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Original article

The role of osteopontin in children with systemic inflammatory response syndrome and sepsis

Introduction: *Sepsis is a leading cause of morbidity and mortality in critically ill children despite the use of modern antibiotics and resuscitation therapies. Sepsis must be distinguished from non-infection systemic inflammatory response syndrome (SIRS) induced by agents such as trauma and ischemia causing extensive tissue injury to establish appropriate treatments in critically ill patients. Osteopontin acts as an extracellular matrix component or soluble cytokine in inflamed tissues. Its exact role in immune response and sepsis remains to be elucidated.* ***Objective:*** *This study investigated the level of osteopontin in SIRS and sepsis to assess its involvement in the acute inflammatory diseases and its possible role as a marker differentiating children with SIRS from those with sepsis.* ***Methods:*** *Prospective, observational study at pediatric ICU at the children's Hospital, Zagazig University, Egypt, from October 2013 to December 2014. Forty-four patients with SIRS or sepsis and 44 healthy subjects were enrolled. All the children were subjected to detailed medical history, Clinical examination, laboratory estimation for CBC, blood cultures, serum osteopontin and IL-6 determination was performed by sandwich enzyme immunoassay technique.* ***Results:*** *Serum osteopontin levels were significantly higher in patients than in controls and in sepsis than in SIRS, and decreased during the resolution of both the disorders. A receiver operating characteristic curve identified that osteopontin level of 1040 ng/ml has discriminative power between SIRS and sepsis patients with 82.6% sensitivity and 70.4% specificity, area under curve was 0.833. Osteopontin levels directly correlated interleukin-6 levels and clinical severity scores.* ***Conclusion:*** *Osteopontin is strongly up-regulated during SIRS and sepsis and correlate with IL6 and clinical severity scores.*

Key Words: Sepsis, Inflammation, Osteopontin, IL-6, Cytokines.

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INTRODUCTION

Sepsis is a leading cause of morbidity and mortality in critically ill children despite the use of modern antibiotics and resuscitation therapies.[1] Sepsis was defined as the presence of the symptoms and signs of systemic inflammatory response syndrome (SIRS) associated with infection. Sepsis must be distinguished from non-infection SIRS induced by agents such as trauma and ischemia causing extensive tissue injury to establish appropriate treatments in critically ill patients, since therapies and outcomes greatly vary in patients with and without infection.[2] Recent attention has been directed towards the study of the role of cytokines e.g. tumor necrosis factor- alpha (TNF-α), interleukin-1β (IL-1β), and interleukin -6 (IL-6) in the regulation of inflammation and host responses to infection.[3] Osteopontin is a phosphoprotein with adhesive and cell signaling function; it can act either as an extracellular matrix component in mineralized tissue or as a soluble cytokine in inflamed tissue and serum.[4] During inflammation, osteopontin is expressed by macrophages and T lymphocytes.[5] It acts as a pro-inflammatory cytokine by chemoattracting monocyte, macrophage and lymphocyte. Also it modulates T cell function by affecting the differentiation of T lymphocytes into Th1 and Th 2 cells, regulating the balance between Th1 and Th 2 and participating in the cell induced immunological response. At the same time, osteopontin stimulates B lymphocytes to express multi-clone antibodies. [6] Moreover it plays a role in the protection against herpes virus and bacterial infections through the activation of the Th1 response and induction of Th1 cell-mediated immunity.[7] Interleukin -6 (IL-6) is produced mainly by T cells, macrophages and endothelial cells. IL-6 induces the synthesis of acute phase proteins in the liver and stimulates the production of neutrophils. It

also stimulates growth and proliferation of B lymphocytes.[8]

This study investigated the level of osteopontin in SIRS and sepsis to assess its involvement in the acute inflammatory diseases and its possible role as a marker differentiating children with SIRS from those with sepsis.

METHODS

Study population

This prospective observational study was performed on 88 children, 44 children were patients group with SIRS or sepsis (31 males and 13 females). Their ages ranged between 3 month and 10 years of age. These patients were admitted to the pediatric ICU at the Children's Hospital, Zagazig University, Egypt, from October 2013 to December 2014.The study was approved by the Institutional Ethical Committee of Zagazig University; written informed consent was obtained from the parents of the patients involved in the study as recommended by the ethics committee and in accordance with the Helsinki declaration.

