

Original article

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

Running head: mtDNA haplogroups and sepsis

Authors: M^a Angeles Jiménez-Sousa ^{a(*)}, Eduardo Tamayo ^b, María Guzmán-Fulgencio ^a, María Heredia ^b, Amanda Fernández-Rodríguez ^a, Esther Gómez ^b, Raquel Almansa ^c, José I Gómez-Herreras ^b, Mónica García-Álvarez ^a, Sandra Milena Gutiérrez ^b, Jesús F Bermejo-Martin ^c, Salvador Resino ^{a(*)} on behalf of the Spanish Sepsis Group (SpSG).

(a) Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología. Instituto de Salud Carlos III, Majadahonda, Spain. Email addresses: majimenezsousa@yahoo.es, mguzman@isciii.es, amanda.fernandez.r@gmail.com, monica_gap@yahoo.es, sresino@isciii.es.

(b) Departamento de Anestesiología y Reanimación, Hospital Clínico Universitario, Valladolid, Spain. Email addresses: tamayo@med.uva.es, maria_her_05@hotmail.com, esthergzam@hotmail.com, jgomez012001@yahoo.es, milegij@yahoo.com.

(c) Unidad de Investigación Médica en Infección e Inmunidad. Hospital Clínico Universitario-IECSCYL, Valladolid, Spain. Email addresses: ralmansa@saludcastillayleon.es, jfbermejo@saludcastillayleon.es

(*) Corresponding author.

Correspondence and requests for reprints:

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

Background: Mitochondrial DNA (mtDNA) variants may play an important role for predicting clinical outcome in sepsis. The aim of this study was to analyze whether mitochondrial DNA haplogroups are associated with severe sepsis and mortality after major surgery in European populations.

Methods: We carried out a case-control study on 240 septic patients (severe sepsis or septic shock, Case-group) and 267 patients with systemic inflammatory response syndrome (SIRS, Control-group). Furthermore, a longitudinal substudy for analyzing the survival was performed in septic patients. mtDNA genotyping was performed by Sequenom's MassARRAY platform.

Results: Regarding cardiac surgery, patients with clusters JT and haplogroup J had higher likelihoods of sepsis than patients with clusters HV (OR=2.76 (95%CI=1.27; 6.02); p=0.010) and haplogroup H (OR=3.68 (95%CI=1.17; 11.54); p=0.026), respectively. No significant association was found for abdominal surgery. When analyzing the survival, 45.4% patients died with a survival median of 39 (95%CI=31.4; 46.62) days. When the clusters were analyzed for all patients, 41% (55/134) patients within cluster HV died versus 71.4% (10/14) patients within cluster IWX (p=0.018). The adjusted Cox regression showed that patients within cluster IWX had a higher risk of dying than patients within clusters HV (hazard ratio (HR)=2.24 (95%CI=1.10; 4.56) p=0.027). No significant association between haplogroups and mortality was found when patients were stratified by the type of surgery.

Conclusions: European mitochondrial haplogroups are associated with sepsis development in patients who underwent major cardiac surgery, but not in patients underwent major abdominal surgery. Besides, mtDNA haplogroups have also influence on mortality. Haplogroups HV and H were related to low risk of sepsis and death, while JT and J were related to high risk of sepsis, and IWX was associated with death.

Key words: mitochondrial haplogroups; mtDNA, sepsis; septic shock; severe sepsis; survival

INTRODUCTION

Sepsis is defined by a systemic inflammatory response syndrome (SIRS) secondary to bacterial infection [1], which is caused by the uncontrolled immune system's 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, is 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 O₂ 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, mitochondrial DNA (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 analyze 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. Furthermore, a longitudinal substudy for analyzing 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 and the Institutional Ethics Committee approved it.

Initially, 247 patients with severe sepsis or shock septic (Case-group) and 280 patients with systemic inflammatory response syndrome (SIRS, Control-group) were included. Subjects from the control-group were patients who underwent cardiac or abdominal surgery and they 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 preexisting conditions such as diabetes, chronic obstructive pulmonary disease, hypertension, chronic kidney disease, cancer, liver disease and cardiomyopathy. 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 analyzed 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 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. We defined SIRS and different levels of sepsis: severe sepsis, and septic shock.

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 **Additional file 1**).

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 $p < 0.05$.

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.

