

# Relation of Heart Rate Variability to Serum Levels of C-Reactive Protein in Patients with Severe Sepsis and Septic Shock

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## Abstract

*In this study we tried to quantify the instantaneous and longitudinal correlations between different heart rate variability (HRV) indices and daily measured C-reactive protein (CRP) serum levels in a group of patients with severe sepsis.*

*Metrics used were performed daily in 20 consecutive septic patients and included serum levels of CRP, power spectral analysis in both the time and frequency domain and the two values of standard deviations (SD1, SD2) obtained from the Poincaré plot. CRP blood levels exhibited significant negative correlations with LF, LF/HF and SDNN and positive correlations with HF and SD1/SD2. SDNN, LF and HF power values were the most significant predictors of increasing CRP levels and proved to be significantly different between survivors (n=16) and non-survivors (n=4).*

## 1. Introduction

Systemic inflammation is a normal response to altered homeostasis caused by severe infection or trauma and is characterized by the endocrine release of different cytokines such as tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) and interleukin-1 (IL-1), IL-4, IL-6 and IL-10, normally confined to paracrine regulation of a local inflammatory response. As part from their involvement in local and systemic inflammation, cytokines may induce activation of brain-derived neuroendocrine immunomodulatory responses. Neuroendocrine pathways, such as hypothalamo-pituitary-adrenal axis (HPA) and both the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS) are powerful modulators of inflammation, typically through an anti-inflammatory balancing mechanism [1].

The pathophysiological link to the above hypothesis is the capability of the brain to monitor and affect at the same time the immune status. The first mechanism relies upon activation of vagus nerve afferent fibers from different cytokines that signal the brain that inflammation is occurring. The brain can affect the immunological

status through the activation of the HPA axis and the increased outflow of sympathetic (SNS) and parasympathetic nervous system. The SNS activation induces stimulation of  $\beta_2$ -subtype adrenoreceptor that is associated with an inhibition of pro-inflammatory cytokines production. It has also been demonstrated that acetylcholine decreases TNF production by endotoxin-stimulated human macrophage cultures, through  $\alpha 7$ -subunit of the nicotinic acetylcholine receptor. The vagus nerve cholinergic signaling interacts with the above receptor on immune cells in the spleen and inhibits TNF production and release into the circulation [2].

In conclusion, there is strong evidence that the Central Nervous System controls body's systemic responses to inflammation. The aim of this study is thus to investigate whether there is a link between different physiological parameters estimating indirectly ANS outflow and being extrapolated from heart rate signals, with various biomarkers, such as CRP serum levels and relate them with final outcome in a cohort of septic patients.

The best such 'physiomarkers' that can be studied through analysis of heart rate signals are the heart rate variability (HRV) that is the variability of R-R series in the electrocardiogram (ECG), and its frequency components that estimate the sympathovagal balance. Since HRV is an index of ANS outflow and a measure of an end-organ response to central neural centers, it can be assumed that loss of variability and altered LF/HF ratio is associated with a failure of CNS-derived inflammation-suppressing mechanisms. There is small evidence that a strong association exists between low measures of HRV and increased inflammatory response in patients who survived hospitalization for acute myocardial infarction, in those with severe sepsis (high IL-6) and after unstable angina pectoris (high CRP) [3,4].

## 2. Methods

A total of 20 consecutive septic patients admitted to the Intensive Care Unit (ICU), from October 2007 to April 2008, with a mean Acute Physiology and Chronic Health Evaluation (APACHE) II score upon admission 18

(5.6) were enrolled in the study, after approval by the Institutional Ethics Committee of Alexandroupolis University Hospital (Greece). Twelve patients were diagnosed with severe sepsis (infection of either respiratory or abdominal origin + Systemic Inflammatory Response Syndrome [SIRS]) and 8 with septic shock, according to ACCP/SCCM criteria [5]. The mean Specific Organ Failure Assessment Score (SOFA) during the whole stay in ICU was 9 (2.5). Individuals with previous history of atrial flutter or fibrillation, ventricular ectopic beats and use of anti-arrhythmic medication, severe brain injuries and acquired immunodeficiencies were excluded from the study, as there was an inherent alteration in HRV parameters. All patients were studied in the supine position. Since different levels of anesthesia and respiratory effort may influence HRV, we decided to study patients only during heavy sedation and controlled mechanical ventilation. The study period was approximately 6 days from admission to the ICU.

