Homocysteine plasma levels as a marker of clinical severity in septic patients

Nível plasmático de homocisteína: marcador de gravidade em pacientes sépticos?

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ABSTRACT

Objective: Homocysteine and sepsis are both associated with inflammation and endothelial activation. Therefore this study was aimed to evaluate if the plasma homocystein level is related with the septic patient clinical severity.

Methods: Severe sepsis or septic shock patients, with less than 48 hours from organ dysfunction start, were admitted to this prospective observational study. Homocysteine levels were determined by the time of study admission and then on the Days 3, 7 and 14. The homocysteine association with the Sequential Organ Failure Assessment (SOFA) score was evaluated using the Sperman test, and its association with mortality using the Mann-Whitney test. A $p<0.05$ value was considered statistically significant.

Results: Twenty one patients were enrolled, and 60 blood samples were collected to measure total homocysteine [median 6.92 (5.27 – 9.74 µmol/L)]. The Sperman correlation test showed no association between homocysteine and SOFA (r=0.15 and p=0.26). Also no correlation was found for the homocysteine level by the study admission time and the difference between the Day 3 SOFA score versus by study admission (deltaSOFA) (r=0.04 and p=0.87). Homocysteine variation between the Day 3 and the study admission (deltaHmc) and SOFA score variation in the same period were not correlated (r=-0.11 and p=0.66). Homocysteine by the study admission was not correlated with death in intensive care unit rate (p= 0.46) or in-hospital death rate (p = 0.13). This was also true for deltaHmc (p=0.12 and p=0.99, respectively).

Conclusion: Baseline homocysteine levels and its variations within the first dysfunction days were not related with septic patients' worsened organ function parameters or mortality.

Keywords: Homocysteine; Sepsis; Organ dysfunction; Mortality

INTRODUCTION

Homocysteine (Hmc) is an intermediate amino acid containing a sulphydryl radical. It is formed by methionine amino acid conversion into cysteine. About 70 to 80% of plasma Hmc is protein-bound, mainly albumin.[1] Only 7% free homocysteine appears as the reduced form.[2] This amino acid is considered a risk marker for cardiovascular, particularly coronary, diseases.[3-5] The mechanisms involved on Hmc vascular damage are controversial, however they include increased oxidative stress,[6] enhanced thrombogenisis,[7] endothelial dysfunction,[8] and smooth muscle cells mitosis interference.[9,10] For at least forty years, changed homocysteine plasma levels have been indicative of a systemic disease.

Recently more attention was drawn to Hmc due to the growing body of evidence published on this amino acid connection with atherosclerotic disease.[3-5,11-13] Intima (endothelial layer) degenerative processes, as inflammation and thrombogenesis, are listed among the possible Hmc-related endothelial injury pathophysiology.[14,15]

The atherosclerotic process endothelial dysfunction, related with Hmc plasma levels changes, has similarities with the sepsis' endothelial dysfunction. Sepsis is a systemic syndrome associated with increased inflammatory mediators as cytokines, aracdonic acid derivatives, oxygen reactive species, nitric oxide, intracellular adhesion molecule (ICAM), and endothelial leucocyte adhesion molecule (ELAM), and endothelial function changes are a relevant part of its pathophysiology.[16] Therefore, such a hypermetabolic, pro-inflammatory and pro-thrombotic condition as sepsis would be an appropriate scenario for Hmc levels changes.

Given this, the possible relationship between sepsis severity (i.e., the organ dysfunction degree) and Hmc plasma levels could be questioned. Therefore this study was aimed to evaluate Hmc plasma levels in septic patients and investigate its correlation with organ dysfunction both presence and severity.

METHODS

This was a prospective observational clinical trial conducted in the intensive care unit of a tertiary university hospital. The study was approved by the institution's Ethics Committee, and patients were enrolled upon informed consent form signature.