Categorical data and proportions were analyzed 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 or septic shock (Case-group vs. Control-group). All logistic regression analyses were adjusted for age, gender, prior or preexisting conditions (comorbidities), and type of surgery (cardiac or abdominal).

Kaplan-Meier and Cox regression analyses were used to analyze the survival time in patients with severe sepsis or septic shock (Case-group). Cox regression was performed to analyze the mortality risk associated to mtDNA haplogroups among patients with severe sepsis or septic shock (Case-group). All Cox regression analyses were adjusted for age, gender, APACHE-II score, prior or preexisting conditions (comorbidities), type of surgery (cardiac or abdominal), and microbiologically confirmed infection.

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 ($p = 0.045$), liver disease ($p = 0.030$), and abdominal surgery ($p < 0.001$), while Control-group had higher percentage of patients with hypertension ($p = 0.005$), cancer ($p = 0.002$), cardiomyopathy ($p = 0.041$), 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).

mtDNA haplogroups and 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**). When mtDNA haplogroups were analyzed according to type of surgery, Case-group 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 regarding cardiac surgery. (**Figure 1B**). However, we did not find any significant differences in mtDNA haplogroup frequencies for abdominal surgery (**Figure 1C**).

When comparing each cluster or haplogroup against the cluster HV or haplogroup H (**Figure 2**), respectively; we found haplogroup J had higher odds for severe sepsis development than haplogroup H (OR= 2.16 (95%CI= 1.01; 4.63); $p = 0.048$) for all patients (**Figure 2A**). Regarding cardiac surgery, patients with clusters JT and haplogroup J had higher likelihoods of severe sepsis than patients with clusters HV (OR= 2.76 (95%CI= 1.27; 6.02); $p = 0.010$) and haplogroup H (OR= 3.68 (95%CI= 1.17; 11.54); $p = 0.026$) respectively (**Figure 2B**). However, we did not find any significant association for abdominal surgery (**Figure 2C**).

mtDNA haplogroups and death

Case-group had higher values of mortality than Control-group (109/240 (45.4%) vs. 9/267 (3.4%), $p < 0.001$). The study of association between haplogroups and death was performed only in the Case-group.

For septic patients, we did not find any significant differences in mtDNA haplogroup frequencies among patients alive and dead (see **Additional file 2A**). Note that deceased patients with cardiac surgery had higher frequencies of cluster IWX, but differences were not significant ($p = 0.075$) (**Additional file 2B**).

The survival of patients within 90 days after the diagnosis of severe sepsis or septic shock (Case-group) was calculated by Kaplan-Meier analysis (**Figure 3**). Out of 240 patients, 109 (45.4%) died with a survival median of 39 (95%CI= 31.4; 46.62) days. When the clusters were analyzed for all septic patients, 41% (55/134) patients within cluster HV died versus 71.4% (10/14) patients within cluster IWX ($p = 0.018$) (**Figure 3A**). Furthermore, patients within cluster JT had a faster death than cluster HV ((16 (95%CI= 0.5; 36.06) vs. (43 (95%CI= 34.9; 51.01) days, respectively), but the

difference was not significant ($p= 0.143$). Moreover, we did not find any significant differences when patients were split by the type of surgery (data not shown).

Figure 4 shows the adjusted mortality risks by Cox regression according to mitochondrial clusters and haplogroups. Patients within cluster IWX had a higher risk of dying than patients within clusters HV (hazard ratio (HR)= 2.24; $p= 0.027$).

DISCUSSION

The differences in mitochondrial energetic production among haplogroups could potentially affect the development of 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, 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 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 disease 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]. Thus, the molecular mechanism underlying the functional differences between mitochondrial haplogroups could be the reason for different clinical outcomes (sepsis and death) in our patients who underwent major surgery.

There is an association between mortality and mitochondrial dysfunction in sepsis, due to the direct consequence of mitochondrial dysfunction is a significant fall in the level of ATP, as observed in the tissues of model animals and septic patients [9, 32]. Moreover, non-survivors of sepsis exhibit an impaired capacity to increase tissue oxygen consumption in response to the augmentation of oxygen delivery [33]. In this context, patients with mitochondrial haplogroup H might be more efficient in energetic metabolism than patients with other haplogroup variants; and the presence of H haplogroup might be a protective factor for the mortality. In fact, previous evidence suggests that haplogroup H is a strong independent predictor of

outcome in sepsis, and contributes to an increased chance of survival compared with other haplogroups [18]. By contrast, in our study, patients within the cluster IWX had a lower survival than patients with cluster HV. This association between cluster IWX and mortality among patients with severe sepsis 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. [34]. 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. Furthermore, we could not perform functional experiment to determinate the energetic efficiency in isolated mitochondria or tissue homogenates of patients.

