Title page

Type of manuscript: Article

Title: Mitochondrial DNA haplogroups are associated with severe sepsis and mortality in patients who underwent major surgery

Short running title: mtDNA haplogroups and sepsis

Authors: Maria Angeles Jiménez-Sousa, PhD [a (*)], Eduardo Tamayo, PhD [b], María Guzmán-Fulgencio, PhD [a], María Heredia, MD [b], Amanda Fernández-Rodríguez, PhD [a], Esther Gómez, M.D. [b], Raquel Almansa, PhD [c], José I Gómez-Herreras, M.D. [b], Mónica García-Álvarez, PhD [a], Sandra Gutiérrez-Junco, M.D. [b], Jesús F Bermejo-Martin, PhD [c], Salvador Resino, PhD [a (*)] on behalf of the Spanish Sepsis Group (SpSG).
(*) Corresponding author.

Current affiliations: (a) Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología. Instituto de Salud Carlos III, Majadahonda, Spain. (b) Departamento de Anestesiología y Reanimación, Hospital Clínico Universitario, Valladolid, Spain. (c) Unidad de Investigación Médica en Infección e Inmunidad. Hospital Clínico Universitario-IECSCYL, Valladolid, Spain.

Correspondence and reprint requests:
Salvador Resino; Centro Nacional de Microbiología, Instituto de Salud Carlos III (Campus Majadahonda); Carretera Majadahonda- Pozuelo, Km 2.2; 28220 Majadahonda (Madrid); Telf.: +34 918 223 266; Fax: +34 915 097 946; e-mail: sresino@isciii.es
Maria Angeles Jiménez Sousa; Centro Nacional de Microbiología, Instituto de Salud Carlos III (Campus Majadahonda); Carretera Majadahonda- Pozuelo, Km 2.2; 28220 Majadahonda (Madrid); Telf.: +34 918 2223278; Fax: +34 915 097 946; e-mail: majimenezsousa@yahoo.es

ABSTRACT

Objective: To analyse whether mitochondrial DNA (mtDNA) haplogroups are associated with severe sepsis and mortality after major surgery.

Methods: We performed a case-control study on 240 cardiac or abdominal surgery patients developing severe sepsis (Case-group) and 267 cardiac or abdominal surgery patients without severe sepsis and with systemic inflammatory response syndrome (SIRS, Control-group). Furthermore, a longitudinal substudy was performed for analysing the survival in septic patients. Only European white patients within the N macro-cluster were included.

Results: Case-group underwent cardiac surgery had lower frequencies of cluster HV (p=0.005) and haplogroup H (p=0.005) and higher frequencies of cluster JT (p=0.028) than Control-group; but no significant differences were found for abdominal surgery. Besides, both cluster HV and haplogroup H were associated with decreased odds of severe sepsis (odds ratio (aOR)=0.45 (95%CI=0.25; 0.82); p=0.009) and aOR=0.48 (95%CI=0.26; 0.87); p=0.015), respectively) among patients underwent cardiac surgery. In Case-group, 45.4% (109/240) patients died with a survival median of 39 (95%CI=31.4; 46.62) days. When the clusters were examined, 41% (55/134) patients within cluster HV died versus 71.4% (10/14) patients within cluster IWX (p=0.018). Additionally, patients within cluster IWX had an increased risk of death (adjusted hazard ratio (aHR)=2.22; (95%CI=1.14; 4.34); p=0.019).

Conclusions: European mitochondrial haplogroups might be related to the onset of severe sepsis in patients who underwent major cardiac surgery, but not in patients underwent major abdominal surgery. Besides, mtDNA haplogroups could have influence on mortality in septic patients.

Key words: mitochondrial haplogroups; mtDNA; genetic polymorphism; severe sepsis; systemic inflammatory response syndrome (SIRS); survival

INTRODUCTION

Sepsis is defined by a systemic inflammatory response syndrome (SIRS) secondary to bacterial infection (1), which is caused by an uncontrolled immune response to microbial antigens (2). Sepsis may lead to generalized hypoperfusion, multiorgan failure, and finally death. Thus, severe sepsis is defined as a sepsis with sepsis-induced organ dysfunction or tissue hypoperfusion; and whether low blood pressure persists after the administration of intravenous fluids, it is considered as a septic shock (3). Sepsis may occur around 6-30% of all intensive care unit (ICU) patients, with substantial variation due to the heterogeneity between ICUs (4-6). In general, sepsis is predominantly caused by intra-abdominal infection among surgery ICU patients (7). Furthermore, more than 50% of severe sepsis patients require intensive care services and mortality among themselves may range up to 50% (4, 8).

Mitochondrial dysfunction plays a crucial role in the pathophysiology of sepsis (9). Many studies have indicated that the cellular O_2 utilization is altered in sepsis (10), which leads to excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (11). These metabolites produce several negative effects: induce mitochondrial dysfunction and inflammation, a significant fall in the level of ATP, and deleterious effects on various biological molecules and structures; leading to cell dysfunction, which may result in organ failure and death (9, 12). Besides, mitochondrial dysfunction could lead to bioenergetic failure of key leukocytes acting in sepsis (13, 14), altering its function and thus, decreasing the host´s ability to combat the infection.

The question why, under similar circumstances, some patients eliminate more easily an invading microorganism, whereas other patients develop sepsis and evolve to more advanced stages of the disease, remains unresolved. Differences in mitochondrial function could have an important role in this regard. In fact, mitochondrial DNA (mtDNA) is highly polymorphic, and its variations might contribute to these differences in function. Evolutionarily, human population has been subdivided into a number of discrete mitochondrial clades or haplogroups, which are defined on the basis of specific mtDNA polymorphisms acquired throughout human history (15). In this setting, mtDNA variants may play an important role for predicting clinical outcome in sepsis. In Chinese Han population, the mtDNA macrolineage R has been associated to the outcome of severe sepsis (16, 17). In European Caucasians, this macrolineage includes 4 major haplogroups or clusters (HV, U, JT, and IWX) and several minor haplogroups (H, V, pre-V, J, T, Uk, W, X, I, etc.) (15). In septic patients, haplogroup H has been associated with increased chance of survival (18). Furthermore, controversial findings have been published about cluster JT and mortality. Whereas some authors have found that JT cluster confers an increased risk for complications and death in septic patients (19), others have described a higher survival compared with other haplogroups (20).

Due to these controversial findings, the aim of this study was to analyse whether mtDNA haplogroups are associated with severe sepsis and mortality after major surgery in European populations.

MATERIALS AND METHODS

Patients

We carried out a case-control study on patients older than 18 who underwent major cardiac or abdominal surgery at the Hospital Clínico Universitario of Valladolid (Spain) between April 2008 and November 2012. Major surgery was considered as an operative procedure in which the patient was under general anaesthesia and respiratory assistance because the patient was not able to breathe independently. Furthermore, a longitudinal substudy for analysing the survival was performed in septic patients. The study was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent for the study. The Ethics Committee of Hospital Clínico Universitario (Valladolid) and Instituto de Salud Carlos III (Majadahonda) approved this study.

Initially, 247 cardiac or abdominal surgery patients developing severe sepsis or shock septic (Case-group) and 280 cardiac or abdominal surgery patients without severe sepsis or septic shock and with systemic inflammatory response syndrome (SIRS, Control-group) were included. Subjects from the control-group were patients who had age and gender similar to the Case-group. Next, a patient from Case-group and five patients from Control-group were excluded because it was not possible to determine their mtDNA haplogroups. Additionally, to make this study more uniform, only patients with European ancestry were included. Thus, 14 patients who did not have a European "N" mtDNA macro-haplogroup (six in Case-group and eight in Control-group), which is ancestral to almost all European and many Eurasian haplogroups (15), were excluded. Finally, 240 cases and 267 controls were included in this study.

