

# Circadian Rhythms in Septic Shock Patients

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## Research

**Keywords:** Clock genes, septic shock, sepsis, circadian rhythm, pathophysiology

**Posted Date:** August 21st, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-61571/v1>

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# Abstract

**Background:** Although intensive efforts to improve diagnosis and therapy of sepsis over the last decade, the mortality of septic shock remains high and causes substantial socioeconomical burden of disease. The function of immune cells is time-of-day-dependent and is regulated by several circadian clock genes. This study aims to investigate whether the rhythmicity of clock gene expression is altered in patients with septic shock.

**Methods:** This prospective pilot study was performed at the university hospital Charité – Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK). We included 20 patients with septic shock between May 2014 and January 2018, from whom blood was drawn every 4 hours over a 24-hour period to isolate CD14-positive monocytes and to measure expression of 17 clock and clock-associated genes. Of these patients, 3 whose samples expressed fewer than 8 clock genes were excluded from the final analysis. Expression data were compared to data of a healthy study population and a rhythmicity score  $S_p$  was calculated, which comprises values between -1 (arrhythmic) and 1 (rhythmic).

**Results:** Overall, the rhythmicity scores for septic shock patients were significantly ( $p < 0.0001$ ) lower ( $0.23 \pm 0.26$ ) compared to the control group (12 healthy young men,  $0.70 \pm 0.18$ ). 77% of the measured clock genes were classified as having inconclusive rhythms, i.e. neither rhythmic nor arrhythmic. The clock genes *NR1D1*, *NR1D2* and *CRY2* were the most rhythmic, while *CLOCK* and *ARNTL* were the least rhythmic. In addition, the expression of clock genes *CRY1*, *NR1D1*, *NR1D2*, *DBP*, and *PER2* was suppressed in septic shock patients and *CRY2* was significantly upregulated compared to controls.

**Conclusion:** Compared to young healthy men, molecular rhythms in immune cells of septic shock patients were substantially decreased. The decrease in rhythmicity was clock gene-dependent. The loss of rhythmicity and downregulation of clock gene expression might be caused by sepsis and might further deteriorate immune responses and organ injury, but further studies are necessary to understand underlying pathophysiological mechanisms.

Clinical trial registered with [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT02044575) on 24 January 2014.

## Background

Estimates suggest that sepsis affects more than 19 million people worldwide every year and might cause up to 5 million deaths [1]. In adult intensive care units (ICUs), sepsis and septic shock are the leading causes of death [2]. Even though a multitude of studies provided a substantial improvement in understanding the complex pathophysiology of sepsis, several treatments have failed in clinical trials [2]. The clinical outcome as well as host-response-patterns correlate with 4 different clinical phenotypes ( $\alpha$  -  $\delta$  depending on severity of septic manifestation and organ failure), which may help to understand the heterogeneous treatment effects in future sepsis therapy [3]. Nevertheless, with a rising overall incidence

and fatal mortality rates, there is an urgent need towards targeted sepsis therapy addressing individual disease trajectories [4].

Conventionally, sepsis' serious clinical picture would mainly be a result of an exaggerated, hyperinflammatory response. However, current data indicate more complex mechanisms, such as a suppressed inflammatory response, metabolic changes and dysregulated coagulation [5]. A dysregulated immune response leads to the formation of systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS) at the same time [6]. In the further course of sepsis, either a recovery to immune homeostasis, or the persistence of CARS with severe immunosuppression is present [6–8]. In the phase of severe immunosuppression, viral reactivations or opportunistic infections occur frequently and account for the majority of all sepsis-related deaths, which can also occur years after hospitalization [2, 9]. As a reliable biomarker for immune function and immunosuppression serves the human leucocyte antigen-D related on monocytes (mHLA-DR), as it correlates with immune competence, occurrence of nosocomial infections and mortality [2, 6, 9, 10].

Immune cell function is dependent on a 24-hour circadian rhythm that is regulated by the expression of clock genes [11]. Key cell types comprise macrophages and monocytes, as they may have a robust intrinsic circadian clock and a high expression of clock genes [11]. Molecular circadian rhythms are generated by autoregulatory transcription-translation feedback loops [12]. The transcription factors *CLOCK* and *BMAL* induce the expression of their negative regulators *PER1-3* and *CRY1-2* [12]. *PER-CRY* complex formation triggers its nuclear translocation and interacts with the *BMAL1-CLOCK* complex, which inhibits *PER* and *CRY* transcription [12]. When the expression of the *PER-CRY* complex decreases due to its proteasomal degradation, the cycle restarts [12].