CONCLUSIONS

European mitochondrial haplogroups are associated with sepsis development and mortality in patients who underwent major cardiac surgery but not in patients who underwent major abdominal surgery. Haplogroups HV and H were related to low risk of sepsis and death, while JT and J were related to high risk of severe sepsis or septic shock, and IWX was associated with death.

COMPETING INTERESTS

The author(s) declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

ET, JFBM and SR have made substantial contributions to conception and design of the study. MH, SMG, JIGH and EG have participated in patient selection and recruitment. MH, EG and RA have contributed to sample collection and acquisition of clinical data. MAJS and SR have been involved in statistical analysis, interpretation of data and drafting the manuscript. MGF, AMR and MGA have contributed to data interpretation and have revising the manuscript critically for important intellectual content. ET, JFBM and SR have given final approval of the version to be published.

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Table 1. Baseline characteristics of patients with septic shock/severe sepsis (case group) and SIRS (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 |
| Severity of disease score | | | |
| APACHE II score | 9 (1) | 15 (6.8) | <0.001 |
| SOFA score | 3 (0) | 8 (4) | <0.001 |
| Prior or preexisting conditions | | | |
| Diabetes | 22 (8.2%) | 21 (8.8%) | 0.837 |
| Chronic obstructive pulmonary disease | 26 (9.7%) | 35 (14.6%) | 0.09 |
| Hypertension | 163 (61%) | 117 (48.8%) | 0.005 |
| Chronic kidney disease | 15 (5.6%) | 25 (10.4%) | 0.045 |
| Cancer | 85 (31.8%) | 48 (20%) | 0.002 |
| Liver disease | 3 (1.1%) | 10 (4.2%) | 0.030 |
| Cardiomyopathy | 116 (43.4%) | 83 (34.6%) | 0.041 |

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

FIGURE LEGENDS

Figure 1. Frequencies of mtDNA haplogroups according to 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.

Figure 2. Association between mtDNA haplogroups and sepsis according to the type of surgery. A, all patients; B, cardiac surgery; C, abdominal surgery. Odds ratio (OR), 95% of confidence interval (95%CI), and p-values were calculated by adjusted logistic regressions.

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

Figure 4. Adjusted mortality risk in patients with septic shock/severe sepsis (case group) according to mitochondrial haplogroups. HR and p-values were calculated by an adjusted Cox regression.

Abbreviations: HR, hazard ratio; 95%CI, 95% of confidence interval; p-value, level of significance.

ADDITIONAL FILES

File name: Additional file 1.

File format: .pdf

Title of data: Summary of European mitochondrial DNA (mtDNA) haplogroups with their defining polymorphisms.

File name: Additional file 2.

File format: .pdf

Title of data: Frequencies of mtDNA haplogroups among septic patients (Case-group) according to mortality and type of surgery.

Description of data: A, all patients; B, cardiac surgery; C, abdominal surgery. P-values were calculated by Chi-square test or Fisher's exact test.