Considering the fact that HRV parameters are influenced by many factors it is important to maintain standard conditions (same time and period of measurements). ECG was recorded on a daily basis (early in the morning when the workload was minimal) for all patients. Analog ECG signals were obtained from monitors (Marquette 8000, GE Milwaukee, Wis, USA) with a low-pass filter at 100 Hz. Data were collected and analyzed using an HP Pavilion 6181, 2GHz PC. Sampling rate for data collection was done at 250Hz. The ECG signal was recorded for 600 secs from a standard lead II ECG in order to satisfy the requirements of data stationarity (stable signal statistical properties during the measurement period), that cannot be fulfilled during longer ( $\geq 1$  hour) periods of measurement and especially in the ICU setting. From each 600-s data set, a 128-s time series that was artifact free was chosen for off-line analysis by someone who was blind to the patient's diagnosis. The spectral power of each spectrum ( $\text{msec}^2/\text{Hz}$ ) was calculated at high [0.15-0.4Hz] and low frequency [0.04-0.15 Hz], using a software for advanced HRV analysis for Windows, that was developed from the Department of Physics of the University of Kuopio, Finland. Fast periodicities (HF) are largely due to the influence of the respiratory phase on vagal tone whereas low-frequency periodicities (LF) are produced by baroreflex feedback loops, affected by both sympathetic and parasympathetic modulation of the heart [6]. The ratio of low to high frequency (LF/HF) was used to assess sympathovagal balance.

The software also calculated normalised units of the frequency domain variables (n.u), time domain metrics such as the standard deviation of NN intervals (SDNN), as a measure of variability of the signals and SD1/SD2 ratio, as indicator of the dispersion of RR points from the constructed Poincaré plots.

A venous blood sample was collected every day and after centrifugation, serum samples were frozen at  $-80^{\circ}\text{C}$  until assayed at a core laboratory. CRP levels were measured with a high sensitivity nephelometric method (Dade Behring GmbH, Marburg, Germany).

Data are expressed as mean (standard deviation). Heart rate power data and CRP blood levels were logarithmically transformed to satisfy the requirements of normal data distribution. Linear regression and correlation analysis with Pearson's test was performed in order to assess whether HRV variables were independent predictors of CRP and for evaluation of trends over time. Differences between survivors and non-survivors were evaluated with analysis of variance (ANOVA). Furthermore, CRP blood levels were categorized into 3 quartiles: 1. including values  $< 20$  mg/dL ( $n=11$ ) 2. including values between 21-30 mg/dL ( $n=5$ ) and 3. including values  $> 30$  mg/dL ( $n=4$ ) and HRV variables were compared across CRP quartile groups with ANOVA. Tests were performed with SPSS Software Version 13 and values of  $p<0.05$  were considered to be significant.

### 3. Results

Patients' characteristics are listed in Table 1.

Table 1. Patients' characteristics.

Parameter	Value [mean (SD)]
APACHE II score	18 (5.6)
SOFA score (mean)	9 (2.5)
Age	57.8 (10.5)
Sepsis/septic shock	12/8
Sex (M/F)	13/7
LF n.u	36.13 (18.2)
HF n.u	63.86 (18.2)
LF/HF	0.7 (0.4)
SDNN (sec)	0.016 (0.014)
CRP (mg/dL)	22.4 (13.5)
Outcome (survivors/non survivors)	16/4
Length of stay (LOS)	9.5 (6)

Non-survivors did not differ from survivors in terms of age or gender. Their diagnosis of admission was severe septic shock of abdominal origin. Furthermore, they were more severely ill with higher APACHE II score of admission [25 (6.7) vs 17 (9.3)] and mean SOFA score [14 (3.2) vs 7 (3.3)], during their stay in ICU ( $p<0.05$ ). They had also higher values of CRP during the study period [34 (12.4) vs 19 (11.8) mg/dL] ( $p<0.05$ ) related to survivors.

CRP blood levels exhibited significant negative corre-

lations with LF ( $r=-0.78$ ,  $p<0.01$ ), SDNN ( $r=-0.79$ ,  $p<0.01$ ) and LF/HF ( $r=-0.61$ ,  $p<0.05$ ) and positive correlations with HF ( $r=0.80$ ,  $p<0.01$ ) and SD1/SD2 ratio ( $r=0.66$ ,  $p<0.05$ ).