Patients aged 18 years or more, diagnosed severe sepsis or septic shock and less than 48 hours organ dysfunction, were enrolled. Severe sepsis was defined as a suspected infective focus and at least two 1992 consensus'[17] systemic inflammatory response criteria, plus at least one organ dysfunction. Septic shock was defined as hypotension refractory to volume replacement and requiring vasopressor amines. Dysfunction was considered as hypotension, partial oxygen pressure and inspired oxygen fraction ratio (PaO_2/FiO_2) ≤ 250, metabolic acidosis with lactate changes, platelets count below 100,000 cells/mm^3, total bilirubin above 2 mg/dL, urinary output lower than 0.5 mL/hour although appropriate volume replacement, and depressed consciousness level. These dysfunctions should be clearly secondary to the septic process. Patients developing acute renal failure during their stay were excluded, due to interference with methionine metabolism. Plasma homocysteine level was age-corrected for ≥ 70 year old patients.

The demographic and sepsis characteristics and the Acute Physiological Chronic Health Evaluation II (APACHE II) score[18] were recorded for all patients. The Sequential Organ Failure Assessment (SOFA) score was used for organ dysfunction follow-up.[19] Blood samples for Hmc levels measurement were drawn by the admission and then after 3, 7 and 14 days. The patients were followed from their intensive care unit (ICU) admission to 28 days after enrollment, for mortality assessment.

The samples were drawn from a peripheral vein or artery, with the patient laying in supine position and less than 3 minutes tourniquet, after 8 hours fasting. Following collection the samples were placed in EDTA tubes and packaged in ice and immediately transferred to the laboratory. Special attention was given to fast transference, in order to prevent red blood cells to change Hmc levels. After separated, the plasma was frozen at -20ºC until the dosage time.

Plasma Hmc levels were measured with a high performance liquid chromatography method [Pfeiffer et al.[20]]. All chemicals were obtained from Sigma Chemical Co. The results are expressed as µmol/L.

Hyper-homocysteinemia was categorized as: moderate (15 to 30 µmol/L), intermediate (30 to 100 µmol/L), and severe (> 100 µmol/L).[21] The correlation between homocysteine and SOFA variables was checked with the Spearman's test. In addition, homocysteine levels were also expressed as logarithm, and evaluated with the Pearson's correlation test. Additionally, the homocysteine level by the admission time, the between the 3rd and 1st days homocysteine level variation (deltaHmc), the SOFA scores variation between the 3rd and 1st day (deltaSOFA) (both expressed as absolute variation) were related, and this last analyzed both as continuous or categorical (worsened versus improved) variable. Unchanged SOFA score (deltaSOFA = 0) was considered as worsened, as suggests unfavorable clinical outcome.

The categorical results were expressed as percentage, and the continuous variables as mean and standard deviation (minimum-maximum), or median (interquartile 25%-75%). For the survivors versus non-survivors comparison, the Mann-Whitney test was used. $P<0.05$ results were considered statistically significant.

RESULTS

Twenty one patients (11 male, 10 female) mean age 43.9 ±19.38 years (17-78 years) and APACHE II 18.19 ± 8.23 (2-35) were admitted to the study. Pulmonary was the main infective focus, with pneumonia diagnosed in 71.43% patients, followed by abdominal infection (47.62%) and urinary tract infection (33.3%). Severe sepsis was diagnosed in 33.3% of the patients, and septic shock in 66.5%. Mean admission time organ dysfunctions were 2.19 ± 0.87 (1-4 dysfunctions), and 76.19% of the patients had two or more dysfunctions. Mean first day SOFA was 7.48 ± 3.76 (1 to 4). ICU stay mortality rate was 23.8%, and the 28 days mortality 28.6%. Overall patients' data are shown on table 1.

Sixty samples were drawn for Hmc measurement, with a median 6.79 (5.07-9.77) µmol/L for all collected samples. The female subjects median was 9.3045 (5.73-13.41) µmol/L, and male subjects 6.97 (4.76-8.63) µmol/L, p = 0.28. Median Hmc levels for each sampling day, and mean SOFA scores are shown on table 2.