Clinical data

Demographic and clinical data were obtained from medical records: age, gender, type of surgery, prior or pre-existing conditions such as diabetes, chronic obstructive pulmonary disease, hypertension, chronic kidney disease, cancer, liver disease and cardiomyopathy. Cardiopulmonary bypass was carried out in all cardiac surgeries. Acute Physiology and Chronic health Evaluation (APACHE II score) (21) and Sequential Organ Failure Assessment (SOFA score) (22) were calculated within the first 24 hours after diagnosis, in order to evaluate severity of sepsis.

Sepsis diagnosis and outcome variables

Two major outcome variables were analysed in this study: i) severe sepsis: diagnosis of at least severe sepsis (severe sepsis or shock septic); ii) mortality: death within 90 days after diagnosis of severe sepsis.

The diagnosis of SIRS, severe sepsis or septic shock was established according to the criteria laid down by the SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference (1). The presence of infection was either documented or presumed based on clinical findings. In those cases where infection was strongly suspected but not microbiologically confirmed, two experienced clinicians discussed and reached a consensus diagnosis according to physical and laboratory findings.

mtDNA genotyping

Total DNA was extracted from peripheral blood with High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). DNA samples were genotyped for 14 mtDNA polymorphisms (adapted from Hendrickson et al. (23)) at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/). Genotyping was performed by using Sequenom's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system.

Our study only included European white participants within the N macro-cluster that is ancestral to almost all European and many Eurasian haplogroups (15). These subjects (N macro-cluster) were further parsed into the most common European major-haplogroups or cluster (HV, IWX, U, and JT) and haplogroups (H, V, pre-V, J, T, I, W and X) according to 14 polymorphisms in the mtDNA (see **supplemental data (SD) 1**).

The minor haplogroups pre-V, I, X, and W were discarded for the genetic association study because these mtDNA haplogroups had low frequencies, and they were included in broader clusters (HV and IWX) to minimize type I errors in statistical analyses. Thus, the genetic association tests were performed on the clusters HV, U, JT, and IXW; and on the haplogroups H, V, J, and T.

Statistical analysis

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS INC, Chicago, IL, USA). All p-values were two-tailed. Statistical significance was defined as $p<0.05$.

Categorical data and proportions were analysed using the chi-squared test or Fisher´s exact test. Mann-Whitney U t-test was used to compare data between independent groups. Logistic regression was carried out to assess the relationship between mtDNA haplogroups and development of severe sepsis (Case-group vs. Control-group). All logistic regression analyses were adjusted for age, gender, prior or pre-existing conditions (comorbidities), and type of surgery (cardiac or abdominal; emergency or scheduled). Kaplan-Meier and Cox regression analyses were used to analyse the survival time in patients with severe sepsis (Case-group). Cox regression was performed to analyse the mortality risk associated to mtDNA haplogroups among patients with severe sepsis (Case-group). All Cox regression analyses were adjusted for the most important clinical variables, which were selected by a "Stepwise" algorithm (age, gender, APACHE-II score, prior or pre-existing conditions (comorbidities), type of surgery (cardiac or abdominal; emergency or scheduled), and microbiologically confirmed infection were considered for entry or removal with p-value for entry of 0.15 and exit of 0.20).

RESULTS

Characteristics of the study population

Table 1 shows demographic and clinical characteristics of 240 cases (severe sepsis or septic shock) and 267 controls (SIRS). There were no significant differences in age and gender, which allowed us to exclude any bias due to age or gender differences. However, Case-group had higher values of APACHE II and SOFA scores than Control-group (p<0.001). Furthermore, Case-group had higher percentage of patients with chronic kidney disease (p<0.001), and abdominal surgery (p<0.001), while Control-group had higher percentage of patients with cancer (p= 0.001), cardiomyopathy (p<0.001), and cardiac surgery (p<0.001). Moreover, we checked the frequencies for major comorbidities stratified by mitochondrial haplogroups, but there were not significant differences (data not shown).

Table 1. Baseline characteristics of patients with septic shock/severe sepsis (case group) and systemic inflammatory response syndrome (control group).

Characteristics	Control group	Case group	p-value
No. patients	267	240	
Gender (male)	171 (64%)	150 (62.5%)	0.719
Age (years)	72 (13)	73 (17)	0.121
Surgery			
Cardiac	156 (58.4%)	99 (41.3%)	**<0.001**
Abdominal	111 (41.6%)	141 (58.8%)	**<0.001**
Emergency	20 (7.2%)	153 (63.8%)	**<0.001**
Scheduled	247 (92.5%)	87 (36.3%)	**<0.001**
Severity of disease score			
APACHE II score	9 (1)	15 (6.8)	**<0.001**
SOFA score	3 (0)	8 (4)	**<0.001**
Prior or pre-existing conditions			
Diabetes	51 (19.1%)	31 (12.9%)	0.059
Chronic obstructive pulmonary disease	35 (13.1%)	39 (16.3%)	0.317
Hypertension	167 (62.5%)	133 (55.4%)	0.103
Chronic kidney disease	14 (5.2%)	35 (14.6%)	**<0.001**
Cancer	95 (35.6%)	53 (22.1%)	**0.001**
Liver disease	5 (1.9%)	11 (4.6%)	0.081
Cardiomyopathy	163 (61%)	109 (45.4%)	**<0.001**

Values are expressed as median (interquartile range) and absolute count (percentage).
P-values were calculated by Mann-Whitney test and Chi-square test.

SD 2 shows demographic and clinical characteristics of cases according to the type of surgery. Patients underwent abdominal surgery had higher rates of emergency surgery (p< 0.001), APACHE II score (p= 0.005) and mortality (p=0.002) than patients underwent cardiac surgery. Regarding pre-existing conditions, abdominal surgery group had higher percentage of patients with cancer (p< 0.001) and lower percentage of patients with hypertension and cardiomyopathy (p= 0.001 and p< 0.001, respectively) than cardiac surgery group.

Table 2 shows the types of microorganisms causing infection that were microbiologically confirmed at the time of severe sepsis diagnosis. The most commonly isolated pathogens in patients underwent both cardiac and abdominal surgery were gram-negatives (45.7% and 50% respectively). Regarding differences between groups, methicilin-susceptible *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Haemophilus influenzae* had higher frequency among patients underwent cardiac surgery.

Table 2. Summary of microorganisms causing infection that were microbiologically confirmed at the time of severe sepsis diagnosis.

Type of microorganism	Cardiac surgery	Abdominal surgery	*P-value*
Gram-positive			
All	52 (40.3%)	69 (39.2%)	0.845
Staphylococcus aureus			
Methicilin susceptible	13 (10.1%)	7 (4.0%)	**0.038**
Methicilin resistant	4 (3.1%)	6 (3.4%)	1.000
Staphylococcus epidermidis	18 (13.9%)	11 (6.2%)	**0.023**
Other *Staphylococcus*	4 (3.1%)	7 (4.0%)	0.765
Streptococcus sp.	2 (1.5%)	7 (4.0%)	0.311
Enterococcus sp.	7 (5.4%)	18 (10.2%)	0.131
Other Gram-positive	4 (3.1%)	13 (7.4%)	0.133
Gram-negative			
All	59 (45.7%)	88 (50%)	0.461
Klebsiella sp.	14 (10.8%)	13 (7.4%)	0.292
Enterobacter sp.	7 (5.4%)	5 (2.8%)	0.372
Haemophilus influenzae	9 (7.0%)	0 (0%)	**<0.001**
Pseudomonas sp.	3 (2.3%)	8 (4.5%)	0.366
Acinetobacter	3 (2.3%)	7 (4.0%)	0.527
Escherichia coli	11 (8.5%)	28 (15.9%)	0.056
Other Gram-negative	12 (9.3%)	27 (15.3%)	0.119
Fungus			
All	18 (13.9%)	19 (10.8%)	0.404
Candida albicans	13 (10.1%)	12 (6.8%)	0.406
Other *Candida sp*.	4 (3.1%)	5 (2.8%)	1.000
Other fungus	1 (0.8%)	2 (1.14%)	1.000

Values are expressed as absolute number (percentage). Statistically significant differences are shown in bold. P-values were calculated by Chi-square test or Fisher's exact test.
Note that patients may have had more than one organism cultured.

mtDNA haplogroups and severe sepsis

Figure 1 shows the frequencies of mtDNA haplogroups in Case-group and Control-group. For all patients, we did not find any significant differences in mtDNA haplogroup frequencies (**Figure 1A**). However, we found significant differences when mtDNA haplogroups were analysed according to type of surgery. For cardiac surgery, clusters (Chi-square global test= 8.981; p= 0.030) and haplogroups (Chi-square global test= 9.297; p= 0.026) were different between Case-group and Control-group (**Figure 1B**), but no significant differences were found for abdominal surgery (**Figure 1C**). In a detailed analysis, Case-group who underwent cardiac surgery had a tendency to have lower frequencies of cluster HV (p= 0.005) and haplogroup H (p= 0.005); and higher frequencies of cluster JT (p= 0.028) than Control-group (**Figure 1B**).