Various pathological conditions resulting in lethal inflammation and immune dysfunction have been associated with aberrant circadian rhythms [13]. In addition, myocardial infarction, asthma and particularly inflammatory diseases show a day-night-variation in appearance of symptoms and patients' outcomes [14, 15]. In murine models, genetic ablation of the myeloid *Bmal1* gene results in an increased inflammatory response in experimental sepsis [13]. In sepsis patients, the severity of the inflammatory process is directly related to the degree of change in the clock genes *BMAL1* and *PER2* [16]. Coiffard et al. showed impaired circadian rhythms of cytokines, leukocytes and three clock genes (*BMAL1*, *PER2* and *PER3*) in severe trauma patients [17]. However, no study has investigated the circadian system in critically ill patients with septic shock. Therefore, we hypothesized that critically ill patients with septic shock have attenuated circadian rhythms in the expression of clock genes compared to individuals without septic shock.

## Patients And Methods

### Study participants and data acquisition

This prospective observational study was performed at the university hospital Charité – Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK). Between May 2014 and January 2018, we screened five anesthesiological or surgical ICUs, respectively, for patients with septic shock. All patients received guideline-based intensive care treatment in accordance to our standard operating procedures [18]. Patients with septic shock and the need for norepinephrine dosages  $> 0,3 \mu\text{g}/\text{kg}/\text{min}$  for at least 2 hours were included according to the American College of Chest Physicians (ACCP) consensus definition [19].

Age below 18 years, pregnant or lactating female patients, acute leukemia, severe leukocytosis ( $> 50.000/\text{nL}$ ) or thrombocytopenia ( $< 5.000/\text{nL}$ ), other immunosuppressing conditions (acute leukemia, organ transplantation, systemic prednisolone treatment, interferon therapy, chemotherapy or radiotherapy) or participation in an interventional study, acute pulmonary embolism or acute myocardial infarction within the last 72 hours and cardiopulmonary resuscitation within the last 7 days were defined as exclusion criteria. The patient or legal guardian had to consent in the study participation. Primary outcome measure was the assessment of circadian expression of clock genes over 24 hours. Secondary outcome measures were clinical scoring systems as Charlson Comorbidity Index (CCI), Sequential Organ Failure Assessment Score (SOFA), Simplified Acute Physiology Score II (SAPS II) and Therapeutic Intervention Scoring System (TISS-28), lactate, body temperature, time of mechanical ventilation, ICU and hospital length of stay and mortality.

For clock gene measurement, 5 mL blood in an EDTA vial and 5 mL blood in a Serum vial were drawn each 4 hours in 24 hours starting the day after inclusion at 8:00 a.m. (Fig. 1). The core body temperature (CBT) was measured hourly using the indwelling urinary catheter. Data was taken from the charts for the 24 hour period of clock gene measurement.

## Measurement of clock and clock-associated genes

The serum samples were centrifuged and the serum was conserved at  $-80 \text{ }^\circ\text{C}$ . CD14-positive monocytes were isolated from the EDTA samples using an Auto-MACS Pro Separator (Miltenyi Biotec GmbH, Bergisch Gladbach). The isolated monocytes were stored at  $-80 \text{ }^\circ\text{C}$  until transport.

A panel of 20 clock and clock-associated genes – *ARNTL*, *ARNTL2*, *BHLHE40*, *BHLHE41*, *CIART*, *CIPC*, *CLOCK*, *CRY1*, *CRY2*, *CSNK1D*, *CSNK1E*, *DBP*, *NFIL3*, *NPAS2*, *NR1D1*, *NR1D2*, *PER1*, *PER2*, *PER3*, *RORA* - were quantified using Nanostring™ PlexSet at each time point for each patient. For a given patient, a clock gene time series was included if the gene was detected in at least 6 of the 7 time points. Only patients with at least 8 expressed clock genes were used for further study. Finally, a gene was retained in the final dataset only if it was expressed in at least 6 patients. The final dataset contained time series data from 17 patients on up to 15 genes. All expression data are  $\log_2$  transformed in accordance with Nanostring™ analysis guidelines. The septic shock patients are labeled “SCXX”.

## Determination of circadian rhythms

The gene expression patterns of clock genes across all patients were subject to harmonic regression analysis with a fixed oscillation period of 24 hours; very short time series (consisting of only 7 points herein) do not permit accurate estimation of periods. We characterized the amplitude of circadian rhythms in gene expression using confidence intervals (CI) (instead of a single estimate of amplitude). The CI represents the range of circadian amplitudes of gene expression that are consistent with the measurements at the 95% level (i.e.,  $p < 0.05$ ). The amplitude was defined as the fold-change in expression between the peak and trough of the rhythm.

It is generally understood that only rhythms with a sufficiently large amplitude, defined as a greater than 1.4-fold (equal to 0.5 change in  $\log_2$  scale) increase, are biologically relevant. Using this threshold, three classes of clock genes in the dataset could be defined. Clock genes with a CI falling completely above the threshold have biologically meaningful gene expression rhythms with adjusted- $p < 0.05$ . Similarly, clock genes with CIs that fall completely within the threshold and zero have no rhythms with adjusted- $p < 0.05$ . Finally, clock genes with CIs that include the threshold are inconclusive, i.e., the data are insufficient to determine rhythmicity or a lack thereof.

