomarkers to mortality prediction.

Abbreviations

APACHE: Acute Physiology and Chronic Health Evaluation; aPTT: activated partial thromboplastin time; COPD: Chronic Obstructive Pulmonary Disease; FIO_2: fraction inspired of oxygen; GCS: Glasgow Coma Scale; ICU: Intensive Care Unit; INR: International normalized ratio; PaO_2: Pressure of arterial oxygen/fraction inspired oxygen

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Authors'contributions

LL conceived, designed and coordinated the study, participated in acquisition and interpretation of data, and drafted the manuscript. MMM, RS, LR, MA, JSV, JJC, VGM participated in acquisition of data. PAG participated in blood determination levels. AJ participated in the interpretation of data. All authors revised the manuscript critically for important intellectual content, made the final approval of the version to be published, and were agreed to be accountable for all aspects of the work.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was performed with the Institutional Review Board approval of the six participating hospitals and with the written informed consent of patient legal guardians. This study was carried out in the Intensive Care Units of the following hospitals: H. General de La Palma from Breña Alta, H. Universitario Dr. Negrín from Las Palmas de Gran Canaria, H. Insular from Las Palmas de Gran Canaria, H. Universitario Nuestra Señora de Candelaria from Santa Cruz de Tenerife, H. Clínico Universitario de Valencia from Valencia, and H. Universitario de Canarias from La Laguna. The study adheres to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n. La Laguna –, 38320 Santa Cruz de Tenerife, Spain. [2]Intensive Care Unit, Hospital Universitario Nuestra Señora de Candelaria, Crta del Rosario s/n, 38010 Santa Cruz de Tenerife, Spain. [3]Deparment of Phisiology, Faculty of Medicine, University of the La Laguna, 38320 Santa Cruz de Tenerife, La Laguna, Spain. [4]Deparment of Radiology, Hospital Universitario de Canarias, Ofra, s/n. La Laguna –, 38320 Santa Cruz de Tenerife, Spain. [5]Intensive Care Unit, Hospital General La Palma, Buenavista de Arriba s/n, 38713 Breña Alta, La Palma, Spain. [6]Intensive Care Unit, Hospital Clínico Universitario de Valencia, Avda. Blasco Ibáñez n°17-19, 46004 Valencia, Spain. [7]Intensive Care Unit, Hospital Universitario Dr. Negrín, CIBERES, Barranco de la Ballena s/n, 35010 Las Palmas de Gran Canaria, Spain. [8]Intensive Care Unit, Hospital Insular, Plaza Dr. Pasteur s/n, ia-35016 Las Palmas de Gran Canar, Spain. [9]Research Unit, Hospital Universitario de Canarias, Ofra, s/n. La Laguna –, 38320 Santa Cruz de Tenerife, Spain. [10]Deparment of Neurosurgery, Hospital Universitario de Canarias, Ofra, s/n. La Laguna –, 38320 Santa Cruz de Tenerife, Spain.

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