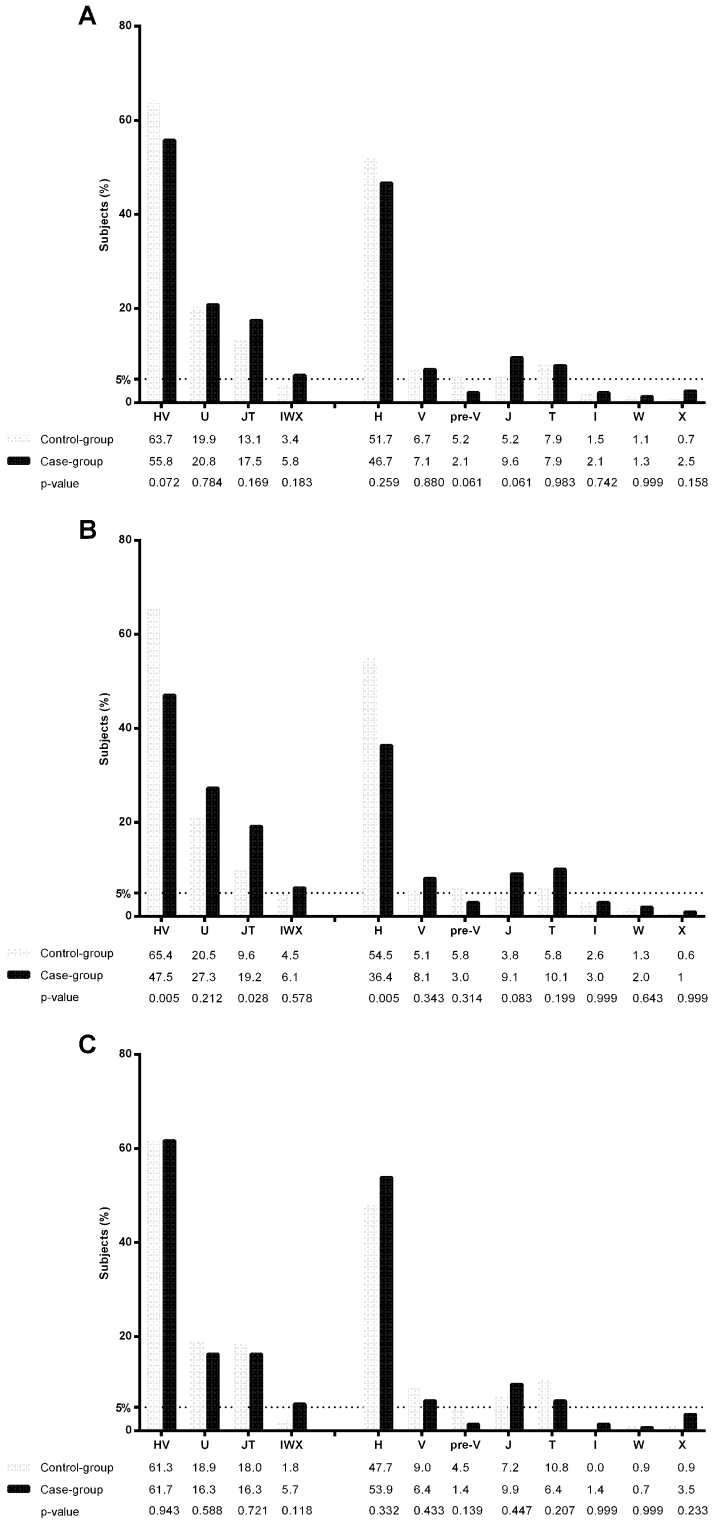


Figure 1. Frequencies of mtDNA haplogroups in Control-group and Case-group according to type of surgery. P-values were calculated by Chi-square test or Fisher's exact test.
Abbreviations: A, all patients; B, cardiac surgery; C, abdominal surgery.

Figure 2 shows the likelihood of severe sepsis according to the type of surgery. When the analysis was performed according to one cluster/haplogroup versus the other clusters/haplogroups, patients carrying cluster HV had lower odds of severe sepsis than others (odds ratio (aOR)= 0.60 (p= 0.029). When patients were stratified by the type of surgery, cluster HV and haplogroup H carriers who underwent cardiac surgery

had lower odds of severe sepsis (aOR= 0.45 (p= 0.009) and aOR= 0.48 (p= 0.015), respectively) (**Figure 2B**). Besides, haplogroup J carriers had higher odds of severe sepsis (aOR= 3.66; p= 0.032). However, we did not find any significant association for abdominal surgery (**Figure 2C**).