In order to better characterize the rhythmicity of the entire panel of clock genes in each patient, we defined a rhythmicity score which was defined as  $\frac{r - a}{r + a}$ .  $r$  is the difference between the fraction of genes that are rhythmic and the fraction of genes that are arrhythmic in that patient. The score takes a value 1, when all genes are rhythmic and a value -1 if all the genes are arrhythmic. To characterize the rhythmicity of a clock gene across patients, we defined a rhythmicity score for a clock gene similar to the one above but the averaged value across the number of patients it is observed in, i.e.,  $\frac{1}{n} \sum \frac{r_i - a_i}{r_i + a_i}$ .

## Data from Body Time study (BOTI)

To determine whether the circadian clocks in septic shock patients are different from healthy adults, we compared clock genes from our study against clock genes from the BOTI study [20]. We used gene expression time series measured from CD14+ monocytes of 12 young, healthy, male subjects kept under constant routine using Nanostring™; details on the study participants and design can be found in Wittenbrink et al. [20]. Subjects in this study were sampled every 3 hours resulting in 9 samples over 24 hours. Therefore, we dropped two time points 12 hours apart in order to have the same 7 samples over 24 hours as in septic shock patients. This was important, since the computed confidence intervals (CI) also incorporate information on the number of time points available to estimate amplitude. Furthermore, only 7 clock genes – *PER1*, *PER2*, *NR1D1*, *NR1D2*, *DBP*, *CRY1*, *CRY2* - were measured in common between the two studies. Therefore, we restricted our comparisons of the two studies to these 7 clock genes. The subjects from the BOTI study are labeled “BXX”.

We first calculated CI for the clock genes in BOTI subjects, which were then compared to septic shock patients. We next computed rhythmicity scores for each septic shock patient and for each BOTI subject

based on only the 7 common clock genes. We compared the rhythmicity of the common clock genes in septic shock patients and BOTI subjects using rhythmicity scores.

## Measurement of HLA-DR

The expression of mHLA-DR on monocytes was measured using flow cytometric measurement and was taken from routine laboratory results [21]. The time point of mHLA-DR measurement was the day of inclusion; if not available, we took the closest measurement from the charts.

## Statistical analysis

The data was analyzed using R statistical software (ver. 3.6.2) and SPSS 26.0 (IBM Corporation, Armonk, NY). The  $lm$  function was used to fit the function  $y = a \sin(bx + c) + d$  to the data.

A parametric bootstrap [22] provided CI for the amplitude of oscillations ( $a$ ). The chosen base statistical level for the CI was 0.05, which was Bonferroni-corrected to account for multiple gene testing within each patient. Results are expressed in medians  $\pm$  quartiles or percentage, respectively. A p-value  $< 0.05$  or adjusted p-value  $< 0.05$  (as the case may be) was considered statistically significant.

## Results

### Study population

During the study period, 112 patients were screened, of whom 20 patients could be included (Fig. 2). Of these, 3 patients had to be excluded from further analysis, since their samples expressed fewer than 8 clock genes, leaving 17 patients for final analyses. The basic patient characteristics and outcome parameters are shown in Table 1.

Table 1  
Patient characteristics and secondary outcome parameters.

Parameter	Value (N = 17)
Age (years)	66 (51.5–74)
Male sex, n (%)	7 (41.2)
BMI (kg/m <sup>2</sup> )	27.7 (23.6–33.4)
TISS-28	42 (37-49.5)
CCI	6 (5–8)
SOFA	17 (11.5–17.5)
SAPS II	69 (57-79.5)
Hemoglobine (g/dL)	8.6 (7.9–10)
Leukocytes (/nL)	14.8 (8.7–19.5)
INR	1.31 (1,27-1.53)
Thrombocytes (/nL)	104 (65–218)
mHLA-DR (antigens/cell)*	5632 (3322–10192)
Lactate (g/dL)	15 (12-42.5)
PTT (sec)	40.6 (37.5–57.9)
Hemodialysis, n (%)	14 (82.4)
ECMO, n (%)	3 (17.6)
Ventilation n (%)	17 (100)
Time of mechanical ventilation (hours)	471 (331–910)
ICU length of stay (days)	25 (15.5–40)
Hospital length of stay (days)	29 (15.5–42.5)
In-hospital mortality, n (%)	7 (41.2)

*Values at the first point of clock genes measurement, continuous quantities in median with quartiles; BMI = Body Mass Index; CCI = Charlson Comorbidity Index; ECMO = extracorporeal membrane oxygenation; ICU = Intensive Care Unit; INR = International Normalized Ratio; mHLA-DR = human leucocyte antigen-D related on monocytes; PTT = partial thromboplastin time; SAPS II = Simplified Acute Physiology Score II; SOFA = Sequential organ failure assessment score; TISS = Therapeutic Intervention Scoring System; \*mHLA-DR was measured in 14 patients and median 0,5 (-3,25 - 0,25) days before inclusion*

## Mixture of rhythmic and arrhythmic genes in septic shock patients

The gene expression patterns of clock genes across all patients are shown in Figure S1. Classification of clock genes in the dataset revealed a complex picture (Fig. 3). Some patients (e.g., SC009 and SC011) had robust rhythms in several clock genes, while others (e.g., SC013 and SC016) showed a few arrhythmic clock genes and no conclusively rhythmic genes. The gene expression profiles (Figure S1) are color-coded to reflect this classification of the clock genes. About 75% of the measured clock genes were classified as having inconclusive rhythms. This suggests that a substantial number of clock genes have dampened rhythms or that the number of measurements is insufficient to obtain enough statistical power.