Figure 1 shows parallel alterations over time of both CRP blood levels and HF n.u., where there is a progressive upward shift in the two variables from day 1 until day 4, with an interval of decrease during the 3<sup>rd</sup> day of study and subsequently, a downward shift in both metrics until day 6. Figures 2, 3 and 4 display inverse changes between CRP and both LF, LF/HF and SDNN respectively. It seems that from day 1 to day 4 there is a progressive increase in CRP values with concomitant inverse changes in both LF and LF/HF ratio, however, the most apparent alterations concern the upward shift of CRP during the 4<sup>th</sup> day and its subsequent decrease until the 6<sup>th</sup> day of study, paralleled with opposite alterations in LF, LF/HF and SDNN.

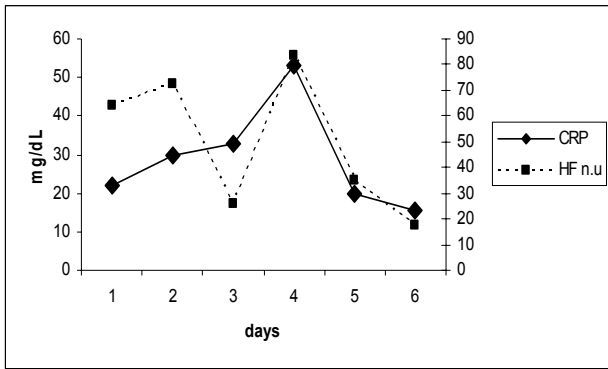


Figure 1. Longitudinal trends over time of mean values of CRP and HF normalized units (n.u) for the whole patients group.

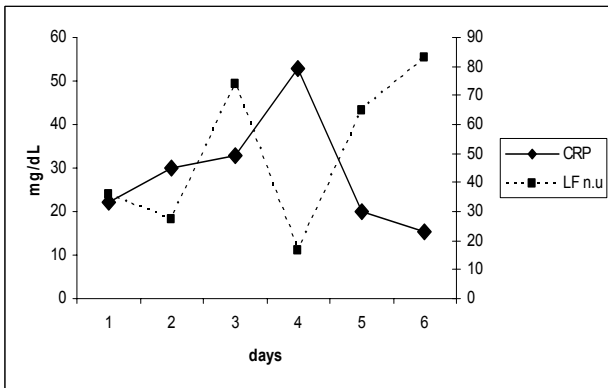


Figure 2. Longitudinal trends over time of mean values of CRP and LF normalized units (n.u) for the whole patients group.

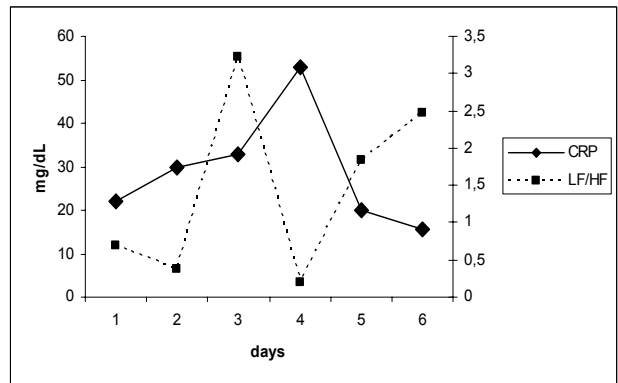


Figure 3. Longitudinal trends over time of mean values of CRP and LF/HF ratio for the whole patients group.

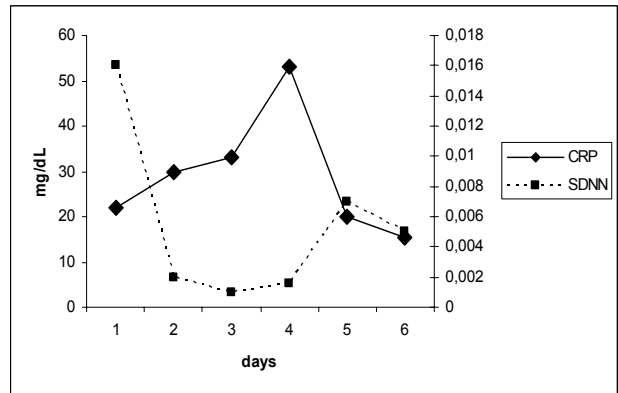
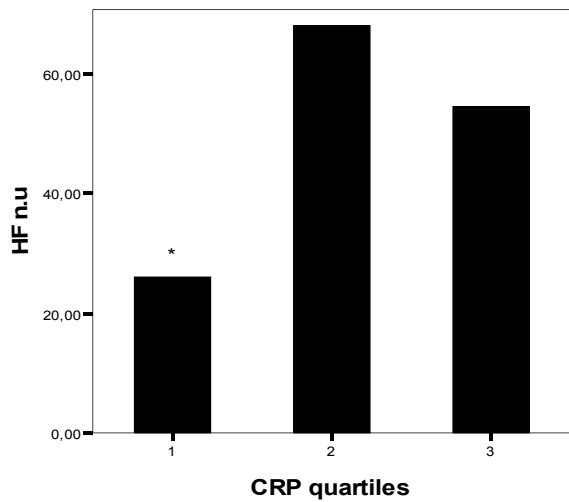


