

## References

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## P41

### Early heat shock protein 72 and 90a intracellular and extracellular responses in patients with severe sepsis or systemic inflammatory response syndrome

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**Introduction** Heat shock proteins (HSPs) have intracellular cytoprotective actions, while they act extracellularly as inducers of cytokines and stimulants for immune cells during stress. Their induction constitutes a highly conserved cellular defense mechanism against all kinds of stress. Our objective was to determine the intracellular as well as extracellular levels of HSP72 and HSP90a in patients with severe sepsis (SS) or systemic inflammatory response syndrome (SIRS) admitted to a general ICU, compared with those of healthy individuals; to correlate their expression with severity of illness.

**Methods** Eighty-two consecutively admitted patients in the ICU (35 SIRS, 47 SS) as well as 35 healthy controls (H) were finally enrolled in the study. Patients' demographic characteristics, laboratory examinations and Acute Physiology and Chronic Health Evaluation (APACHE II) score were recorded on admission. HSP levels were determined intracellularly using four-color flow cytometry. Mean fluorescence intensity (MFI) values for each HSP were measured and analyzed. Extracellular levels of HSPs were determined via ELISA.

**Results** HSP expression differed significantly between groups (Kruskal-Wallis), both intracellularly (HSP72 lower in SS,  $P < 0.001$ ), and extracellularly (higher levels of HSP90a ( $P < 0.001$ ) and HSP72 ( $P = 0.003$ ) in SS). HSP72 and HSP90a intracellular expression was inversely correlated to severity of illness, as expressed by APACHE II score (Spearman's,  $P = 0.003$  and  $P = 0.025$  respectively). Intracellular HSP72 was correlated to mortality when confounding factors were excluded from the analysis (logistic regression,  $P = 0.05$ ). Extracellular HSP90a levels correlated with prolonged PT ( $P = 0.021$ ) and INR ( $P = 0.008$ ). Finally, in the SIRS group, intracellular levels of HSP90a were higher in nonsurvivors ( $P < 0.001$ ).

**Conclusion** SS is characterized by high levels of extracellular HSPs. Intracellular HSP72 is highly expressed during the acute phase of stress in SIRS, while being downregulated in SS. HSP72 and HSP90a intracellular expression and extracellular level variations correlate with severity of illness and mortality.

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## P42

### Heat shock proteins 70/90 and associations with immunosuppression along with sepsis: preliminary data

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**Introduction** CD14/HLADR is an index of immune suppression. Heat shock proteins (hsp) regulate cell response to oxidative stress. We evaluated the relationship of CD14/HLADR and hsp70/90 in patients with SIRS and severe sepsis versus healthy volunteers.

**Methods** We evaluated 31 patients with SIRS or severe sepsis against a group of sex-matched healthy volunteers. Demographic data were obtained for all patients. APACHE score was calculated upon admission. Blood samples were collected upon diagnosis of SIRS or severe sepsis.

To evaluate the %HLA-DR expression on monocytes, the fresh whole blood was stained with anti-CD14-FITC, anti-HLA-DR-PE and CD45-PC5 while staining with anti-CD33-PE, anti-CD45-PC7, anti-hsp70-FITC and anti-hsp90-PE allowed evaluation of the MFI expression of hsp on CD33<sup>+</sup> monocytes. Cells were then analyzed using flow cytometry. ANOVA with *post hoc* tests was used to compare CD14/HLADR cell counts and hsp70 and hsp90 levels among the three groups.

**Results** Nineteen controls, six SIRS patients and 25 severe sepsis patients were studied. The percent expression of HLADR on CD14<sup>+</sup> monocytes was significantly different between the three groups showing progressive decrease from controls (mean  $90.5 \pm 3.8\%$ ) to SIRS (mean  $61.2 \pm 5.9\%$ ) to severe sepsis (mean  $39.2 \pm 5.5\%$ ) patients (controls vs. severe sepsis,  $P < 0.001$ ; controls vs. SIRS,  $P = 0.006$ ; SIRS vs. severe sepsis,  $P = 0.03$ ). hsp70 and hsp90 MFI were significantly different between controls (mean  $49.5 \pm 4.9$  and  $33.5 \pm 3.4$  respectively), SIRS (mean  $69.9 \pm 16.5$  and  $46.5 \pm 5.7$  respectively) and severe sepsis patients (mean  $33.3 \pm 4.5$  and  $21.7 \pm 2.7$  respectively) ( $P < 0.05$  for all comparisons). Notably, the hsp level rose from controls to SIRS and fell from SIRS to severe sepsis patients. APACHE score increased significantly ( $P = 0.023$ ) in septic patients compared with SIRS.