Patients were enrolled when they met the criteria of SIRS or severe sepsis and septic shock (SS/SS).[2] Diagnosis of SIRS was made based on the presence of two or more of the following criteria (1) temperature (core or rectal) $>38.5^0$C or $<35.5^0$C. (2) tachypnea (respiratory rate >95 percentile for age breaths/min or $PaCO_2$ <32 mmHg) (3) tachycardia (heart rate >95 percentile for age beats/min) (4) white blood cells count >12,000 or <4,000cells/mm^3 or the presence of more than 10% immature neutrophils (band cells). Patients had sepsis when they developed SIRS as a result of infection which was proved clinically and by blood culture.[2] Septic patients with organ dysfunction were considered to have severe sepsis and those with persisting hypotension, requiring vasopressor treatment were considered to have septic shock.[9] Pediatric risk of mortality (PRISM) scores were calculated for all patients at admission and the Sequential Organ Failure Assessment(SOFA) scores were conducted by physicians blinded to the experimental results to assess the clinical severity and progression of the cases.[10,11]

The two groups consisted of 21 patients with SIRS and 23 with SS/SS. We also studied; (1) 44 healthy children (29 males and 15 females) defined as controls, matched for gender and age. (2) 17 patients in resolution from SIRS (10 patients) and SS/SS (7 patients).The resolution group comprised patients who no longer met the inclusion criteria of SIRS.

Children were excluded if they had chronic systemic inflammatory disease, primary or acquired immunodeficiency diseases, were on corticosteroids or nonsteroidal anti-inflammatory drugs.

Sample collection

All blood samples were obtained within 6 hours of meeting the criteria of SIRS.[2] For patients analyzed during the resolution phase, blood withdrawal was performed on the first day in which they lost the inclusion criteria.

All patients were subjected to the following laboratory investigations; complete blood pictures (CBC) were performed on Sysmex-Kx-21 (Sysmex corporation–Japan), C-reactive protein (CRP), liver function tests were done on Cobas 6000 (Roche diagnostics-Switzerland) and blood cultures were done on Bact Alert analyser and organism identification were done by the Vitek MS system (bioMe´rieux).

Osteopontin and Interleukin -6 were measured using a sandwich enzyme immunoassay technique, Osteopontin (ELISA kit) provided by (Glory Science Co., Ltd, USA), IL-6 assayed by Assay Max Human Interleukin-6 ELISA Kit (catalog Number EI1006-1). Blood samples were collected from all subjects into vacutanier tubes and centrifuged at 2500 g for 15 minutes then the sera were stored at -80^OC until assay according to manufacturer's instructions. The lowest sensitivity limits for the detection was 2 ng/ml for osteopontin and 10 pg/ml for IL-6.

Statistical methods

Mann–Whitney U-test and Wilcoxon's signed rank were used to analyze the unpaired and paired data respectively. Comparison between groups was performed using Kruskal–Wallis test. Correlations were tested with *Spearman's coefficient*. A p value <0.05 was considered statistically significant. Data were analyzed by sensitivity (percent of positives detected correctly identified) and specificity (percent of negatives detected correctly identified) and area under the curve (AUC) derived from the receiver operating characteristic curve (ROC).[12] Statistical analysis was performed SPSS (Statistical package for social sciences) version 19.

RESULTS

We studied 21 patients with SIRS and 23 with SS/SS the pediatric ICU at the Children's Hospital, Zagazig University, Egypt, and 44 healthy matched for gender and age, as controls. Moreover 17 patients were also analyzed on the first day in which they lost the inclusion for SIRS. Baseline

clinical features of the patients are summarized in Table 1. Clinical severity was evaluated using Pediatric risk of mortality (PRISM) scores and the Sequential Organ Failure Assessment(SOFA) scores .Both scores were significantly different in SS/SS and SIRS patients($p< 0.001$).

Regarding microbiological finding, the primary focus of infection in the patients with SS/SS was the lung in 12 (52.2%), central nervous system in 5 (21.7%), intestine in 4 (17.4%) and blood in 2 (8.7%). It was possible to isolate microorganisms in the blood of 23 patients with SS/SS. In 14 (60.9%) patients, gram-negative organisms were identified, in 7 (30.4%) cases there were gram-positive and in another two (8.7%) patients the infection was polymicrobial.

Serum osteopontin levels were strikingly higher in patients than in the controls. Moreover, they were significantly higher in SS/SS than in SIRS) and seems to be associated with the clinical outcome as they were significantly higher in non-survivors than in survivors as revealed in Table 2 and displayed a direct correlation with clinical severity scores (PRISM: $r = 0.684$, $p< 0.001$; SOFA: $r =0.677$, $p < 0.001$). Furthermore, during the resolution phase osteopontin levels markedly decreased (median 420 ng/ml) compared to the acute phase of the disease (median 1004 ng/ml, $p<0.001$), but still threefold higher than controls (Fig.1).