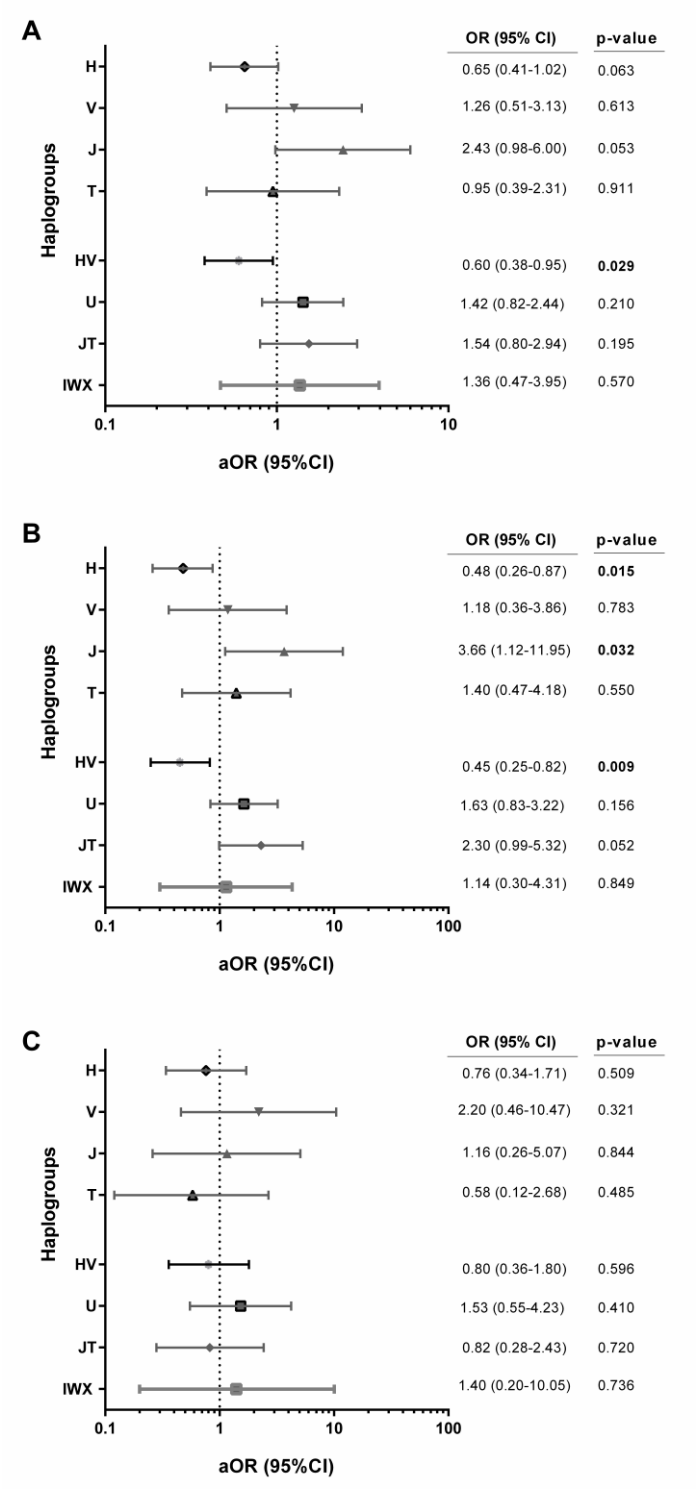


Figure 2. Association between mtDNA haplogroups and severe sepsis according to the type of surgery. Statistically significant differences are shown in bold. P-values were calculated by logistic regression analysis adjusted by the most important clinical and epidemiological characteristics (see **statistical analysis** section). Abbreviations: A, all patients; B, cardiac surgery; C, abdominal surgery; aOR, adjusted odds ratio; 95%CI, 95% of confidence interval; p-value, level of significance.

The associations were also examined by comparing each cluster or haplogroup against the cluster HV or haplogroup H (as reference) (see SD 3). Regarding cardiac surgery, we found that patients carrying clusters U and JT had higher odds of severe sepsis (cluster U, aOR= 2.03 (p= 0.050); cluster JT, aOR= 2.88 (p=0.018)) than patients with cluster HV. Besides, higher odds of severe sepsis was observed in patients with haplogroup J (aOR= 5.21; p= 0.010) compared to patients with haplogroup H (see **SD 3B**).

mtDNA haplogroups and death in septic patients

No significant differences in mtDNA haplogroup frequencies were found between patients alive and dead (see **SD 4**), even when patients were split by the type of surgery. Note that deceased patients with cardiac surgery had higher frequencies of cluster IWX, but differences were not significant (p= 0.075) (see **SD 4B**).

Figure 3 shows the survival of patients within 90 days after the diagnosis of severe sepsis by Kaplan-Meier analysis. Out of 240 patients, 109 (45.4%) died with a survival median of 39 (95%CI= 31.4; 46.62) days. The log-rank test showed that the survival was different according to the clusters (Chi-square global test= 9.092; p=0.028). When the clusters were analysed, 41% (55/134) patients within cluster HV died versus 71.4% (10/14) patients within cluster IWX (p=0.018) (**Figure 3A**). However, we did not find any significant differences among individual haplogroups (**Figure 3B**).

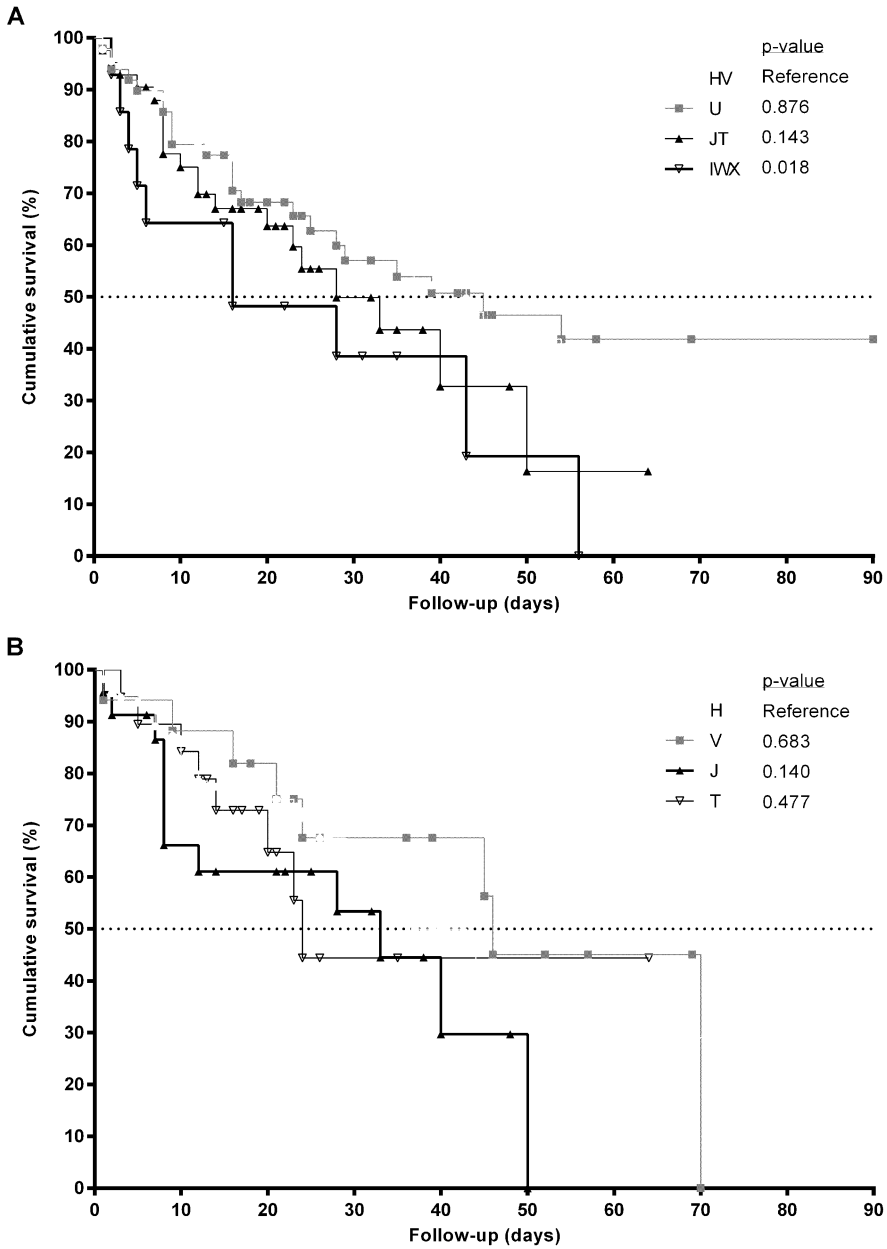


Figure 3. Kaplan-Meier curves of cumulative survival according to European haplogroup in patients with severe sepsis. P-values were calculated by log-rank test. Abbreviations: A, Clusters (HV, IXW, U and JT); B, Haplogroups (H, V, J and T).

Figure 4 shows the adjusted mortality risks by Cox regression according to mitochondrial clusters and haplogroups. When the analysis was performed according to one cluster/haplogroup versus the other clusters/haplogroups, cluster IWX showed a higher risk of death (adjusted hazard ratio (aHR)= 2.22; p= 0.019) (**Figure 4**).