**Conclusion** There were a significant difference in CD14/HLADR, a marker of immune paralysis, between controls and patients with SIRS or severe sepsis. hsp70 and hsp90 showed an initial stimulation followed by exhaustion as sepsis progressed.

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## P43

### Prospective immune profiling in critically ill adults: before, during and after severe sepsis and septic shock

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**Introduction** Rethinking the host's defense mechanisms during severe infection has led to the use of flow cytometry (FCM) and to the current concept of sepsis-induced immunosuppression. However, organ dysfunctions that develop in the period preceding severe sepsis as a consequence of surgery, trauma or burn might also trigger immune reprogramming predisposing to overwhelming infection. Our aim was to look for correlation of specific phenotypes among four commonly encountered populations of patients and the later occurrence of severe sepsis and septic shock.

**Methods** In total, 114 non-infected patients were prospectively screened via FCM on days 1 (T1) and 3 (T2) of elective cardiac surgery, trauma, acute neurologic dysfunction and prolonged ventilation (>48 hours). A third sample was drawn when infection was diagnosed (Tx) and 7 days later (Tx + 7). Exclusion criteria included use of immunosuppressive agent(s). The broad panel of cell-specific antibodies focused on B, T lymphocytes (Tregs, Th17, NKT), NK cells, monocytes and neutrophils. Plasmatic levels of IL-2/IL-6/IL-7/TNF $\alpha$ /IFN $\gamma$  were also determined.

**Results** Ninety-nine patients were included in the final analysis. Eighteen patients developed severe sepsis or septic shock. They presented with significantly higher levels of intermediate (CD14<sup>+</sup>CD16<sup>-</sup>) and CD62L<sup>-</sup> monocytes and lower IL-2 levels at T1 compared with patients who did not get septic. ROC AUC for association of these parameters with the occurrence of sepsis were 0.78 (95% CI: 0.63 to 0.91), 0.72 (0.62 to 0.82) and 0.73 (0.65 to 0.82), respectively. High counts of these monocytic cells were also associated with increased 90-day mortality ( $P < 0.01$ , ROC AUC = 0.87 (0.77 to 0.95), 0.79 (0.66 to 0.9)). Kaplan-Meier survival curves showed significantly higher mortality after stratification based on these cell counts at T1 (CD14<sup>+</sup>CD16<sup>-</sup>: cutoff >236.8 cells/ $\mu$ l, HR = 23.6 ( $P = 1.24 \times 10^{-5}$ ); CD62L<sup>-</sup>: cutoff >95.4 cells/ $\mu$ l, HR = 6.67 ( $P = 7.6 \times 10^{-4}$ )). Multivariate logistic regression analysis using



clinical scores indicated that addition of IL-2 levels at T1 significantly improved prediction of sepsis (OR = 0.834,  $P = 0.02$ ).

**Conclusion** Predisposition to sepsis in selected critically ill medico-surgical adults can be identified on day 1 of admission based on high counts of circulating intermediate and CD62L<sup>-</sup> monocytes and low levels of IL-2 (the latter provide incremental prognostic information). High counts of these specific monocytes correlate with higher 90-day mortality.

#### P44

##### Macrophage phenotype in sepsis immunosuppression

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**Introduction** Sepsis is followed by profound, yet poorly characterized, innate immune system suppression. While low monocyte HLA-DR expression is observed in septic patients, its clinical significance has not been established [1]. *In vitro*, repeated LPS stimulation induces a tolerant or M2 macrophage phenotype, characterized by decreased cytokine production [2], which could contribute to sepsis immunosuppression. The present study examines macrophage phenotype in a mouse model and in patients with sepsis immunosuppression.

**Methods** Sepsis was induced in C57Bl6 mice by cecal ligation and puncture (CLP) followed by intratracheal instillation of *Pseudomonas aeruginosa*. Bronchoalveolar lavage fluid (BALF), cells and serum, collected 12 hours after lung infection, were analyzed for bacterial load, cytokine levels and the classical M1 marker, iNOS. Peripheral blood monocytes isolated from septic adult patients admitted to the ICU on the 1st and 7th day after admission were analyzed by flow cytometry for the expression of HLA-DR and CD86 (co-stimulatory molecule and M1 marker), and for the M2 markers, CD163 and CD206. Additional blood samples from patients and healthy volunteers were exposed *ex vivo* to LPS prior to isolation and analysis of monocyte markers.