The rhythmicity scores for the septic shock patients were (Fig. 2). The clustering of the rhythmicity score close to zero reflects the large fraction of clock genes classified as inconclusive. Moreover, the few patients with negative scores have more arrhythmic clock genes than rhythmic ones.

We also tested whether the rhythmicity scores were associated with demographic or secondary clinical outcome parameters of the septic shock patients. However, we could not identify any statistically significant linear association or correlation between the demographic (age, gender, BMI) and secondary outcome parameters (SAPS II, SOFA, TISS-28, lactate, body temperature, time of mechanical ventilation, ICU length of stay, hospital length of stay, mortality) and the rhythmicity score.

Core body temperature (a secondary outcome parameter) is regulated by the central circadian clock and shows circadian rhythms with an amplitude of about 0.5° C in healthy humans. The core body temperature of septic shock patients showed strong trends with 7 patients each having increasing and decreasing trends (Figure S2A). On detrending, core body temperature did show variation of the order of 0.5° C or more, but with no stable 24-h rhythmicity and minima at different times of the day (Figure S2B).

## Specific clock genes are rhythmic (or arrhythmic) across septic shock patients

We next enquired whether specific clock genes were more or less rhythmic across the set of septic shock patients. The rhythmicity scores for the clock genes were clustered similarly to the rhythmicity score of the patients, 4 (Fig. 4). The clock genes *NR1D1*, *NR1D2* and *CRY2* were the most rhythmic across patients, while *CLOCK* and *ARNTL* were the least rhythmic (with non-positive scores). The high score of *NR1D1* must be regarded here with caution as reliable expression was detected in only 11 out of the 17 patients. In summary, the positive regulators of the transcriptional-translation feedback loop [23] (*CLOCK* and *ARNTL*) were the least rhythmic, while negative regulators (*NR1D1*, *NR1D2*, *CRY2*) were the most rhythmic in septic shock patients.

## Clock gene expression levels are altered in septic shock patients

We first compared mean expression levels of the clock genes to determine if clock gene expression was altered in septic shock patients. Six of the 7 clock genes showed significantly altered average expression

in septic shock patients compared to healthy, young men from the BOTI study (Fig. 5). While *CRY1*, *NR1D1*, *NR1D2*, *DBP*, *PER2* were suppressed in septic shock patients, *CRY2*, surprisingly was significantly upregulated compared to healthy young men. Moreover, the most differentially expressed clock genes were *NR1D2* and *CRY2*, which were 12-fold up-regulated and 7-fold down-regulated, respectively.

## Clocks of septic shock patients are less rhythmic than young, healthy adults

CI for the clock genes in BOTI subjects are shown in Fig. 6A. In comparison to septic shock patients, most of the 7 clock genes were classified as robustly rhythmic in all BOTI subjects (Fig. 6B). We found the rhythmicity scores for septic shock patients ( $0.23 \pm 0.26$ ) were lower (one-sided Wilcoxon rank-sum test,  $p < 10^{-4}$ ) compared to young healthy men ( $0.70 \pm 0.18$ ) (Fig. 6C). Nevertheless, some caution is warranted as the two groups were not age-matched and the scores were calculated using a small set of clock genes.

## Discussion

In this prospective observational pilot study, we examined the rhythmicity of the 15 most common clock and clock-associated genes in 17 patients with septic shock and compared them with young, healthy men. We were able to show a decreased mean gene expression of almost every clock gene in patients with septic shock, except for *CRY2*, which was upregulated. The positive regulators *CLOCK* and *ARNTL* (*BMAL1*) had the lowest rhythmicity score and were most arrhythmic, while the negative regulators *NR1D1*, *NR1D2* and *CRY2* were the most rhythmic. No association with secondary clinical parameters was found. To the best of our knowledge, no other study investigated a comparable amount of clock genes in septic shock patients.

