Title: Common TNF-alpha, IL-1beta, PAI-1, uPA, CD14 and TLR4 polymorphisms are not associated with disease severity or outcome from Gram negative sepsis

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Author's response to reviews: see over
Dear Anita,

Thank you very much for the opportunity to revise our manuscript. We’ve read the constructive criticism from the reviewers with great interest and have listed our responses (in bold) to their suggestions below. Changes in the manuscript are marked with bold red. We hope that you find our revision satisfactory.

We have made the corrections suggested by the editorial office.

Kind regards,

Thomas Benfield

Reviewer 1
Major Compulsory Revisions
Was reason for admission to ICU and underlying illness / illness severity adjusted for? Yes, we did collect information on ICU admission and underlying illness. By univariate Cox proportional regression analysis, ICU admission but not comorbidity was associated with outcome. However, in multivariate analysis, only age, polymicrobial infection and haemoglobin levels were associated with outcome. The analysis is now included in the results section: “Multivariate analysis…” and the conclusion has been added to the abstract.

When was blood drawn (was micro-chimerism a potential confounding issue)? Blood was drawn on admission. Unfortunately, we did not collect information on blood transfusions. However, since DNA was sampled around the time of admission, because leukocyte-depleted erythrocyte suspensions are used for transfusions, and PCR amplifies majority gene populations, we do not find it likely that micro-chimerism has confounded our study.
Was interaction between multiple loci investigated in a formal manner? **We did perform an analysis of SNPs and outcome after stratification for other variables associated with outcome. The (negative) results of this analysis are now described in the last paragraph of the discussion. We did not investigate for interactions between loci because the six studied SNPs are located on six different chromosomes.**

eg: logistic regression with interaction terms, mutational load model, etc?

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**Minor Essential Revisions**

Was logistic regression attempted? **No, we chose to use Cox regression analysis because we have complete follow up and can include time in the analysis. However, we repeated all analysis with logistic regression analysis and did not detect any associations that were not detected by Cox regression analysis.**

Was censoring conducted in Cox and Kaplan-Meier analysis? **In survival analysis, patients were censored after 3 months.**

If so, how was censoring conducted?

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**Discretionary Revisions**

Please add figure depicting Cox and Kaplan-Meier time to event data/curves. **A K-P survival curve has been included as figure 1.**

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**Reviewer 2:**

**Major Compulsory Revisions**

1. My main concern is that the study is underpowered both for disease severity (given that the subgroups with severe sepsis and septic shock are very small) and for outcome (given that mortality is only 19% and that the TLR4 SNP is extremely rare). Thus for example the doubling of mortality in those with the TNF -308 homozygous SNP and the higher mortality in the TLR4 SNP heterozygotes do not reach significance, perhaps because of the small numbers. The authors must justify and discuss analysing such a small patient population. **We fully agree. Sample size and statistical power is certainly a concern that we have mentioned but clearly not sufficiently explicative. We have more clearly stated that our study is underpowered to detect associations with the lesser frequent homozygous and heterozygous SNPs.**

2. The authors must explain more clearly the basis on which they selected the genes and SNPs to be included in their analysis. Why did they select only one SNP for each gene? **We included only SNPs that had been shown in other studies to have either clinical or experimental associations with sepsis outcome through the inflammatory and coagulation pathways. Thus, only one SNP was chosen per gene. We have stated this more precisely in the introduction.**

3. The authors should include a description of the procedures they used for the quality control of genotyping. **Yes. We have specified the procedure.**

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**Minor Essential Revisions**

1. Table 2, column 4 should be headed "septic shock". **Thanks.**

2. The authors might like to cite another negative TNF/LTA SNP association study (Gordon et al. Genes and Immunity 2004). **Naturally.**