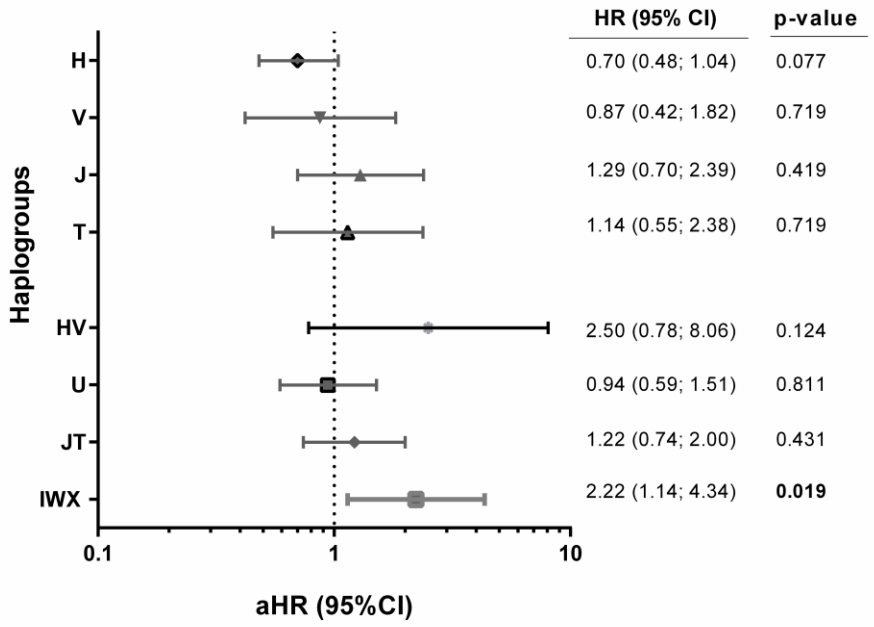


Figure 4. Adjusted mortality risk in patients with septic shock/severe sepsis (case group) according to mitochondrial clusters and haplogroups. Statistically significant differences are shown in bold. P-values were calculated by Cox regression analysis adjusted by the most important clinical and epidemiological characteristics (see **statistical analysis** section). Abbreviations: aHR, adjusted hazard ratio; 95%CI, 95% of confidence interval; p-value, level of significance.

When comparing each cluster or haplogroup against the cluster HV or haplogroup H (as reference, respectively), patients within cluster IWX had a higher risk of dying than patients within clusters HV (aHR= 2.38 (p= 0.014) (see **SD 5**).

DISCUSSION

The differences in mitochondrial energetic production among haplogroups could potentially affect the development of severe sepsis and its survival. In this study, we examined the association of European mtDNA haplogroups with the development of severe sepsis or septic shock in patients who underwent cardiac or abdominal major surgery. Our data show that, in patients who underwent major cardiac surgery, HV and H haplogroups showed lower risk of severe sepsis than JT and J haplogroups. Besides, cluster IWX was associated with death. However, no significant association was found in patients who underwent major abdominal surgery.

Regarding the association found for cardiac surgery subgroup, cardiopulmonary bypass may actually engender a state of low global systemic oxygen delivery and reduced tissue oxygen saturation (24). Under these circumstances, the energetic efficiency of cells could be modified and thus, the effects of certain haplogroups could be enhanced. Moreover, cardiac surgery is associated with increased vascular levels of ROS in conjunction with altered endothelial cell and smooth muscle cell function. These alterations potentially lead to restenosis, thrombosis, or endothelial dysfunction in the treated artery (25). Thus, the differences between surgeries could be partly explained by the biggest constraint of oxygen in cardiac surgery patients. In addition, a high variability of mitochondrial function among organs and tissues should not be ruled out (26). For example, whereas in cardiac muscle of rodents a reduced enzymatic activity of certain complexes of respiratory chain has been described, a higher efficiency of respiration was observed in liver (26). In our study, it is important to point out that sites of infection were different depending on the type of surgery carried out, as expected. Therefore, differences in mitochondrial function among organs and tissues could lead to different ability to respond against an invading organism at the site of infection and thus, increase or decrease the susceptibility to sepsis after surgery. Besides, these differences in mitochondrial function among organs could be responsible for the different influence of mtDNA haplogroups on severe sepsis development found for different types of surgeries in this study.

Mitochondrial haplogroups are defined by specific sequence variants within the population, which have an important role in adaptation to environmental conditions (27). Besides, these mtDNA polymorphic variants are not silent. They are known to modulate certain mitochondrial functions. For example, haplogroup H has demonstrated higher activity in the electron transport chain, producing higher quantities of ATP and ROS than other haplogroups, such as J, which exhibits lower energy efficiency (28-30). In this setting, functional differences in mitochondrial efficiency, ATP production, heat generation, and/or oxidative damage among haplogroups could lead to differences in sepsis susceptibility (31). Although the functional role of mitochondrial haplogroups is still a matter of controversy, it is possible that the effects of mitochondrial haplogroups may emerge under special conditions such as sepsis (9). However, we could not perform functional experiments to determinate the energetic efficiency in isolated mitochondria or tissue homogenates of patients with sepsis. Thus, although mitochondrial haplogroups seem to have influence on severe sepsis development and death, further studies studying functional parameters would be needed to reach a better understanding of the molecular pathophysiology of sepsis in relation to mitochondrial function.

There is an association between mortality and mitochondrial dysfunction in sepsis. In our study, patients within the cluster IWX had increased risk of death. IWX cluster has been described as protective factor for several diseases such as AIDS (32). However, several studies have also found that I, W or X haplogroups are associated with development of certain diseases such as coronary artery disease (33), metabolic disease (34) and cancer (35). It is probable that mitochondrial haplogroups exert positive or negative effects depending on the molecular mechanisms underlying the specific disease. Regarding sepsis, the association between cluster IWX and mortality is novel and quite intriguing; since there is little biological evidence to explain this observed association. Cluster IWX is relatively uncommon in persons of European descent (~5%), and has not been previously associated with sepsis; possibly due to its low level in the population, making it difficult to detect associations. Within the IWX cluster, haplogroups are characterized by non-synonymous and synonymous polymorphisms at mtDNA positions related to *ribosomal RNA* gene; *cytochrome C oxidase subunit II* gene; *NADH dehydrogenase* gene, *ATPase6* gene etc. (36). In this regard, some of these polymorphisms could have influence on certain mitochondrial functions, leading to the

observed higher risk of death in our study. However, further studies are needed to improve the understanding of the influence of IWX cluster on mortality.

Several points should be taken into account for the correct interpretation of the results. Although this work showed significant results, one limitation of the present study is the lack of a replication study in another related population. Furthermore, the study design and the limited sample size might be responsible of the lack of statistical association in some comparisons, i.e. in patients who underwent abdominal surgery. Further studies with higher sample size would be interesting in order to corroborate the association found in our study and to check other possible associations. Finally, it is clear that both aetiology and pathology of sepsis is complex, and the mitochondrion plays a critical role in this process. Mitochondrial haplogroups may act synergistically with other nuclear genetic factors, proteins and environmental components, which are all epistatic factors contributing to ischemic cardiomyopathy.

In conclusion, European mitochondrial haplogroups might be related to the onset of severe sepsis in patients who underwent major cardiac surgery, but not in patients underwent major abdominal surgery. Haplogroups HV and H were related to decreased risk of severe sepsis, while JT and J were related to increased risk of severe sepsis. Besides, mtDNA haplogroups could have influence on mortality in septic patients. In this study, IWX cluster was associated to increased risk of death.

ACKNOWLEDGEMENTS

The authors thank the Spanish National Genotyping Center (CeGen) for providing the SNP genotyping services (http://www.cegen.org).

COMPETING INTERESTS

The authors do not have a commercial or other association that might pose a conflict of interest.

FINANCIAL SUPPORT:

This work has been supported by grants given by Fondo de Investigacion de Sanidad en España (FIS) [Spanish Health Founds for Research] [grant number FIS PI10/01362], "Gerencia de Salud, Consejería de Sanidad, Junta de Castilla y Leon" [grant number GRS 463/A/10], and PFIZER [grant number CT25-ESP01-01]. MGF, MAJS are supported by "Instituto de Salud Carlos III" [grant numbers RD12/0017/0024 and CD13/00013, respectively].

