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Septic patients with mitochondrial DNA haplogroup JT have higher respiratory complex IV activity and survival rate☆,☆☆

Leonardo Lorente, MD, PhD [a,*,1], María M. Martín, MD, PhD [b], Ester López-Gallardo, PhD [c], José Ferreres, MD [d], Jordi Solé-Violán, MD, PhD [e], Lorenzo Labarta, MD [f], César Díaz, MD [g], Alejandro Jiménez, PhD [h], Julio Montoya, PhD [i], Eduardo Ruiz-Pesini, PhD [j,1]

[a] Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n, La Laguna-38320, Santa Cruz de Tenerife, Spain
[b] Intensive Care Unit, Hospital Universitario Nuestra Señora Candelaria, Crta Rosario s/n, Santa Cruz Tenerife-38010, Spain
[c] Departamento de Bioquímica y Biología Molecular y Celular, Centro de Investigaciones Biomédicas En Red de Enfermedades Raras (CIBERER) and Instituto de Investigación Sanitaria de Aragón, Universidad de Zaragoza, Zaragoza-50013, Spain
[d] Intensive Care Unit, Hospital Clínico Universitario de Valencia, Avda, Blasco Ibáñez n°17-19, Valencia-46004, Spain
[e] Intensive Care Unit, Hospital Universitario Dr. Negrín, Barranco de la Ballena s/n, Las Palmas de Gran Canaria-35010, Spain
[f] Intensive Care Unit, Hospital San Jorge de Huesca, Avenida Martínez de Velasco n°36, Huesca-22004, Spain
[g] Intensive Care Unit, Hospital Insular, Plaza Dr. Pasteur s/n, Las Palmas de Gran Canaria-35016, Spain
[h] Research Unit, Hospital Universitario de Canarias, Ofra, s/n, La Laguna-38320, Santa Cruz de Tenerife, Spain
[i] Departamento de Bioquímica y Biología Molecular y Celular, Centro de Investigaciones Biomédicas En Red de Enfermedades Raras (CIBERER) and Instituto de Investitación Sanitaria de Aragón, Universidad de Zaragoza, Zaragoza-50013, Spain
[j] Departamento de Bioquímica y Biología Molecular y Celular, Centro de Investigaciones Biomédicas En Red de Enfermedades Raras (CIBERER), Instituto de Investitación Sanitaria de Aragón and Fundación ARAID, Universidad de Zaragoza, Zaragoza-50013, Spain

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ABSTRACT

Objective: The influence of mitochondrial deoxyribonucleic acid (mtDNA) haplogroup or oxidative phosphorylation system (OXPHOS) function on survival of septic patients has been scarcely studied. However, the association between mtDNA haplogroup, OXPHOS capacity at diagnosis of severe sepsis, and survival has been not previously reported, and that was the objective of the present study.
Methods: This was a prospective, multicenter, observational study. Blood samples from 198 patients at diagnosis of severe sepsis were analyzed to determine mtDNA haplogroup and platelet respiratory complex IV (CIV) specific activity. The end point of the study was 30-day survival.
Results: Septic patients with mtDNA haplogroup JT showed higher 30-day survival than those with mtDNA haplogroup non-JT (31/38 [81.6%] vs 99/160 [61.9%]; $P = .02$). Septic patients with mtDNA haplogroup JT showed higher platelet CIV specific activity than those with mtDNA haplogroup non-JT ($P = .002$).
Conclusions: The main novel finding of our study, including the largest series providing data on platelet CIV specific activity according to mtDNA haplogroup in severe septic patients, was that those with mtDNA haplogroup JT showed higher survival and higher platelet CIV specific activity at diagnosis of severe sepsis than patients with mtDNA haplogroup non-JT.