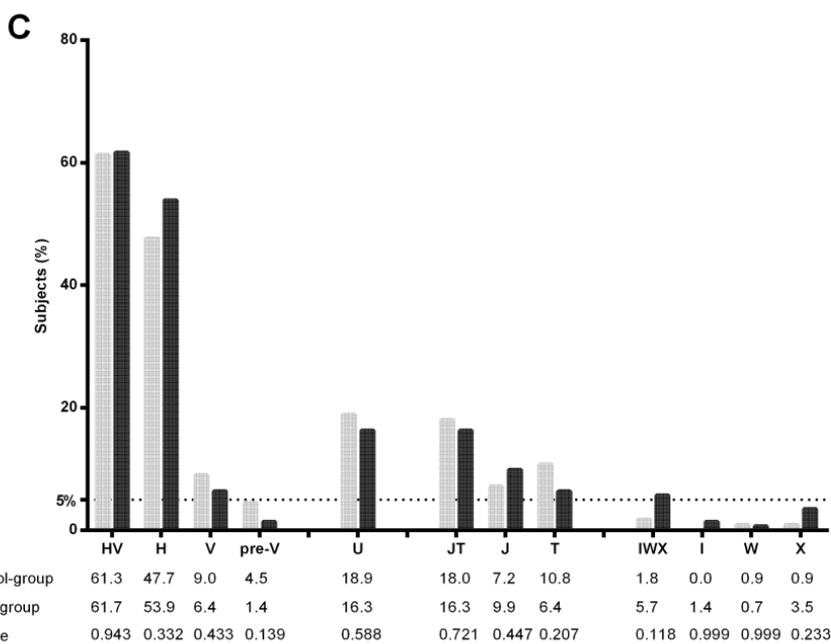
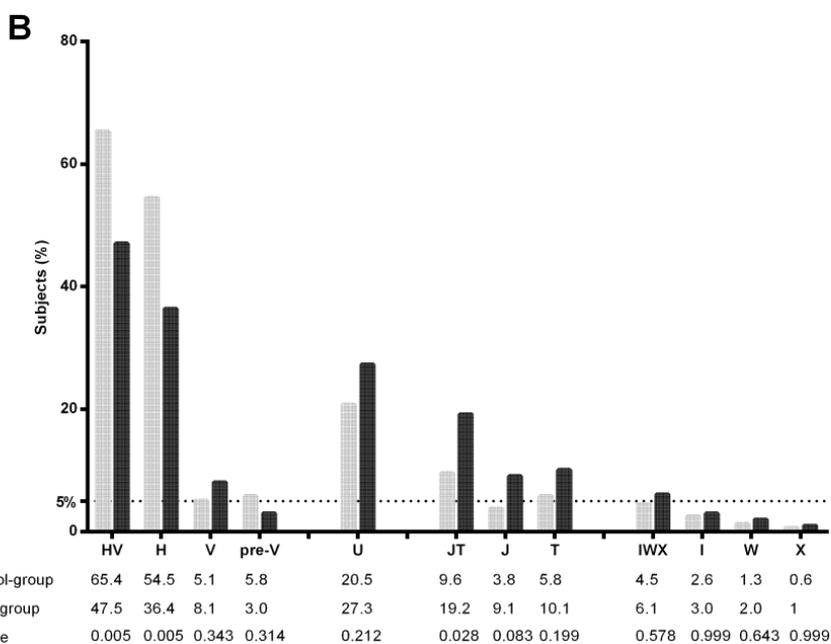
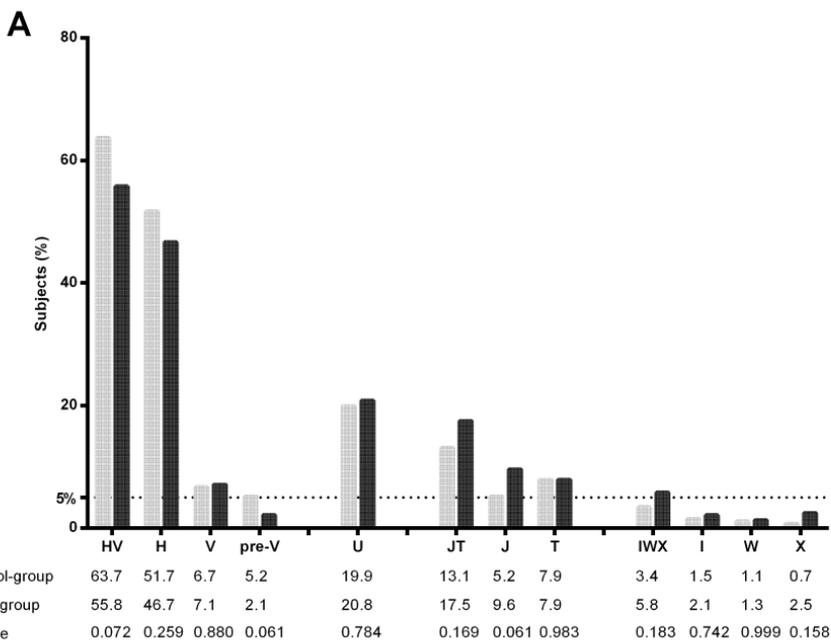