Other studies also investigated the circadian rhythm in critically ill patients. Coiffard et al. included 38 severe trauma patients, of whom at least 13 developed a septic episode [17]. All trauma patients had disrupted rhythms of cortisol, leukocytes and cytokines. Of the three measured clock genes *BMAL1*, *PER2* and *PER3* only the last two were down-regulation. Diaz et al. investigated the circadian rhythm in 11 patients in neurological intensive care units [24]. On the day of admission, all patients had a rhythmic expression of *CLOCK*, *BMAL1*, *CRY1* and *PER2*, whereas after one week the clock gene *CLOCK* showed a disrupted circadian rhythm [24]. A recent study of Acuna-Fernandez et al. compared *BMAL1*, *CLOCK*, *PER2* and *CRY1* expression in healthy volunteers, ICU and sepsis patients [16]. In line with our results, the authors found disrupted circadian rhythms in sepsis patients. In addition, they showed that conditions in ICUs, such as permanently high light levels, do not disrupt circadian rhythms in sepsis patients, because non-sepsis ICU-patients expressed clock genes rhythmically. In comparison to these studies, we investigated a larger panel of clock genes and were able to show a downregulation of *BMAL1* and *PER2* as well. Additionally, we found that *NR1D1*, *NR1D2*, *CRY1*, *CRY2*, *PER1*, *PER2* and *DBP* had altered rhythms in at least some patients with septic shock.

Secondary outcome parameters including SOFA, SAPS II and TISS-28, lactate, time of mechanical ventilation, ICU and hospital length of stay and mortality did not show a linear correlation with rhythmicity scores. The core body temperature measurements coincident with clock gene measurements were highly variable with strong trends and therefore, could not be associated with clock gene expression amplitudes. As already known, body temperature varies rhythmically with its peak in the evening and trough in the morning hours [15]. However, the core body temperature troughed at different times during the day in different patients, which is in agreement with our finding of disrupted circadian rhythmicity. Generally, due to the small number of patients, associations of rhythmicity scores with outcome parameters might have gone undetected. Larger studies should examine whether disrupted rhythmicity is associated with mortality.

As another clinical secondary outcome parameter, we investigated the correlation between circadian rhythmicity and the expression of mHLA-DR on monocytes, which is one of the most studied markers of immunosuppression. A decreased mHLA-DR expression is associated with higher mortality in critically ill as well as sepsis patients [25]. Due to the robust intrinsic circadian rhythm of monocytes [11], we hypothesized that in patients with septic shock the grade of immunosuppression as measured by mHLA-DR expression is associated with a loss of circadian rhythm. In our study population, 11 out of 14 patients had levels of HLA-DR of 15.000 molecules per monocyte or less, which is associated with immunosuppression and immunoparalysis [21]. However, HLA-DR expression on monocytes did not show an association or significant correlation with the rhythmicity score. Nearly all patients showed immunosuppression and no rhythmicity in the expression of some clock genes. This could be attributed to the small study population not capturing the multitude of factors influencing clock gene expression.

It is recognized that melatonin helps restoring and maintaining circadian homeostasis [26]. Additionally, melatonin was reported to have immunomodulatory and antioxidative effects [27]. Lorente et al. found that melatonin levels in non-surviving sepsis patients were higher than in survivors as high melatonin levels correlate with worse clinical conditions of the patients [28]. The increased melatonin levels are compatible with the reported disruption of circadian rhythmicity in critically ill patients [29]. Melatonin is used as a supporting drug in septic infants and could be a promising therapeutic option in sepsis patients to promote stable circadian rhythms, improve immunity and outcomes [30]. However, further research is needed to analyze the impact of the circadian rhythm on sepsis mortality and its translational potential to find promising treatment options for improving sepsis care [31]. Understanding circadian rhythmicity in sepsis and the implications from and on the immune response are fundamental to develop new diagnostic and therapeutic tools and to individualize sepsis treatment.

This study has several limitations. First, only septic shock patients were included in our study. Thus, no conclusion can be drawn as to whether the disruption of the circadian rhythm favors sepsis or whether sepsis is the cause of the arrhythmia. Second, the inability of the analysis to classify clock genes as rhythmic or arrhythmic was due to a limited number of samples in the time series, i.e. we measured only 7 time points for each subject. When the rhythms are partially disturbed as is the case here, more data points are needed to detect arrhythmicity than to show rhythmicity. Third, the differences in mean

expression levels of clock genes can both cause the changes in rhythmicity observed in septic shock patients and affect our statistical ability to quantify rhythms. Finally, samples from septic shock patients and BOTI patients were measured using slightly different Nanostring™ technologies, which – although unlikely - may result in differences in quantified expression levels.

## Conclusion

In conclusion, our study showed impaired circadian rhythmicity in patients with septic shock compared to a group of young, healthy men. The positive regulators of the transcriptional-translation feedback loop (*CLOCK* and *ARNTL*) were the least rhythmic, while negative regulators (*NR1D1*, *NR1D2*, *CRY2*) were the most rhythmic. In addition, the expression of the clock genes *CRY1*, *NR1D1*, *NR1D2*, *DBP*, and *PER2* were suppressed in septic shock patients, whereas *CRY2* was significantly upregulated compared to healthy young men. These results strengthen the relationship between circadian disruption, a restricted immune response, and sepsis.