1. Introduction

The influence of mitochondrial deoxyribonucleic acid (mtDNA) haplogroup [1–4] or oxidative phosphorylation system (OXPHOS) function [5–10] on survival of septic patients has been scarcely studied. Regarding the influence of mtDNA haplogroup on survival of septic patients, in a study by Baudouin et al [2] including 150 septic patients in England, patients with mtDNA haplogroup H showed higher 6-month survival than those with other haplogroups. In a study involving 181 septic patients in China, Yang et al [1] found that patients with mtDNA macrolineage R showed higher 6-month survival than those with other macrolineages. In a study by our team including 96 severe septic patients in Spain, patients with mtDNA haplogroup JT had higher 1-month survival than those with other mtDNA haplogroups [3]. In a

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* Corresponding author at: Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n, La Laguna-38320, Santa Cruz de Tenerife. Spain. Tel.: +34 922678000; fax: +34 922647112.
E-mail addresses: lorentemartin@msn.com (L. Lorente), mar.martinvelasco@gmail.com (M.M. Martín), esterlop@unizar.es (E. López-Gallardo), blanquer_jos@gva.es (J. Ferreres), jsolvio@gobiernodecanarias.org (J. Solé-Violán), llabarta@salud.aragon.es (L. Labarta), incaicos@yahoo.es (C. Díaz), ajimenezsosa@gmail.com (A. Jiménez), jmontoya@unizar.es (J. Montoya), eduruiz@unizar.es (E. Ruiz-Pesini).
[1] These authors equally contributed to this article.

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later study of 292 Spanish patients with severe septic sepsis, we found that mtDNA haplogroup JT patients showed increased 30-day and 6-month survival [4]. Regarding the influence of OXPHOS function on survival in septic patients, our team has reported that nonsurviving septic patients showed lower platelet respiratory complex IV (CIV) specific activity than nonsurvivors [5–7]. In addition, a lower mitochondrial inner membrane potential in platelets [8] and in peripheral blood mononuclear cells [9] has been reported in nonsurviving than in surviving septic patients.

In a previous study by our team with 96 severe septic patients, patients with mtDNA haplogroup JT showed higher platelet CIV specific activity at day 4 and day 8 of severe sepsis diagnosis than patients with mtDNA haplogroup non-JT [3]. However, the association between mtDNA haplogroup, platelet CIV specific activity at diagnosis of severe sepsis, and survival has not been previously reported. We hypothesized that septic patients with mtDNA haplogroup JT may have higher platelet CIV specific activity at diagnosis of severe sepsis and higher survival than patients with mtDNA haplogroup non-JT. The objective of the present study was therefore to determine whether there is an association between mtDNA haplogroup, platelet CIV specific activity at diagnosis of severe sepsis, and survival rate.

2. Material and methods

2.1. Design and subjects

This prospective, multicenter, and observational study was carried out in 6 intensive care units of hospitals in Spain. The study was approved by the Institutional Review Boards of each hospital: Hospital Universitario de Canarias (La Laguna, Santa Cruz de Tenerife), Hospital Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife), Hospital Universitario Dr. Negrín (Las Palmas de Gran Canaria), Hospital Clínico Universitario de Valencia (Valencia), Hospital San Jorge (Huesca), and Hospital Insular (Las Palmas de Gran Canaria).

We included 198 patients with severe sepsis diagnosed according to the International Sepsis Definitions Conference criteria [11]. We excluded patients with age <18 years; lactation; pregnancy; platelet transfusion; white blood cell count <1000 cells/μL; human immunodeficiency virus; solid or hematological tumor; and steroid, immunosuppressive, or radiation therapy. Written informed consent from patients (or their family members) was obtained. Part of this patient cohort was previously analyzed by our team [3]. In that study with 96 severe septic patients, we found that those with mtDNA haplogroup JT showed higher platelet CIV specific activity than patients with mtDNA haplogroup non-JT, which was statistically significant at day 4 and day 8 of severe sepsis diagnosis and nonsignificant at day 1 [3]. In addition, there was much early mortality, which prompted us to determine whether there is an association between mtDNA haplogroup, platelet CIV specific activity at diagnosis of severe sepsis, and survival rate.

We recorded the following variables for each septic patient: age, activated partial thromboplastin time (aPTT), Acute Physiology and Chronic Health Evaluation (APACHE) II score [12], bilirubin, bloodstream infection, chronic obstructive pulmonary disease (COPD), creatinine, diabetes mellitus, empiric antimicrobial treatment, female sex, international normalized ratio (INR), infection site, ischemic heart disease, lactic acid, leukocytes, microorganism responsible, pressure of arterial oxygen/fraction inspired of oxygen (Pao_2/Fio_2), platelets, Sepsis-related Organ Failure Assessment (SOFA) score [13], and survival at 30 days (as the end point of the study).