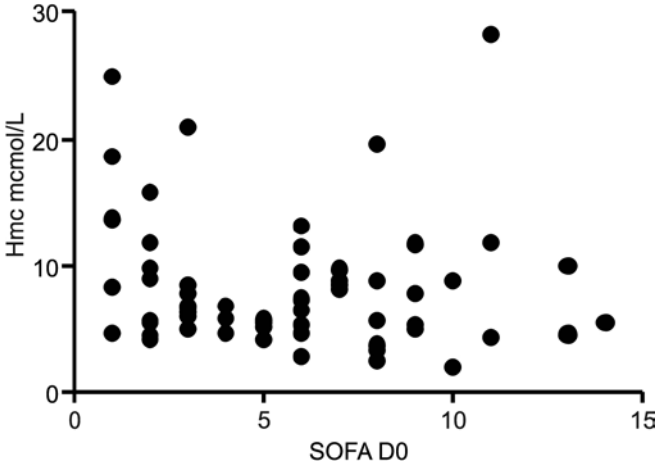
No association was found between the study admission day (D0) homocysteine levels and the outcomes discharge from ICU or death (non-survivors: 5.06 (4.76-9.80) and survivors: 8.06 (5.79-9.86); p=0.46), or in-hospital mortality (5.45 (4.71-8.99) versus 8.64 (6.83-10.03) for non-survivors and survivors, respectively; p = 0.13). Even when deltaHmc was considered, no correlation with the outcome was found, neither for ICU mortality (non-survivors: -7.25 (-7.50-1.05) versus survivors: -0.70 (-3.45- -0.67); p=0.12) nor for in-hospital mortality (non-survivors: -0.77 (-7.25-0.42) versus survivors: -1.09(-6.00-0.30); p=0.99).

Homocysteine levels were not correlated with the sampling day SOFA (r=-0.15 and p=0.26) (Figure 1). This was not changed by logarithmic homocysteine expression (r=-0.15; p=0.23). No correlation was found for the study admission homocysteine and deltaSOFA (r=0.04 and p=0.87) (Figure 2). Even when deltaSOFA was categorically evaluated, no difference was found for favorable outcome (8.19 (5.7-10.0) subjects and those who worsened (7.8 (5.3-10.8),

Table 1 – Population characteristics

Variable	Result
Male gender	52.38
Age (years)	43.9 ± 19.38
APACHE II	18.19 ± 8.23
Admission SOFA	7.48 ± 3.76
SOFA D3	
Categorization	
Severe Sepsis	33.3%
Septic Shock	66.7%
Focus	
Respiratory tract infection	71.43%
Abdominal infection	47.62%
Urinary tract infection	33.3%
Mortality	
ICU	23.8%
28 days	28.6%

Apache – Acute Physiological Chronic Health Evaluation; SOFA – Sequential Organ Failure Assessment; ICU – intensive care unit. Results expressed as percent or mean ± standard deviation.



SOFA – Sequential Organ Failure Assessment.

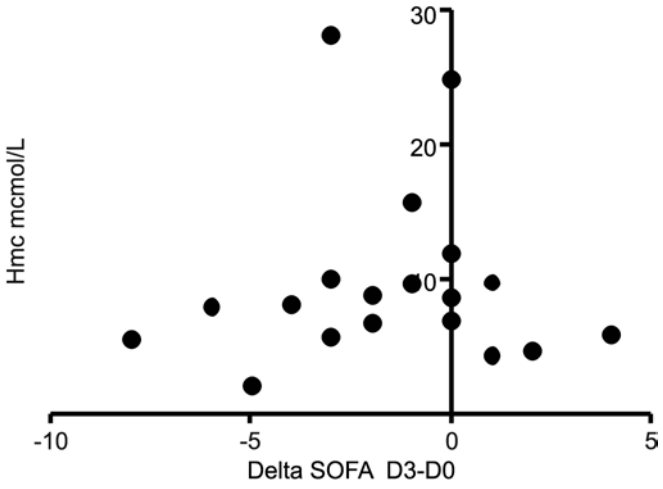
Figure 1- Serum homocysteine levels and Sequential Organ Failure Assessment (SOFA) score correlation, considering all study days. Spearman (r = -0.15 e p = 0.26).