## List Of Abbreviations

ACCP American College of Chest Physicians

BMI Body Mass Index

CARS Compensatory Anti-inflammatory Response Syndrome

CBT Core Body Temperature

CCI Charlson Comorbidity Index

CI Confidence Interval

ECMO Extracorporeal membrane oxygenation

ICU Intensive Care Unit

INR International Normalized Ratio

mHLA-DR human leucocyte antigen-D related on monocytes

PTT Partial Thromboplastin Time

SAPS II Simplified Acute Physiology Score II

SIRS Systemic Inflammatory Response Syndrome

SOFA Sequential organ failure assessment score

## Declarations

**Ethical approval** This study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin (EA1/288/13) and meets the requirements set out by the ICH-GCP and Declaration of Helsinki. **Written informed consent** was obtained from all patients. **Availability of data and materials** Due to legal restrictions imposed by the Ethics Committee and the data protection commissioner of the Charité – Universitätsmedizin Berlin, public sharing of study data with other researchers or entities is not allowed. Requests may be sent to [ed.etirahc@atadhcaeser-iad](mailto:ed.etirahc@atadhcaeser-iad). **Competing interests** The authors declare that they have no competing interests. **Funding** This study was supported by the Charité – Universitätsmedizin Berlin. Gunnar Lachmann is participant of the Berlin Institute of Health (BIH) Charité Clinician Scientist Program funded by Charité – Universitätsmedizin Berlin and BIH. Bharath Ananthasubramaniam is supported by the Deutsche Forschungsgemeinschaft (DFG) grant AN1553/2-1 and SPP 2041. **Authors' contributions** Conceived and designed the experiments: AG, AK, CS. Performed the experiments: VW, LS, BK. Analyzed the data: GL, BA, LS, VW, AK. Wrote the manuscript: GL, BA, VW, LS. Commented on the manuscript: all authors. **Acknowledgements** We thank Dr. rer. medic. Kathrin Scholtz for monitoring the study as well as Anna-Marie Theiss and Felix Siegmeier for their help with acquisition of the data.

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## Figures

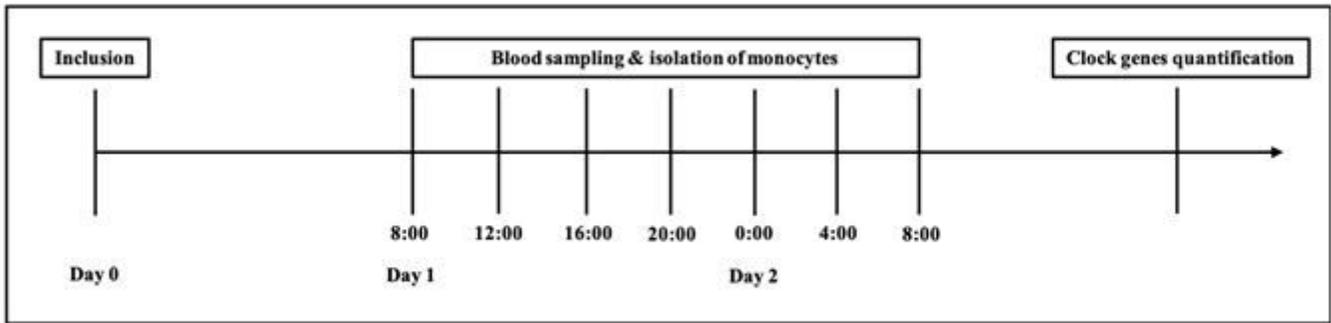


Figure 1

Study schedule from inclusion, over blood sampling and isolation of monocytes to gene quantification.