Figure 1

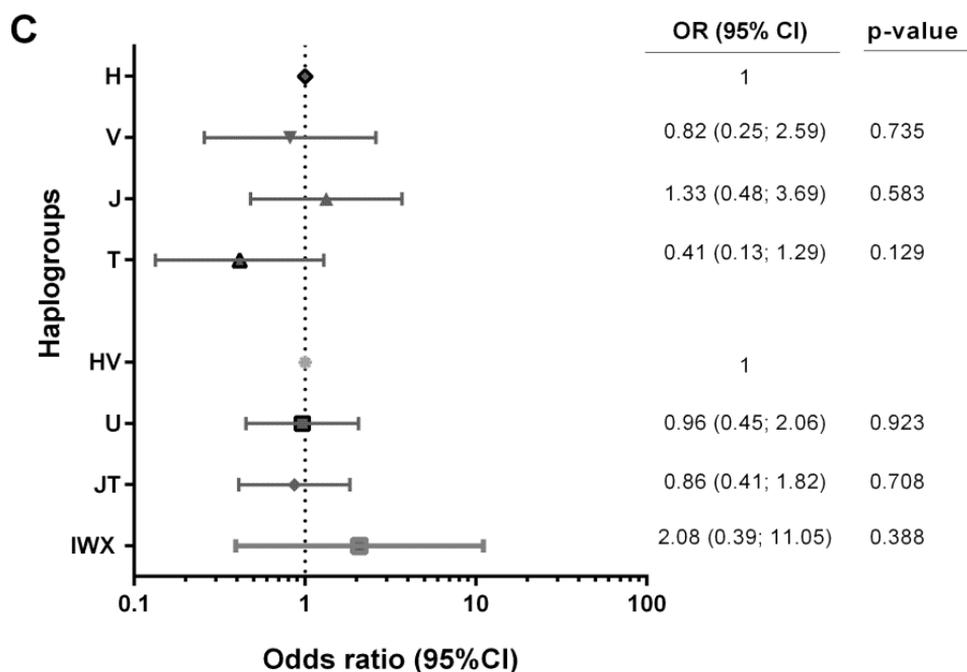
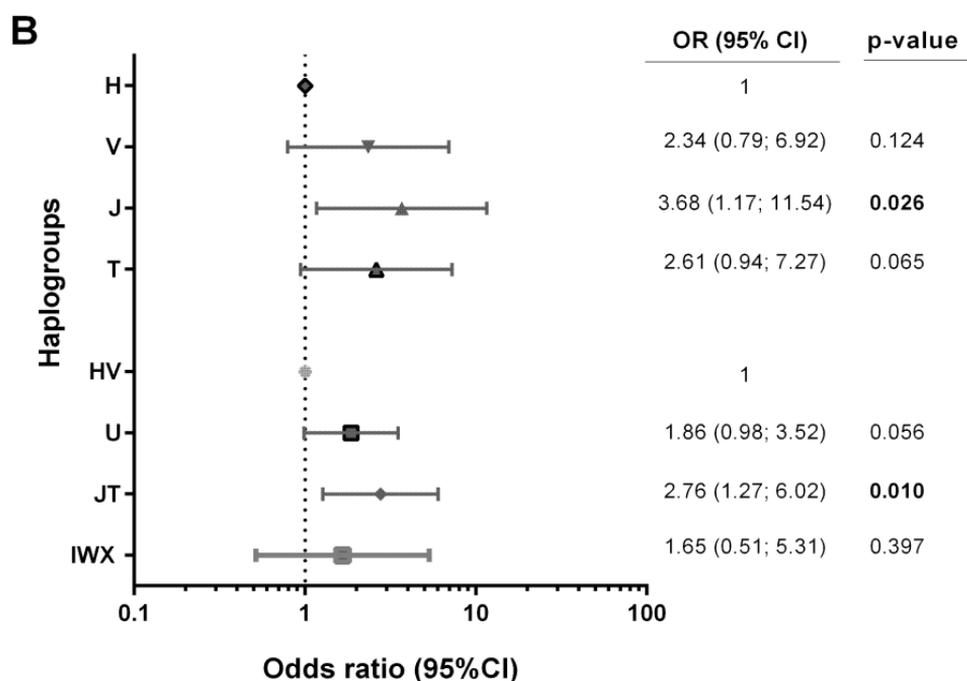
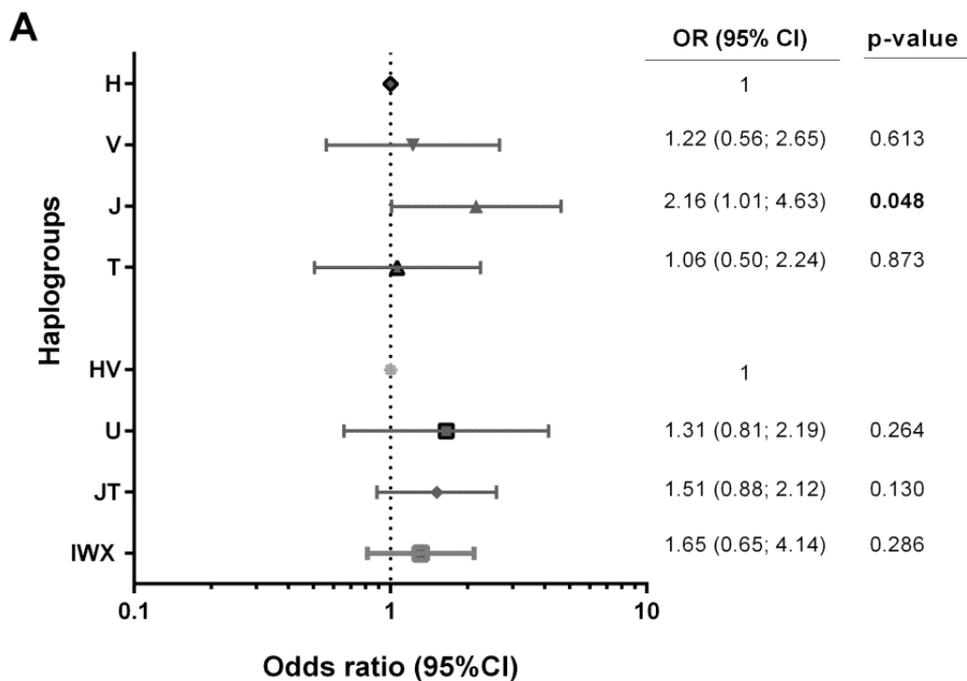