Table 2 - Septic patients' plasma homocysteine levels and severity

Variable	Day 0 (N=21)	Day 3 (N=19)	Day 7 (N=15)	D 14 (N = 5)	Overall
Hmc (µmol/L)	7.94 (5.33 - 9.91)	6.53 (4.36 – 8.94	7.49 (5.12 – 11.62)	5.40 (4.64 – 9.14)	6.79 (5.07 - 9.77)
SOFA	7.62 ± 3.72	5.63 ± 3.05	4.6 ± 2.5	2.80 ± 1.92	

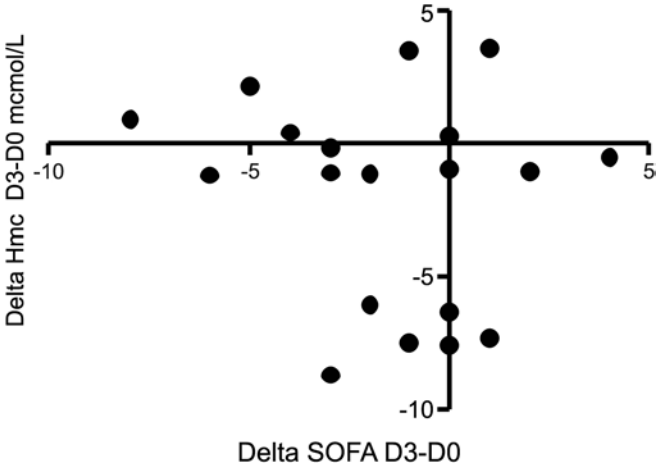
Hmc – homocysteine; SOFA – Sequential Organ Failure Assessment. Results expressed as median (25-75%) and mean ± standard deviation.

p=0.86. Also, no association could be shown for deltaHmc and organ dysfunctions results, as measured by variation of SOFA score in the same period (r=-0.11; p = 0.66) (Figure 3).



SOFA – Sequential Organ Failure Assessment.

Figura 2 – Serum homocysteine levels and Sequential Organ Failure Assessment (SOFA) score variation correlation, between Day 3 and study admission day. Spearman (r = 0.04; p = 0.87).



SOFA – Sequential Organ Failure Assessment.

Figure 3 – Day 3 and study admission day serum homocysteine levels and Sequential Organ Failure Assessment (SOFA) score correlation, for the same period. Spearman (r = -0.11; p = 0.66).

DISCUSSION

No homocysteine and septic disease severity correlation, as evaluated by SOFA score or mortality was shown in this study.

This frustrated our initial expectations. Homocysteine has been correlated with cardiovascular diseases due to atherosclerosis patients' high plasma levels. This pathophysiology appears to involve endothelial dysfunction related to oxidative stress, thrombogenesis and endothelial changes.[14,22] Similarly, endothelial injury has a cornerstone role in sepsis pathophysiology. During the sepsis development, inflammation-coagulation circuit and reduced fibrinolysis are directly related with organ dysfunctions in critical patients. This would be due to intravascular coagulation which would reduce the microcirculatory flow, perpetuating the hypoperfusion endothelial dysfunction and consequently increasing inflammatory response.[16,23] Therefore, the evaluation of plasma total homocysteine levels and its correlation with SOFA values was expected to provide a rational for these parameters connection with organ changes and the possible homocysteine involvement in endothelial function and sepsis pathophysiology.

Although many studies correlate homocysteine with cardiovascular and cerebrovascular diseases,[3,5,10-13,21] little evidence of homocysteine role in septic patients pathophysiology is found in the literature. The septic shock endothelial lesion may lead, among other dysfunctions, to acute respiratory distress syndrome (ARDS) and acute lung injury (ALI), which are frequent in septic patients.[24] Using this study rational, i.e., the correlation between hyperhomocysteinemia and hypercoagulability, Tsangaris et al. conducted a trial where plasma homocysteine levels also failed to correlate with worse outcome ARDS and ALI patients.[25] This agrees with our results.

This failure to find a correlation may be related to the sample size, the studied population, homocysteine metabolism aspects, septic shock metabolic changes, nutritional factors or the study design itself.