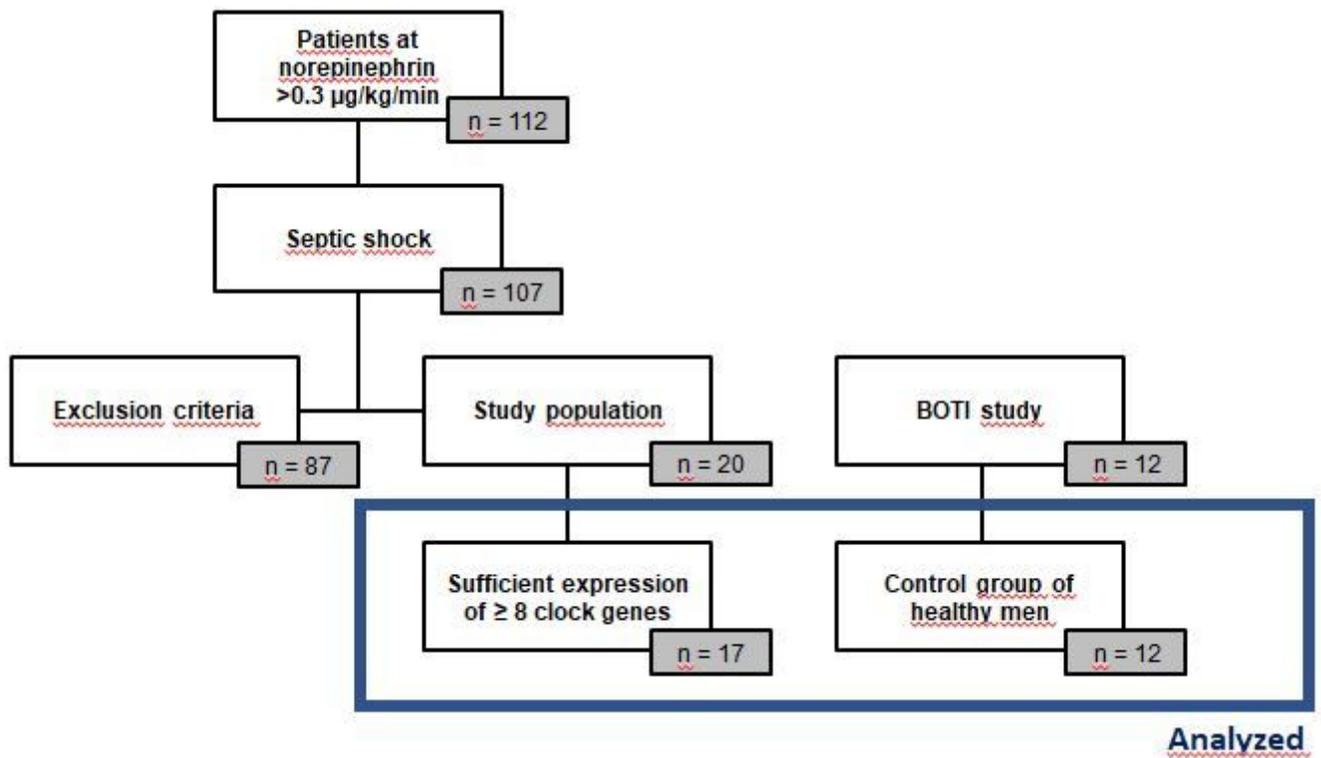
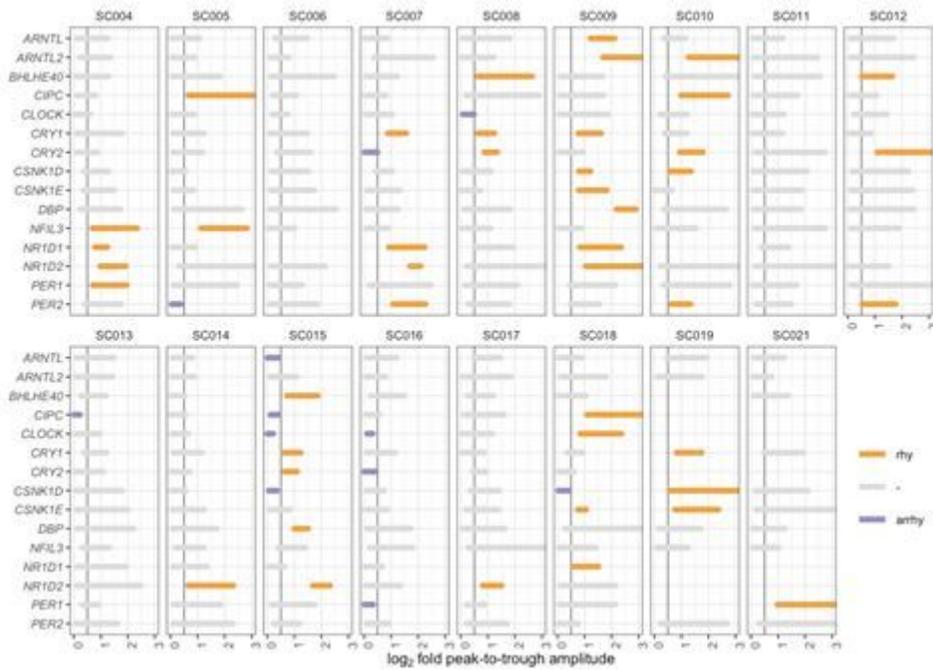


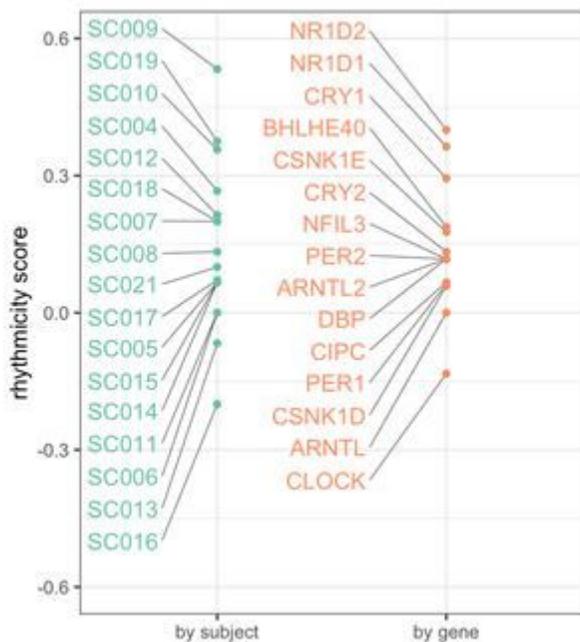
Figure 2

CONSORT diagram, 17 patients were included to the final analyses.