IL6 levels were higher in patients than in the controls .Also IL6 levels were significantly higher in SS/SS than in SIRS. IL-6 levels were higher in non-survivors than in survivors, but without statistical significance as revealed in Table 2. Osteopontin levels displayed a significant direct correlation with IL-6 ($r =0.506$, $p< 0.001$) as seen Fig.2. IL-6 levels decreased during the resolution phase (median 38 pg/ml) compared to the acute phase of the disease (median 70 pg/ml, $p<0.001$), but still higher than controls (Fig.1).

In the view of the significant difference of osteopontin and IL-6 levels in patients with severe sepsis/septic shock (SS/SS) and those with SIRS, we use the receiver operator characteristics curve (ROC) to explore their ability to differentiate SS/SS and SIRS patients. The area under the curve (AUC) was 0.833 (95% CI 0.713-0.954, $P < 0.001$) for osteopontin and 0.717 for IL-6 (95% CI 0.567-0.868, $P = 0.014$). For osteopontin, the optimal cut-off value discriminating between SS/SS and SIRS was 1044 ng/ml and gave a sensitivity of 82.6% and specificity of 70.4% with a positive likelihood ratio of 2.88 and for IL-6 optimal at cut- off value was 87 pg/ml and gave a sensitivity of 73.9% and specificity of 66.7% with a positive likelihood ratio of 2.21(Fig 3).

Table 1. Demographics and clinical features by group

	SIRS (n=21)	**Severe sepsis (n=23)**	**Resolution (n=17)**	**controls**
Age in months Mean±SD	26.2±16	24.6±14.9	27.3±15.33	25±14.3
M/F[a]	15/6	16/7	12/5	29/15
PRISM[b]	11(6-16) [c]	22(18-30)	8(4-12)	-
SOFA[b]	5(3-8)[c]	12(10-16)	4(3-7)	-
ICU mortalit[y]	3(14.2%)	7(34.7%)	0	-

Results are shown as absolute numbers, proportions are in brackets[a]
Results are shown as median, interquartile ranges are shown in brackets[b]
Statistically different from severe sepsis/septic shock [c]

Table 2. Levels of osteopontin and interleukin -6

	osteopontin	Z	P	IL6	Z	P
Controls (n=44)	147 (102-194)	-8.08	<0.0001 (HS)	16 (10-22)	-8.07	<0.0001 (HS)
All patients (n=44)	1140 (350-2260)			88 (30-206)		
SS/SS (n=23)	1380 (960-2260)	-2.3	<0.001 (HS)	103 (70-206)	-3.04	<0.05 (S)
SIRS (n=21)	870 (350-1148)			70 (30-100)		
Non-survivors (n=11)	1940 (1208-2260)	-3.1	p <0.01 (S)	120 (76-206)	-1.04	0.07 (NS)
Survivors (n=33)	1080 (890-1240)			95 (30-166)		

P value was determined by Mann–Whitney U-test.

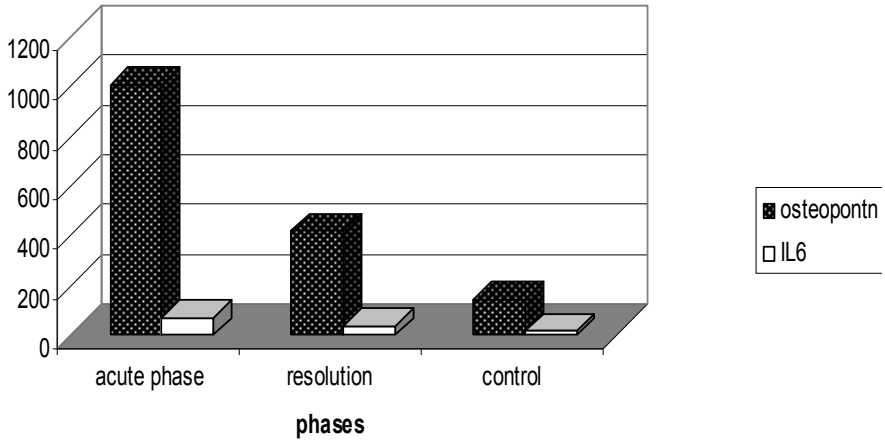

Figure 1. Serum osteopontin and interleukin-6 in acute phase of the disease and in resolution phase, p<0.001, Wilcoxon signed rank test and resolution phase levels still higher than the controls.