AUTHORS' CONTRIBUTIONS

Funding body, ET and SR
Study concept and design: MAJS, ET, and SR.
Sample collection: JFBM and RA.
Patients selection and clinical data acquisition: ET, MH, JIGH, EG, and SGJ.
Sample preparation, DNA isolation and genotyping: MAJS, MGF, AFR, and MGA.
Statistical analysis and interpretation of data: MAJS and SR.
Writing of the manuscript: MAJS and SR.
Critical revision of the manuscript for important intellectual content: ET and JFBM.
Study supervision: SR.
All authors read and approved the final manuscript.

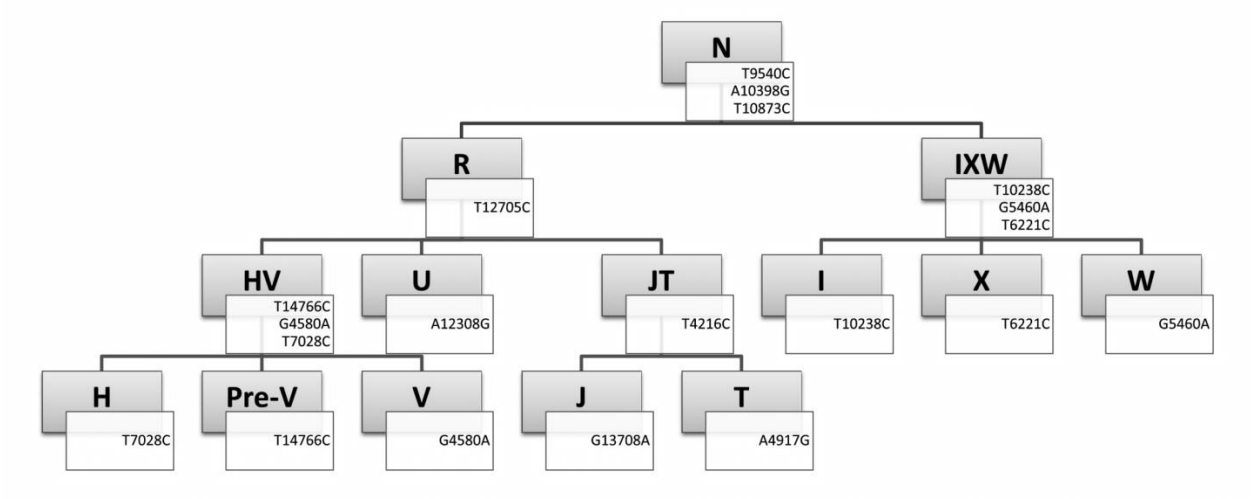
REFERENCES

1. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Critical care medicine. 2003;31(4):1250-6. Epub 2003/04/12.
2. Soong J, Soni N. Sepsis: recognition and treatment. Clin Med. 2012;12(3):276-80. Epub 2012/07/13.
3. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013;39(2):165-228. Epub 2013/01/31.
4. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. Critical care medicine. 2006;34(2):344-53. Epub 2006/01/21.
5. Tamayo E, Alvarez FJ, Martinez-Rafael B, Bustamante J, Bermejo-Martin JF, Fierro I, et al. Ventilator-associated pneumonia is an important risk factor for mortality after major cardiac surgery. J Crit Care. 2012;27(1):18-25. Epub 2011/05/21.
6. Tamayo E, Gualis J, Florez S, Castrodeza J, Eiros Bouza JM, Alvarez FJ. Comparative study of single-dose and 24-hour multiple-dose antibiotic prophylaxis for cardiac surgery. J Thorac Cardiovasc Surg. 2008;136(6):1522-7. Epub 2008/12/31.
7. Moore LJ, McKinley BA, Turner KL, Todd SR, Sucher JF, Valdivia A, et al. The epidemiology of sepsis in general surgery patients. J Trauma. 2011;70(3):672-80. Epub 2011/05/26.
8. Tamayo E, Gomez E, Bustamante J, Gomez-Herreras JI, Fonteriz R, Bobillo F, et al. Evolution of neutrophil apoptosis in septic shock survivors and nonsurvivors. J Crit Care. 2012;27(4):415 e1-11. Epub 2011/11/15.
9. Andrades ME, Morina A, Spasic S, Spasojevic I. Bench-to-bedside review: sepsis - from the redox point of view. Crit Care. 2011;15(5):230. Epub 2011/10/15.
10. Galley HF. Bench-to-bedside review: Targeting antioxidants to mitochondria in sepsis. Crit Care. 2010;14(4):230. Epub 2010/09/02.
11. Dare AJ, Phillips AR, Hickey AJ, Mittal A, Loveday B, Thompson N, et al. A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. Free radical biology & medicine. 2009;47(11):1517-25. Epub 2009/09/01.
12. Garrabou G, Moren C, Lopez S, Tobias E, Cardellach F, Miro O, et al. The effects of sepsis on mitochondria. The Journal of infectious diseases. 2012;205(3):392-400. Epub 2011/12/20.
13. Belikova I, Lukaszewicz AC, Faivre V, Damoisel C, Singer M, Payen D. Oxygen consumption of human peripheral blood mononuclear cells in severe human sepsis. Critical care medicine. 2007;35(12):2702-8. Epub 2007/12/13.
14. Japiassu AM, Santiago AP, d'Avila JC, Garcia-Souza LF, Galina A, Castro Faria-Neto HC, et al. Bioenergetic failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced F1Fo adenosine-5'-triphosphate synthase activity. Critical care medicine. 2011;39(5):1056-63. Epub 2011/02/22.
15. Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, et al. Classification of European mtDNAs from an analysis of three European populations. Genetics. 1996;144(4):1835-50. Epub 1996/12/01.
16. Yang Y, Zhang P, Lv R, He Q, Zhu Y, Yang X, et al. Mitochondrial DNA haplogroup R in the Han population and recovery from septic encephalopathy. Intensive Care Med. 2011;37(10):1613-9. Epub 2011/08/19.
17. Yang Y, Shou Z, Zhang P, He Q, Xiao H, Xu Y, et al. Mitochondrial DNA haplogroup R predicts survival advantage in severe sepsis in the Han population. Genetics in medicine : official journal of the American College of Medical Genetics. 2008;10(3):187-92. Epub 2008/03/18.
18. Baudouin SV, Saunders D, Tiangyou W, Elson JL, Poynter J, Pyle A, et al. Mitochondrial DNA and survival after sepsis: a prospective study. Lancet. 2005;366(9503):2118-21. Epub 2005/12/20.
19. Gomez R, O'Keeffe T, Chang LY, Huebinger RM, Minei JP, Barber RC. Association of mitochondrial allele 4216C with increased risk for complicated sepsis and death after traumatic injury. J Trauma. 2009;66(3):850-7; discussion 7-8. Epub 2009/03/12.