Figure 2

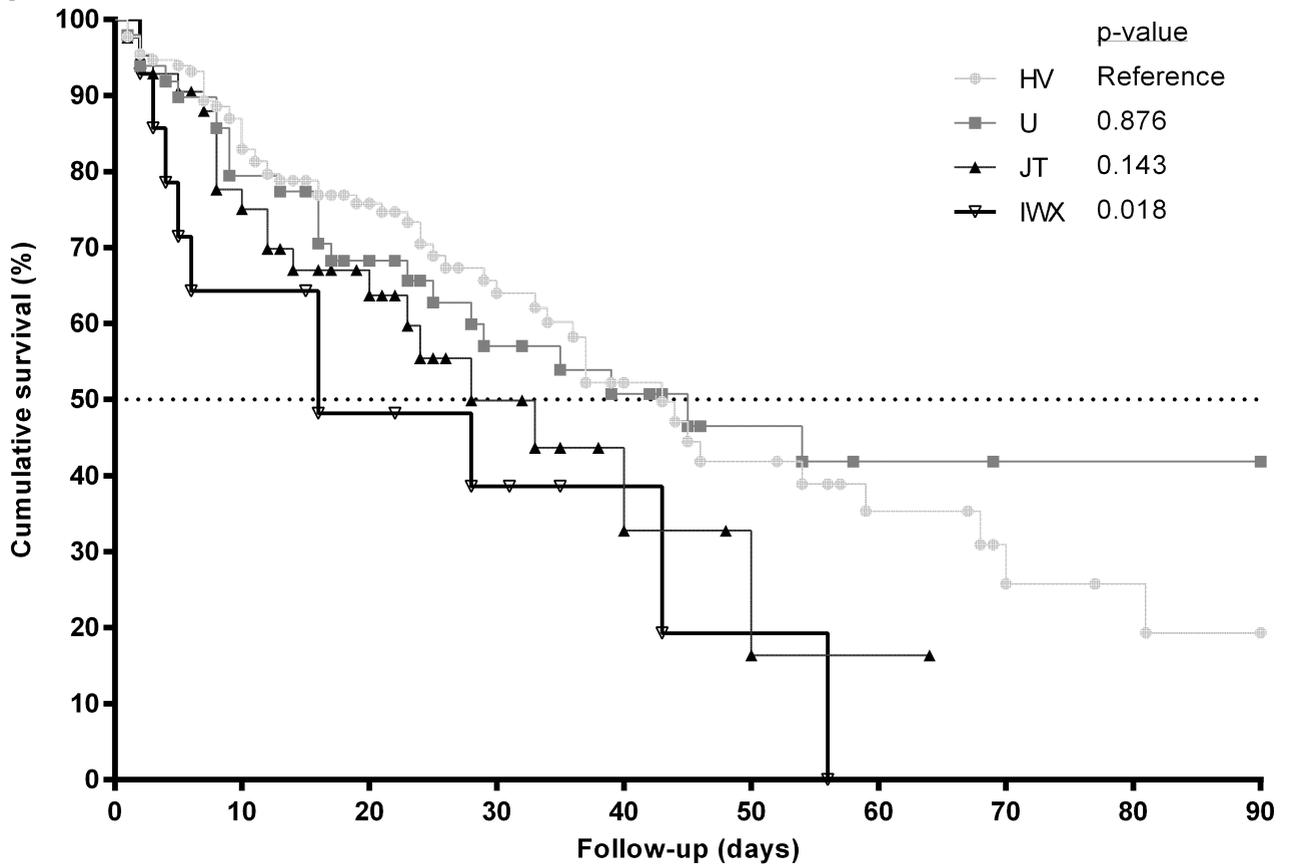