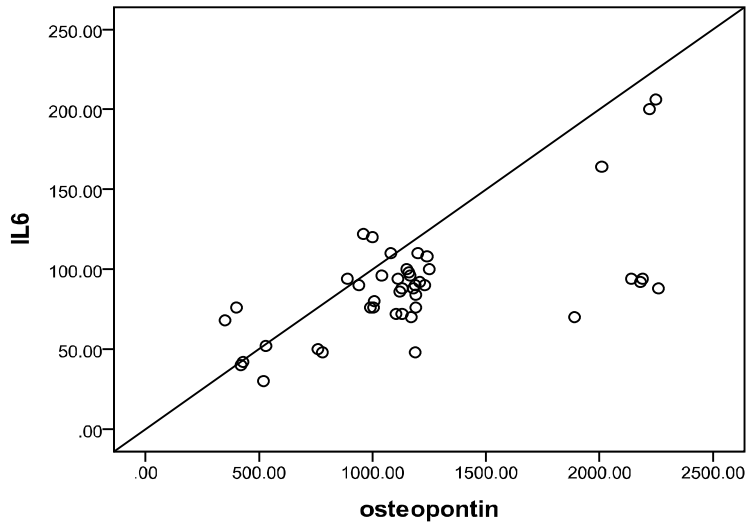

Figure 2. Significant direct correlation between serum osteopontin and interleukin-6 in patients with SIRS and severe sepsis / septic shock (r =0.506, p< 0.001)

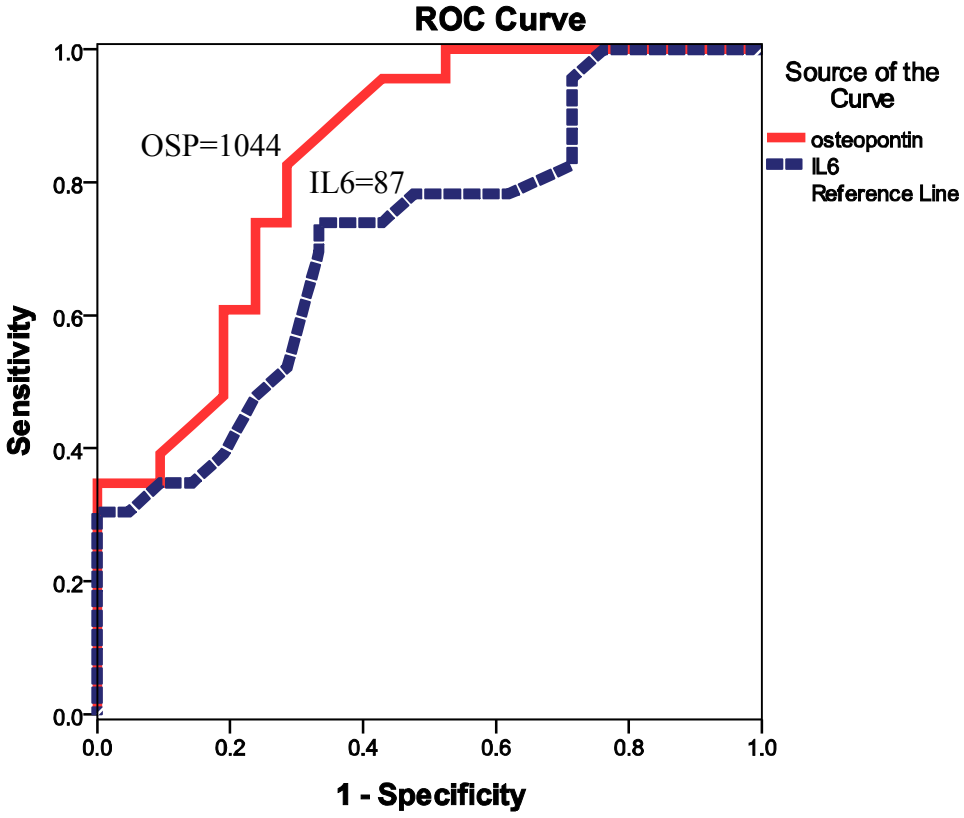

Figure 3. Receiver operator characteristic curve (ROC) evaluating the accuracy of osteopontin and IL-6 to differentiate SS/SS and SIRS patients. A ROC curve identified that a osteopontin level (cut-off value) 1040 ng/ml has discriminative power between SIRS and sepsis patients with 82.6% sensitivity and 70.4% specificity, area under curve was 0.833 (95% CI 0.713 -0.954, P < 0.001) and while IL6 level (cut-off value) 87 pg/ml has discriminative power with sensitivity of 73.9% and specificity of 66.7%, area under curve was 0.717 for IL6 (95% CI 0.567 -0.868, P = 0.014).

