75.037

Cytokine response in severe sepsis — Predicting and modelling the course of illness

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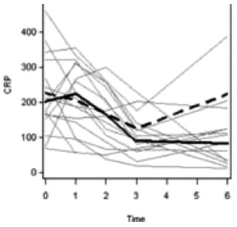
Background: Severe sepsis remains one of the most threatening conditions in intensive care. During the progression of sepsis from early hit to multiorgan failure proinflammatory and anti-inflammatory cytokines are released. Cytokines can be used as a biomarkers to determine the specific patterns of sepsis progression and association with mortality (1). These biomarkers were successfully used as predictors in animal studies (2). Data from humans, especially comparison between children and adults, are limited. Hence, in this study we widely describe systemic cytokine response in this type of patient population.1.Kellum JA et al. Arch Int Med. 2007;167(15):1655-63.3. 2.Osuchowski et al. CCM 2009;37(5):1567-73.

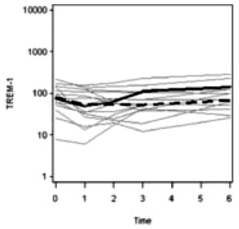
Methods: Prospective study of 37 subjects (20 children, 17 adults) hospitalized with severe sepsis in intensive care. We measured CRP, procalcitonin, TNF, IL-1beta, IL-4, IL-6, IL-8, IL- 10, IL-12, TREM-1. ANOVA models were specified using Proc Mixed. Study was fully approved by an EC.

Results: We identified a correlation of CRP levels with mortality or presence of shock. We found a distinct feature of CRP in adults with pronounced dynamic dichotomy in these subjects. Levels of IL-6 were significantly different in adult patients in the context of shock states. Highest risk of death was in adult patients associated with TREM-1 sustained high after 48 hours after sepsis onset. Otherwise, there was no correlation with death, shock states and SOFA score for PCT, TNF, IL-1beta, IL-4, IL-8, IL-10, and IL-12.



SOFA aduls: Solid line: SOFA 0-12, Dashed line: SOFA 13-24.

Conclusion: Response of circulating factors in patients with severe sepsis is heterogeneous in adults and children population and has some distinct features according to dynamics of CRP, IL-6 and TREM-1. We can find an evident discriminative feature of CRP and TREM-1 value dynamics. Sustained high levels of CRP and TREM-1 48 hours after sepsis onset were predictive of high risk of death. This finding could be related to the progression of illness to multiorgan failure. An activation of proinflammatory cytokines is associated with higher severity of sepsis and is probably related to severity of the initial hit. Supported in part by the Internal Grant Agency of the Ministry of Health NR 9297-3 and NR 9894-4.



Adults, Solid line: Exitus, Dashed line: survived.

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Circulating antigens of *Mycobacterium tuberculosis*: Standardization of immunoenzymatic assay to detect circulating antigens related in the pathology of tuberculosis

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Background: In 2006, an estimated 9.2 million new cases of tuberculosis in worldwide (WHO 2008). WHO has been set goals for global TB control including early detection of infected individuals and the prompt treatment. For this end, it has been encouraged public-private partnerships to find solutions that would drive to the development of alternative diagnostics methods. In attention to WHO recommendations immunological tests are currently being studied and standardized for the diagnosis of the active and latent disease. Based on our laboratory expertise, we have been worked to standardize immunoassays to detect *Mycobacterium tuberculosis* antigens. Objective: To standardize enzyme immunoassay to detect circulating antigens of *M. tuberculosis* in serum samples from patients with active pulmonary tuberculosis.

Methods: 6-kDa early secreted antigen target (ESAT-6), protein low molecular weight, secreted by *M. tuberculosis* and absent in BCG vaccine strain; 16 kDa, heat shock protein, important in the persistence of the bacillus, antigen 85 complex is suggested to be involved in the binding of mycobacteria to specific surface receptors of macrophages, were the antigens studied. For this, plastic plates were coated with monoclonal antibodies anti-ESAT-6, 16 kDa, Complex 85. In order to avoid false results we studied three block solutions (BSA 2%, 5% and 2% skim milk in PBS). Pool of normal human serum was spiked with different concentrations of proteins from H37Rv strain (500 μg/mL to 100 μg/mL) to determine the ELISA analytical sensitivity. The immune reaction was detected using a rabbit anti-*M. tuberculosis* - peroxidase diluted to 1/500.