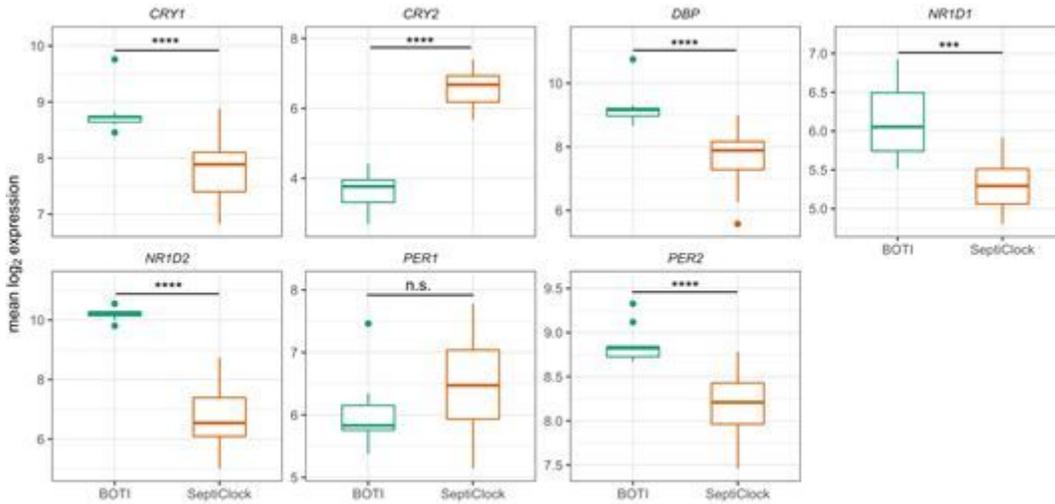
**Figure 3**

Quantification of rhythmicity of clock genes in septic shock patients. Confidence intervals (CI) on the rhythm amplitudes (peak to trough on the log<sub>2</sub> scale) of gene expression based on harmonic regression and bootstrapping are visualized as horizontal bars. The CIs include the true amplitude of the clock gene with a 95% probability. The color of the bars indicates whether the rhythms are present (orange), absent (violet) or data are inconclusive (grey). The vertical line shows the minimum amplitude that is deemed biologically relevant. Bars are missing for certain clock genes that did not satisfy the inclusion criteria.



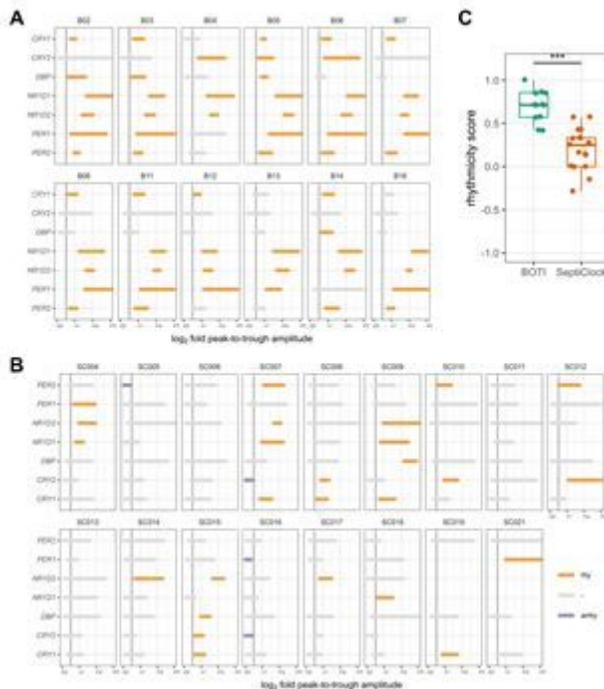
**Figure 4**

Rhythmicity of clock genes in septic shock patients grouped by patient and clock gene. The rhythmicity score takes a value between -1 and 1, with a value of 1 and -1 representing rhythmicity and arrhythmicity, respectively. The scores shown are aggregates of all clock genes in a subject ('by subject'), or of all subjects for a particular clock gene ('by gene').



**Figure 5**

Comparison of average clock gene expression of the 7 common clock genes in septic shock patients and BOTI subjects. The log<sub>2</sub> gene expression average across time points for each septic shock patient (N=17) in the septic shock patients and young, healthy men (N=12) from the BOTI study. P-values for the significance of the difference are based on the non-parametric Wilcoxon rank-sum test.



## Figure 6

Comparison of rhythmicity of common clock genes between septic shock patients and healthy, young men. (A) The confidence intervals (CI) for the clock genes from BOTI subjects used to classify clock genes as rhythmic, arrhythmic or inconclusive (similar to Figure 2). (B) The confidence intervals for the common clock genes in septic shock patients extracted from Figure 2 for comparison with (A). (C) The comparison of the rhythmicity scores between septic shock patients and young, healthy adults based on the classification in (A) and (B). The scores were compared using a non-parametric Wilcoxon rank-sum test.

## Supplementary Files

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