2.2. Blood samples

We obtained blood samples from severe septic patients at diagnosis of severe sepsis; platelets were obtained from blood samples according to a previously described protocol [14] and frozen at −80 °C until the determination of platelet CIV specific activity. In addition, venous blood samples were frozen at −80 °C until determination of mtDNA haplogroup.

2.3. Determination of mtDNA haplogroup

The determination of mtDNA haplogroup was performed as in previous studies by our team [3,4]. We extracted DNA by standard protocols and determined mtDNA haplogroup by real-time polymerase chain reaction. For all the samples, we genotyped three single nucleotide polymorphisms (SNPs) that define very frequent haplogroups: m.4216C (haplogroup JT), m.7028C (haplogroup H), and m.12308G (haplogroup U). In addition, another 14 mtDNA SNPs were analyzed using a hierarchic approach to confirm particular haplogroups: m.1811G, m.3010A, m.4336C, m.4580A, m.4769A, m.9477A, m.10873C, m.13708A, m.14766C, m.14793G, m.14798C, m.15218G, m.15257A, and m.15693C. We used TaqMan reagents (Applied Biosystems, Austin, TX) to perform real-time polymerase chain reaction. We included reagents for the determination of each SNP, including 2 primers around the SNP and 2 probes (a fluorophore VIC-labeled probe specific for one allele and another fluorophore FAM-labeled probe specific for the other allele). We amplified DNA in a final volume of 25 μL, using 12.5 μL of TaqMan Gene Expression Master Mix, 0.9 μmol/L of each primer, 0.2 μmol/L of each probe, and 10 ng of total DNA. DNA amplification was performed by universal conditions.

2.4. Determination of platelet CIV specific activity

Platelet CIV specific activity was determined as in previous studies by our team [5–7] using Mitoprofile Human Complex IV Activity kit from Mitosciences (Invitrogen) according to the manufacturer's instructions. CIV is immunocaptured by this kit, and CIV activity is determined colorimetrically by following the oxidation of reduced cytochrome c as an absorbance decrease at 550 nm. We assayed protein concentrations by previously described protocols [15], expressed in milligrams. We used a NovoStar MBG Labtech microplate instrument for the determinations of CIV activity and protein quantity. CIV specific activity was expressed as milli–optic disc/min per mg of protein multiplied by 100.

2.5. Statistical analysis

Categorical variables are reported as frequencies and percentages; and continuous variables, as medians and interquartile ranges. We used χ^2 test to compare categorical variables between surviving and nonsurviving patients and Wilcoxon-Mann-Whitney test to compare continuous variables. We carried out a survival analysis with Kaplan-Meier curve method using mtDNA haplogroup JT vs non-JT as the independent variable and 30-day survival as the dependent variable, and both curves were compared by log-rank test. Hazard ratios and their 95% confidence intervals were calculated as measures of the association. *P* values lower than .05 were considered statistically significant. We used SPSS v. 17.0 (SPSS Inc, Chicago, IL) and Med Calc v. 10.1.3.0 (Mariakerke, Belgium) programs for the statistical analyses.

3. Results

The study included a total of 198 severe septic patients, and the mtDNA haplogroups were as follows: 80 (40.4%) HV, 51 (25.8%) U, 29 (14.6%) non-R, and 38 (19.2%) JT; thus, 160 (80.8%) had an mtDNA haplogroup non-JT. The survival rate for each mtDNA haplogroup patient group was as follows: 48/80 (60.0%) HV, 33/51 (64.7%) U, 18/29 (62.1%) non-R, and 31/38 (81.6%) JT; thus, the survival rate for patients with mtDNA haplogroup non-JT was 99/160 (61.9%).

Table 1 shows clinical characteristics of patients according to mtDNA haplogroups. We did not find significant differences between patient mtDNA haplogroups JT and non-JT in age, aPTT, APACHE II score, bilirubin, bloodstream infection, COPD, creatinine, diabetes mellitus, empiric