20. Lorente L, Iceta R, Martin MM, Lopez-Gallardo E, Sole-Violan J, Blanquer J, et al. Survival and mitochondrial function in septic patients according to mitochondrial DNA haplogroup. Crit Care. 2012;16(1):R10. Epub 2012/01/19.
21. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Critical care medicine. 1985;13(10):818-29. Epub 1985/10/01.
22. Moreno R, Vincent JL, Matos R, Mendonca A, Cantraine F, Thijs L, et al. The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study. Working Group on Sepsis related Problems of the ESICM. Intensive Care Med. 1999;25(7):686-96. Epub 1999/09/02.
23. Hendrickson SL, Kingsley LA, Ruiz-Pesini E, Poole JC, Jacobson LP, Palella FJ, et al. Mitochondrial DNA haplogroups influence lipoatrophy after highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2009;51(2):111-6. Epub 2009/04/03.
24. van Beest P, Wietasch G, Scheeren T, Spronk P, Kuiper M. Clinical review: use of venous oxygen saturations as a goal - a yet unfinished puzzle. Crit Care. 2011;15(5):232. Epub 2011/11/04.
25. Juni RP, Duckers HJ, Vanhoutte PM, Virmani R, Moens AL. Oxidative stress and pathological changes after coronary artery interventions. Journal of the American College of Cardiology. 2013;61(14):1471-81. Epub 2013/03/19.
26. Jeger V, Djafarzadeh S, Jakob SM, Takala J. Mitochondrial function in sepsis. Eur J Clin Invest. 2013;43(5):532-42. Epub 2013/03/19.
27. Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, et al. Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci U S A. 2003;100(1):171-6. Epub 2003/01/02.
28. Arning L, Haghikia A, Taherzadeh-Fard E, Saft C, Andrich J, Pula B, et al. Mitochondrial haplogroup H correlates with ATP levels and age at onset in Huntington disease. J Mol Med (Berl). 2010;88(4):431-6. Epub 2010/01/29.
29. Martinez-Redondo D, Marcuello A, Casajus JA, Ara I, Dahmani Y, Montoya J, et al. Human mitochondrial haplogroup H: the highest VO2max consumer--is it a paradox? Mitochondrion. 2010;10(2):102-7. Epub 2009/11/11.
30. Marcuello A, Martinez-Redondo D, Dahmani Y, Casajus JA, Ruiz-Pesini E, Montoya J, et al. Human mitochondrial variants influence on oxygen consumption. Mitochondrion. 2009;9(1):27-30. Epub 2008/10/28.
31. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet. 2005;39:359-407. Epub 2005/11/16.
32. Hendrickson SL, Hutcheson HB, Ruiz-Pesini E, Poole JC, Lautenberger J, Sezgin E, et al. Mitochondrial DNA haplogroups influence AIDS progression. AIDS. 2008;22(18):2429-39. Epub 2008/11/14.
33. Haber M, Youhanna SC, Balanovsky O, Saade S, Martinez-Cruz B, Ghassibe-Sabbagh M, et al. mtDNA lineages reveal coronary artery disease-associated structures in the Lebanese population. Annals of human genetics. 2012;76(1):1-8. Epub 2011/10/25.
34. Hulgan T, Haubrich R, Riddler SA, Tebas P, Ritchie MD, McComsey GA, et al. European mitochondrial DNA haplogroups and metabolic changes during antiretroviral therapy in AIDS Clinical Trials Group Study A5142. AIDS. 2011;25(1):37-47. Epub 2010/09/28.
35. Tommasi S, Favia P, Weigl S, Bianco A, Pilato B, Russo L, et al. Mitochondrial DNA variants and risk of familial breast cancer: an exploratory study. International journal of oncology. 2014;44(5):1691-8. Epub 2014/03/08.
36. Li H, Liu D, Lu J, Bai Y. Physiology and pathophysiology of mitochondrial DNA. Advances in experimental medicine and biology. 2012;942:39-51. Epub 2012/03/09.

SUPPLEMENTAL DATA

Supplemental data 1. Summary of European mitochondrial DNA (mtDNA) haplogroups with their defining polymorphisms.



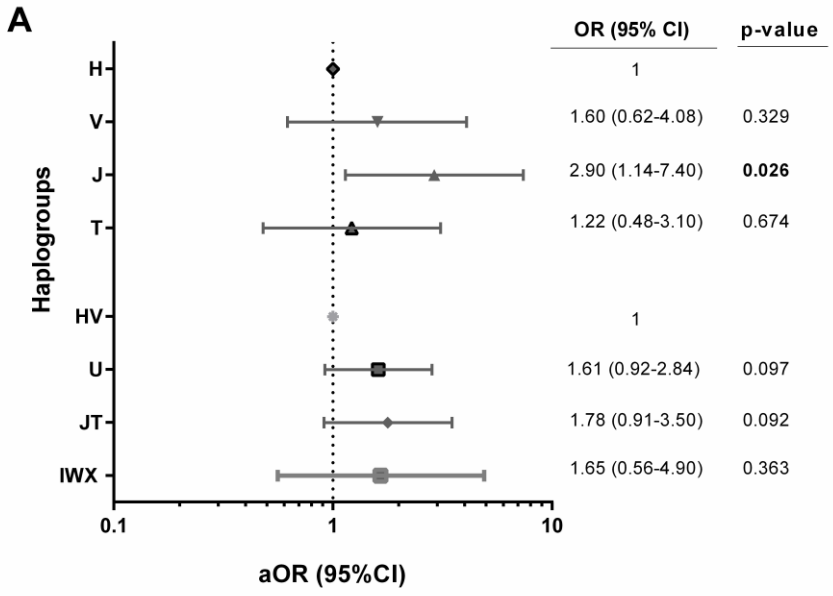
Supplemental data 2. Baseline characteristics of patients with septic shock/severe sepsis (case group) according to the type of surgery.

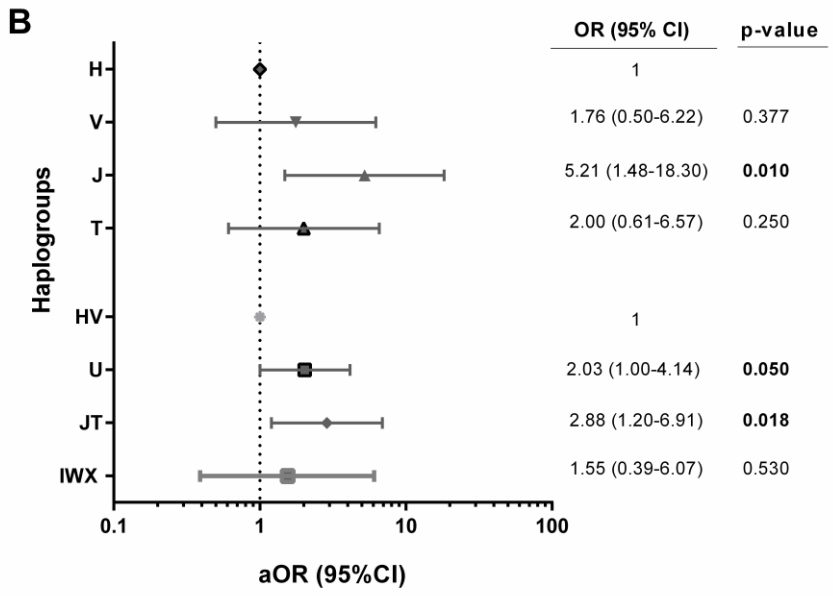
Characteristics	Cardiac surgery	Abdominal surgery	p-value
No. patients	99	141	
Gender (male)	66 (66.7%)	84 (59.6%)	0.264
Age (years)	73 (14)	73 (19.5)	0.709
Type of surgery			
Emergency	34 (34.3%)	119 (84.4%)	**<0.001**
Scheduled	65 (65.7%)	22 (15.6%)	**<0.001**
Severity of disease score			
APACHE II score	13 (8)	16 (6)	**0.005**
SOFA score	8 (4)	8 (4)	0.864
Mortality	33 (33.3%)	76 (53.9%)	**0.002**
Prior or pre-existing conditions			
Diabetes	16 (16.2%)	15 (10.6%)	0.209
Chronic obstructive pulmonary disease	16 (16.2%)	23 (16.3%)	0.975
Hypertension	67 (67.7%)	66 (46.8%)	**0.001**
Chronic kidney disease	14 (14.1%)	21 (14.9%)	0.871
Cancer	8 (8.1%)	45 (31.9%)	**<0.001**
Liver disease	3 (3.0%)	8 (5.7%)	0.335
Cardiomyopathy	69 (69.7%)	40 (28.4%)	**<0.001**