DISCUSSION

Sepsis biomarkers can indicate the presence or absence or severity of sepsis and can differentiate bacterial from viral or fungal infection and systemic sepsis from local infection. Other potential uses of biomarkers include roles in prognosis, guiding antibiotic therapy, evaluating the response to therapy and recovery from sepsis, differentiating Gram-positive from Gram-negative microorganisms as the cause of sepsis, predicting sepsis complications and the development of organ dysfunction (heart, kidneys, liver or multiple organ dysfunction).[3] Osteopontin is implicated in several events, including cell mediated immunity, cell survival and tumor progression. Its vital role in both acute and chronic inflammation is now obvious.[5,6] Its role in inflammatory process through interactions with several integrins, also it mediates cell migration, adhesion and survival in many cell types. Osteopontin encourage Th1 cell-mediated immunity, promotes cell-mediated immune responses.[13,14]

Because of the better sensitivity and specificity, procalcitonin, CRP and IL-6 are commonly used as biomarkers in the diagnosis, while assessing the severity of infection and guiding the use of antibiotics. But meta-analysis found that the sensitivity and specificity procalcitonin and CRP varied in the diagnosis of sepsis.

The sensitivity of IL-6 was not high and declined gradually as the time of infection extended. These findings questioned their ability to distinguish sepsis from SIRS.[15]

Many studies have reported increased osteopontin levels in chronic inflammatory disease such as systemic lupus erythematosus[16] and Crohn's disease.[17] Fewer studies focus on acute inflammatory diseases in pediatric. In all these inflammatory diseases, osteopontin levels seem to be a good marker of the host response, since they increased with the severity of the disease. In this study, we found that serum osteopontin levels were markedly higher in patients with SIRS or SS/SS than in controls.they were also higher in SS/SS than

in SIRS and decreased with the resolution of both conditions.

Osteopontin levels could be used to discriminate between SIRS and sepsis as most septic patients (15/23, 65.2%) displayed higher osteopontin levels than the 75th percentile limits displayed by patients with SIRS (1148 ng/ml).Also the diagnostic accuracy of osteopontin in discriminating SIRS from sepsis, as evaluated from the ROC curve ,was near to that reported in recent meta-analysis for procalcitonin[18,19],i.e the main serum protein markers currently used to monitor systemic inflammation.

IL6 is believed to play a key role in sepsis asIL-6 enhances B cell differentiation into mature plasma cells and secretion of immunoglobulins and shares several activities with Interleukin-1 (IL-1), including production of acute phase proteins (APPs) and induction of pyrexia. IL-6 is the most important inducer of hepatocyte synthesis of APPs). IL-6 levels are significantly elevated during sepsis, and this precedes the appearance of other cytokines. IL-6 has a short half-life, and its sensitivity decreases after 12 to 24 hours. IL-6 as a marker in the early phases of infection has been evaluated in many studies.[20,21]

In this study, we found that serum IL6 levels were markedly higher in patients with SIRS or SS/SS than in controls.they were also higher in SS/SS than in SIRS, and decreased with the resolution of both conditions. However it seem no better than procalcitonin in the diagnosis of sepsis as it displays lower sensitivity and specificity.[19]

The direct correlation between the serum osteopontin and IL-6 levels in SIRS and SS/SS suggest that these cytokines may be functionally related, as their secretion may be coordinated by the responsiveness to the same stimuli, especially since the osteopontin and IL-6 genes share responsiveness to the transcription factor NF-IL6 involved in the acute phase response and macrophage activation.[20] However a study done on adult patients by Vaschetto et al.,[22] suggest that osteopontin may also directly promote IL-6 secretion, since recombinant osteopontin (rOPN) induced mRNA expression and stimulated IL6 secretion by monocytes.

Our study reveal that osteopontin display more sensitivity and specificity than IL6 in the diagnosis of sepsis (82.6% and 70.4% for osteopontin at S value (1044 ng/ml) and 73.9% and 66.7%) for IL6 at s value (87 pg/ml). This result are in agreement with Vaschetto et al.,[22] who found osteopontin has sensitivity and specificity (70% and 79% for osteopontin but at a higher s value (1708 ng/ml) more than IL-6 in the diagnosis of sepsis in adult patients. So osteopontin levels could supplement the data given by IL-6 in the diagnosis of sepsis. The data also showed that osteopontin but not IL6 levels displayed a direct correlation with clinical severity scores (PRISM and SOFA) and seems to be more associated with the clinical outcome of the disease.

In conclusion, serum osteopontin levels are higher in SIRS and SS/SS than controls and the high osteopontin levels could be discriminate between SIRS and SS/SS. Osteopontin levels correlate with IL-6 levels and clinical severity scores (PRISM and SOFA).

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