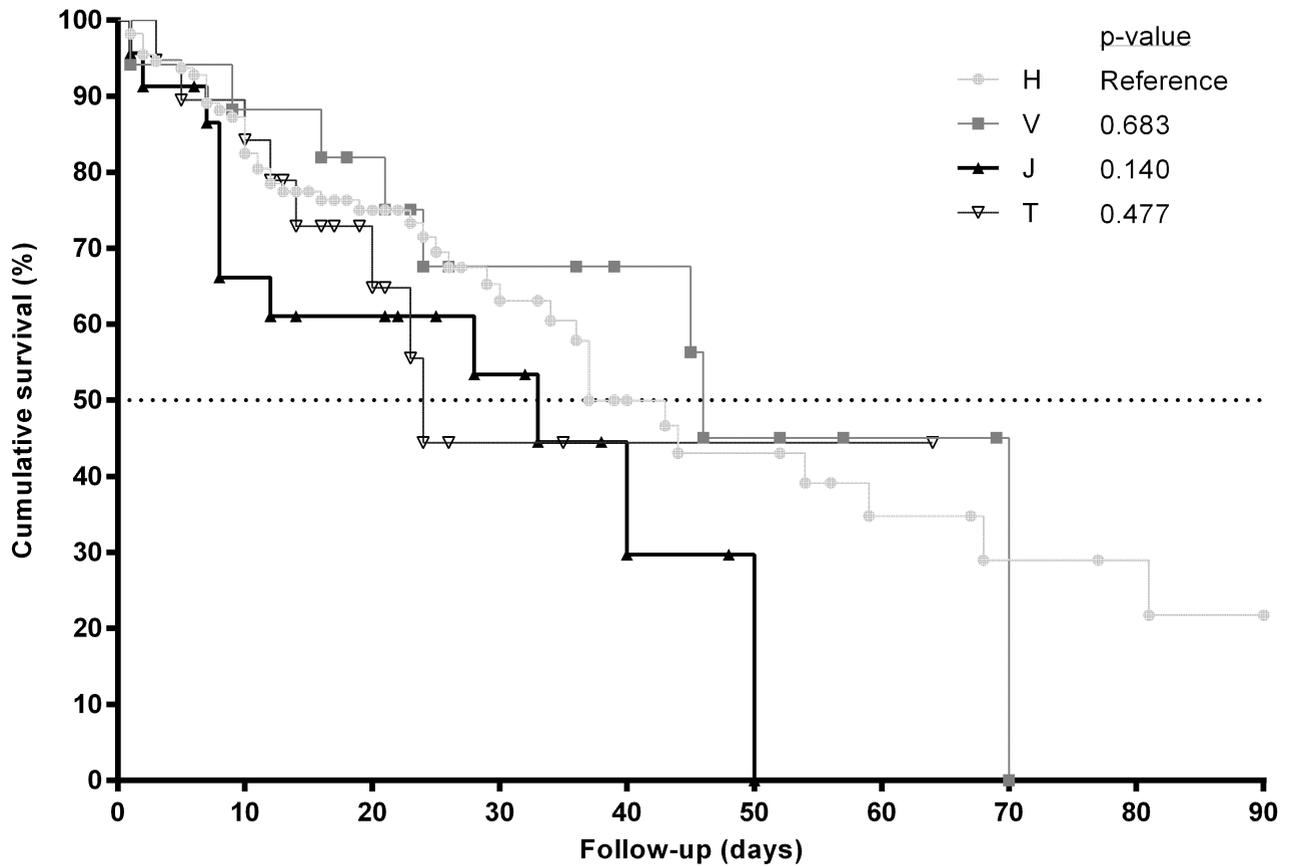
A**B**