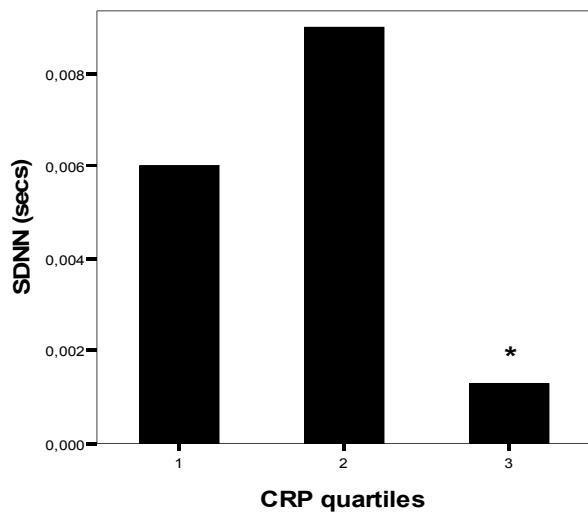
Figure 4. Longitudinal trends over time of mean values of CRP and SDNN for the whole patients group.

The natural logarithms of SDNN, LF and HF proved to be independent predictors of CRP in a univariate linear regression model with beta slope (standard error-SE): -0.28 (0.072), -0.21 (0.014) and 1.24 (0.084) respectively and also were significantly different between survivors and non-survivors ( $p<0.05$  for all comparisons).

The subgroup analysis that was performed after categorizing CRP blood levels into 3 quartiles identified significant differences between HF n.u., LF n.u. and SDNN across different values of CRP. As it is demonstrated in figure 5, the 1<sup>st</sup> quartile ( $CRP<20$  mg/dL) was significantly lower related to the other two in terms of HF n.u. (5A) and significantly higher at the same time in terms of LF n.u. (5C), meaning that the lower the CRP values the lower the HF and the higher the LF components of the power spectrum. Finally, the 3<sup>rd</sup> quartile ( $CRP>30$  mg/dL) proved to be significantly lower than the first and the second in terms of SDNN ( $p<0.001$ ), (5B).



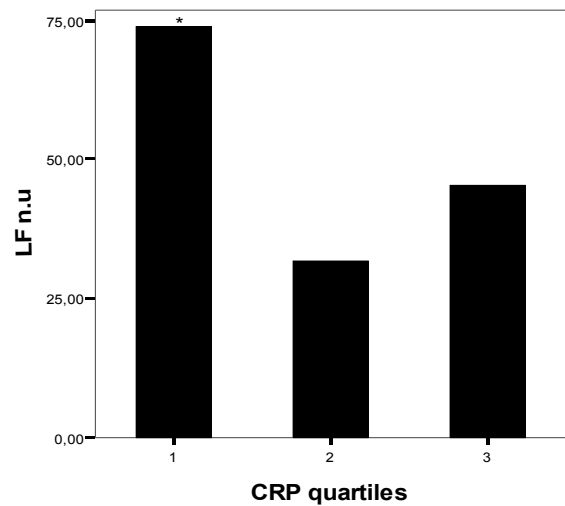
5A.



5B.

#### 4. Discussion and conclusions

In the present series, HRV was shown to be most markedly depressed in the patients with high CRP, indicating that an unopposed inflammatory response is associated with uncoupling between ANS and immune regulated inflammation. Furthermore, HF power spectrum that indicates vagal tone was significantly increased in states of severe sepsis and septic shock. However, association does not mean causality and further studies are needed, including larger patient groups and different clinical settings, in order to unmask possible causal relations



5C.

Figure 5 (A,B,C). Different distribution of HF n.u, SDNN and LF n.u across the three CRP quartiles (bar charts) respectively.

between neuro and immunoregulation.

#### References

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