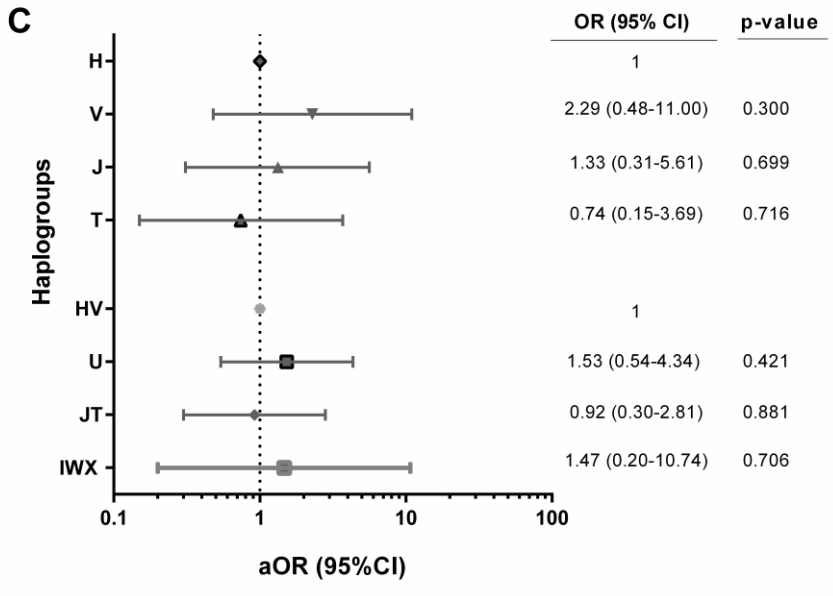
Values are expressed as median (interquartile range) and absolute count (percentage).
P-values were calculated by Mann-Whitney test and Chi-square test.

Supplemental data 3. Association between mtDNA haplogroups and severe sepsis according to the type of surgery. Statistically significant differences are shown in bold. P-values were calculated by logistic regression analysis adjusted by the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: A, all patients; B, cardiac surgery; C, abdominal surgery; aOR, adjusted odds ratio; 95%CI, 95% of confidence interval; p-value, level of significance.

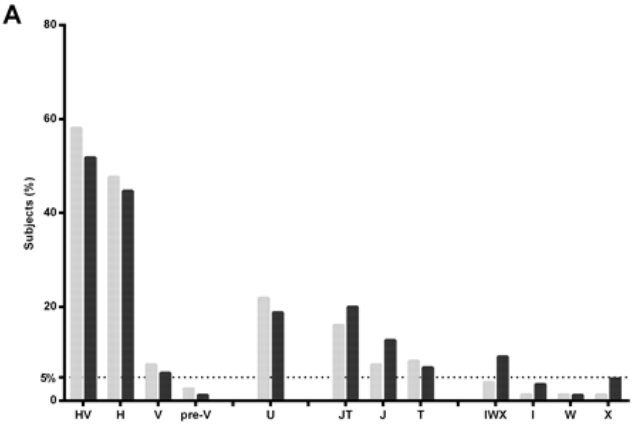

A
OR (95% CI)
p-value
H
V
J
T
HV
U
JT
IWX
1
1.60 (0.62-4.08)
2.90 (1.14-7.40)
1.22 (0.48-3.10)
1
1.61 (0.92-2.84)
1.78 (0.91-3.50)
1.65 (0.56-4.90)
0.329
0.026
0.674
0.097
0.092
0.363
Haplogroups
0.1
1
10
aOR (95%CI)


B
OR (95% CI)
p-value
H
V
J
T
HV
U
JT
IWX
1
1.76 (0.50-6.22)
5.21 (1.48-18.30)
2.00 (0.61-6.57)
1
2.03 (1.00-4.14)
2.88 (1.20-6.91)
1.55 (0.39-6.07)
0.377
0.010
0.250
0.050
0.018
0.530
Haplogroups
0.1
1
10
100
aOR (95%CI)


C
OR (95% CI)
p-value
H
V
J
T
HV
U
JT
IWX
1
2.29 (0.48-11.00)
1.33 (0.31-5.61)
0.74 (0.15-3.69)
1
1.53 (0.54-4.34)
0.92 (0.30-2.81)
1.47 (0.20-10.74)
0.300
0.699
0.716
0.421
0.881
0.706
Haplogroups
0.1
1
10
100
aOR (95%CI)

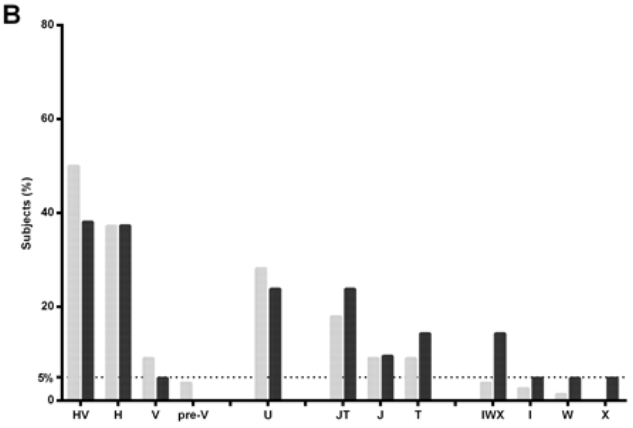
Supplemental data 4. Frequencies of mtDNA haplogroups among septic patients (Case-group) according to mortality and type of surgery. A, all patients; B, cardiac surgery; C, abdominal surgery. P-values were calculated by Chi-square test or Fisher's exact test.

A



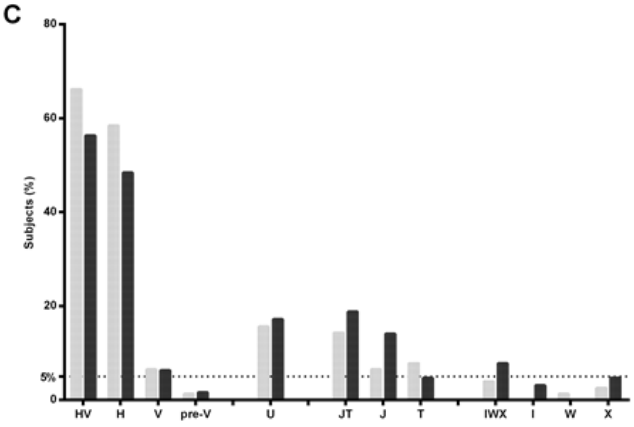
	HV	H	V	pre-V	U	JT	J	T	IWX	I	W	X
Alive	58.1	47.7	7.7	2.6	21.9	16.1	7.7	8.4	3.9	1.3	1.3	1.3
Death	51.8	44.7	5.9	1.2	18.8	20.0	12.9	7.1	9.4	3.5	1.2	4.7
p-value	0.347	0.652	0.591	0.658	0.570	0.450	0.191	0.715	0.080	0.245	0.999	0.189

B



	HV	H	V	pre-V	U	JT	J	T	IWX	I	W	X
Alive	50.0	37.2	9.0	3.8	28.2	17.9	9.0	9.0	3.8	2.6	1.3	0.0
Death	38.1	33.3	4.8	0.0	23.8	23.8	9.5	14.3	14.3	4.8	4.8	4.8
p-value	0.332	0.745	0.530	0.999	0.688	0.545	0.938	0.473	0.075	0.602	0.381	0.212

C



	HV	H	V	pre-V	U	JT	J	T	IWX	I	W	X
Alive	66.2	58.4	6.5	1.3	15.6	14.3	6.5	7.8	3.9	0.0	1.3	2.6
Death	56.3	48.4	6.3	1.6	17.2	18.8	14.1	4.7	7.8	3.1	0.0	4.7
p-value	0.225	0.235	0.953	0.999	0.798	0.475	0.135	0.453	0.317	0.118	0.999	0.659

Supplemental data 5. Adjusted mortality risk in patients with septic shock/severe sepsis (case group) according to mitochondrial clusters and haplogroups. Statistically significant differences are shown in bold. P-values were calculated by Cox regression analysis adjusted by the most important clinical and epidemiological characteristics (see **statistical analysis** section).
Abbreviations: aHR, adjusted hazard ratio; 95%CI, 95% of confidence interval; p-value, level of significance.