Results: Better results were obtained with plates coated with monoclonal anti-ESAT-6 and blocked with 2% BSA in PBS.

Using these conditions our assay is able to detect concentrations of ESAT-6 from 0.1 to 1 μg/mL.

Conclusion: Optimal conditions of the assay are being improved to increase the sensitivity to detect antigenic fractions that may be associated to other serological markers for global evaluation of patients looking for tuberculosis laboratory evidences. The sensitivity level achieved for ESAT-6 is being evaluated in patients with tuberculosis to determine its clinical applicability for TB diagnosis.

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Variation in liver histopathology in chronic HBV-infected individuals with normal liver function tests correlates with HBV replication

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Background: Clinical and laboratory parameters may not reflect disease activity and imaging studies are insensitive indicatos of fibrosis in the precirrhotic liver. The biopsy enables the pathologist and physician to define the extent of disease by grading the necroinflammatory activity and staging the fibrosis and thus determine the progression of liver disease. The aim of this study is to characterize the liver histopathological profiles and their correlation with hepatitis B virus (HBV) replication in chronic HBV-infected individuals with normal liver function tests (LFT).

Methods: We performed a percutaneous liver biopsy in 60 chronic HBV-infected individuals with normal LFT. The biopsied tissue was processed for histological examination including grade of disease activity and stage of fibrosis. HBV markers were detected with ELISA. Serum HBV DNA load was assessed with quantitative real-time polymerase chain reaction.

Results: The histological findings from mild to moderate grade of severity and stage of progression were the most common histological findings. Twenty five cases were with grade 1 stage 0 (G1S0), 25 cases with G1S1, 8 cases with G2S1 and 2 with G2S2. The patients with serum HBV DNA positive had significant severity in the grade of disease activity and stage of fibrosis in liver tissue than those in the patients with HBV DNA negative ($P < 0.05$). In the same way, there was an significant difference between the patients with HBeAg-positive and −negative groups ($P < 0.05$). The patients with the highest serum HBV viral load had the most severe necroinflammatory activity and fibrosis than those in the patients with lower viral load ($P < 0.05$). No significant differences were observed between sex groups and age groups ($P > 0.05$, respectively).

Conclusion: The histological abnormal findings such as hepatic inflammation and fibrosis were common in chronic HBV-infected individuals with normal LFT. The severity of necroinflammatory activity and fibrosis correlates with HBV replication and viral load and HBeAg expression status.

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Short primers for amplification of diverse virus strains

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Background: Due to the high range of viral diversity no universal, highly conserved primer set exists that is able to amplify all sequenced viral targets. We have performed a computational study that predicted that approximately 3,700 18-mers would be necessary to produce amplicons across the sequenced viral database. However, by decreasing the length of the primer from the traditional 18-mer to a 10-mer the number of primers necessary significantly decreases to approximately 1,000. Shortmers show promise for acting as universal primers that can discriminate both DNA and RNA viruses at the serotype level. As a demonstration of the specificity of shortmers for viral identification we designed 10 and 11- mers that were capable of amplifying three serotypes of Blue Tongue Virus in traditional Reverse Transcription PCR (RT-PCR).

Methods: An in-house program, the Multiplex Primer Prediction (MPP) algorithm, was used to identify a primer set capable of amplifying various serotypes of the Blue Tongue Virus (BTV). The RNA of BTV strains 2, 13 and 17 was extracted in-house and selected as the template for Reverse Transcription PCR (RT-PCR) reactions. The primer set was predicted to amplify a 231 bp product from BTV 2, 13 and 17.

Results: Using RT-PCR and gel electrophoresis we visually verified the presence of ∼231 bp products from the singleplex reactions containing the shortmer primer set and individual templates BTV 2, 13 and 17. The target amplicons were then gel extracted and analyzed with using Sanger sequencing, using the forward 11-mer primer. Results indicated that the BTV2 amplicon was 95% homologous to its predicted amplicon, the BTV13 amplicon was 97% homologous for its predicted amplicon sequence and the BTV 17 amplicon was 97% homologous for its predicted amplicon. The percent homology of amplicon to predicted sequence was less than 100% due to gaps in the sequence reads.

Conclusion: We have succesfully predicted short primers that are capable of serotype-level viral detection. As a streamlined version of this analysis, we are currently adapting this assay to run on Luminex platform.

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