Figure 3

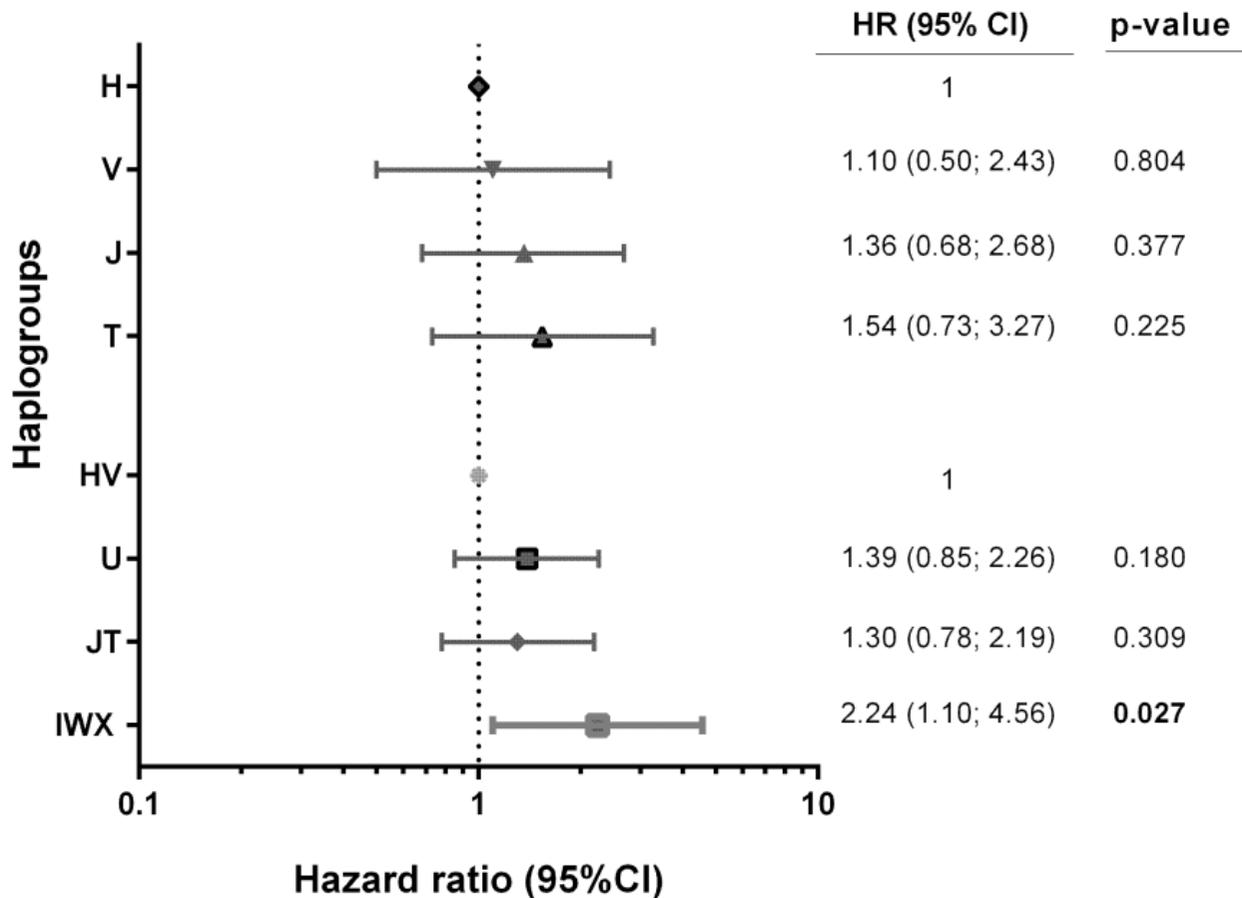


Figure 4

Additional files provided with this submission:

Additional file 1: Additional file 1.pdf, 62K

<http://www.biomedcentral.com/imedia/1130054999110582/supp1.pdf>

Additional file 2: Additional file 2.pdf, 73K

<http://www.biomedcentral.com/imedia/1841947510110582/supp2.pdf>