**Results** CLP-induced sepsis resulted in immunosuppression in mice, indicated by higher BALF bacterial load after infection in CLP than in sham-operated mice, and more severe injury on histology. Serum cytokines TNF and MIP2 were greater in CLP than in sham-operated mice. Although recruitment of CD11c<sup>+</sup> alveolar macrophages post infection was threefold greater in CLP than in sham-operated mice, those macrophages expressed 40% lower levels of iNOS. Evidence of sepsis immunosuppression was present in most patients on the 7th day after ICU admission. Low expression of CD86 and/or HLA-DR was observed in 71% of patients, and increased expression of M2 markers in 15% of patients. Upon LPS stimulation the normal decrease in M2 markers was absent in all patients on day 1, and partially restored in 50% of patients on day 7.

**Conclusion** Sepsis is associated with decreased monocyte expression of M1 markers and increased expression of M2 markers in septic mice and critically ill patients. Therefore, in addition to decreased HLA-DR expression, M2 macrophage polarization appears to be a component of sepsis-induced monocyte dysfunction, and should be considered for immune monitoring and targeted intervention.

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#### P45

##### Expression of mRNA levels of HLA-DRA in relation to monocyte HLA-DR: a longitudinal sepsis study

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**Introduction** Decreased monocyte surface HLA-DR (mHLA-DR) measured by flow cytometry (FCM) is an independent marker of immunosuppression in sepsis. In a previous report we demonstrated

that septic patients display a strong correlation between mHLA-DR and mRNA-levels of HLA-DRA in whole blood [1]. mRNA-based HLA-DR monitoring by PCR would improve the clinical usage and facilitate conduction of multicentre studies. The primary focus in this study was to evaluate the correlation between mHLA-DR and HLA-DRA at different time points during sepsis. In addition, we assessed the dynamic expression of both mHLA-DR and HLA-DRA, in relation to sepsis severity.

**Methods** Study patients ( $n = 54$ ) were included at day 1 to 2 after hospital admission if blood cultures turned positive. Repeated sampling at days 1 to 2, 3, 7, 14 and 28 was performed. mHLA-DR was monitored by FCM and HLA-DRA by quantitative RT-PCR. Mixed models for longitudinal data were used after logarithmic transformation to calculate the interactional effects of time and severity on HLA-DR expression.

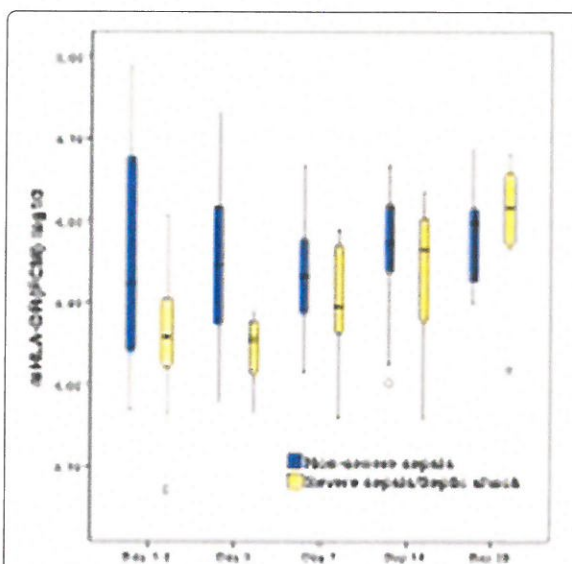


Figure 1 (abstract P45). Box plots of mHLA-DR, measured by flow cytometry.

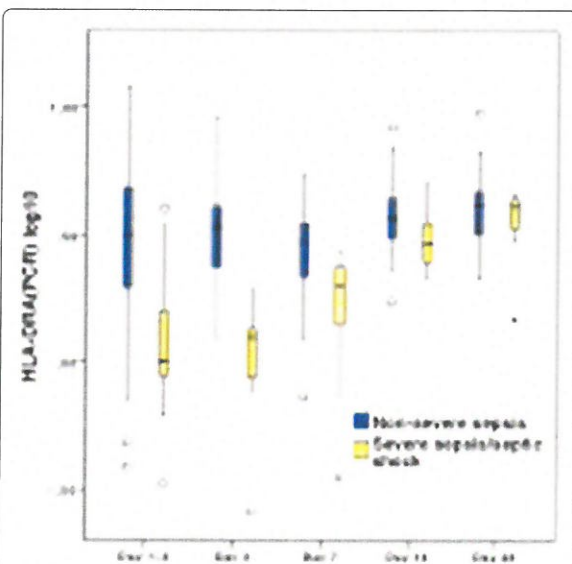


Figure 2 (abstract P45). Box plots of HLA-DRA, measured by qRT-PCR.

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# Erratum: Prospective immune profiling in critically ill adults: before, during and after severe sepsis and septic shock

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