This study has strengths. Serial homocysteine plasma sampling by the admission, 3rd, 7th and 14th days allowed analyzing the results over the time. We should also emphasize that homocysteine values variations could come from long time sampling (above one month), different measurement and drawing techniques, and inter-individual fluctuations. This was prevented by samples draws within up to 15 days, with 3-7 days interval. The same homocysteine evaluation technique was used for all patients, and one single laboratory. The blood draws were always compliant to the consensual techniques for homocysteine analysis. The analysis were independently performed

inter-individually, and dependently in a same subject, thus reducing eventual population variation bias. It should also be considered that the analysis evaluated the homocysteine levels correlation with the organ dysfunction marker, SOFA, and not the isolated homocysteine levels in each subject.

As weaknesses, one should emphasize the small number of patients, inclusion of patients older than 60 years, and restricted to homocysteine plasma analysis. Homocysteine is stable in a control population. In an Australian study, the homocysteine serum levels were shown to have little fluctuation until the sixties, with a subsequent linear 1 µmol/L per decade change. Stable high creatinina values patients (chronic renal failure) also had a linear relationship, 148 µmol/L/(µmol/L).[26] This piece of information was used in this our study to correct total homocysteine levels. This extrapolation may be considered bias. However, this was true only for three patients (age above 60 years) two of them with chronic renal failure (i.e., two of the three above 60 years old patients), corresponding to only 15% of the patients, and 13% of the collected samples. Another possible limitation is that homocysteine is related to its metabolic pathway co-factors levels. Two of these co-factors, which could be changed during the metabolic response to septic disease, would be folates and complex B vitamins, co-factors for both homocysteine methylation (cobalamin) and cystatione (vitamin B6) production.[27-29] Considering that sepsis is a hypermetabolic condition, these complements changes could influence with homocysteine levels. Even nutritional offer of these factors could have a role. Unfortunately, the serum analyses were restricted to homocysteine.

CONCLUSION

Our results suggest no correlation between serum homocysteine levels and SOFA score or mortality. As SOFA is well accepted as a septic patients organ dysfunction marker, we conclude that it was not possible to correlate serum homocysteine levels with worsened organ systems function parameters, likely indicating that homocysteine is not involved in sepsis endothelial dysfunction pathophysiology.

RESUMO

Objetivo: Homocisteína e a sepse estão ambos associados à inflamação e ativação endotelial. O objetivo desse estudo foi verificar se o nível plasmático de homocisteína está relacionado à gravidade do quadro séptico.

Métodos: Estudo clínico, prospectivo e observacional, incluindo pacientes com sepse grave ou choque séptico com menos de 48 horas de instalação da disfunção orgânica. Os níveis de homocisteína foram determinados no dia da inclusão no estudo e nos dias 3, 7, 14. A associação entre homocisteína com o escore *Sequential Organ Failure Assessment* (*SOFA)* foi avaliada pelo teste de Sperman e com mortalidade pelo teste de Mann-Whitney. Os resultados foram considerados significativos se p<0,05.

Resultados: Foram incluídos 21 pacientes e feitas 60 coletas para avaliação da homocisteina total (mediana de 6,92 (5,27 - 9,74 µmol/l). O teste de correlação Spearman não mostrou associação entre homocisteina e SOFA (r = -0,15 e p = 0,26). Também não foi encontrada correlação da medida de homocisteína na data de admissão do estudo e a diferença do SOFA obtido no 3º dia e o SOFA da admissão (deltaSOFA) (r = 0,04 e p = 0,87). A variação da homocisteína do 3º dia e a admissão no estudo (deltaHmc) e a variação do SOFA no mesmo período não estavam correlacionadas (r = -0,11 e p = 0,66). A homocisteina da admissão não se correlacionou com mortalidade na UTI (p=0,46) ou com a mortalidade hospitalar.(p=0,13). Mesmo quando foi utilizado o deltaHmc não houve correlação (p=012 e p=0,99, respectivamente).

Conclusão: O nível basal de homocisteína ou sua variação nos primeiros dias da disfunção não estiveram relacionadas com a piora dos parâmetros funcionais dos sistemas orgânicos ou mortalidade nos pacientes sépticos.

Descritores: Homocisteina; Sepse; Disfunção orgânica; Mortalidade

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