Adipocytokine Profile Reveals a Significant Cytokine Network in the Acute Phase of Sepsis

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Abstract

Background: The cytokines compose a network and play a crucial role in the pathogenesis and prognosis of sepsis. Adipose tissue is noted as an important immune endocrine organ that releases adipocytokines. The aim of this study was to evaluate the adipocytokines in sepsis from a network perspective.

Methods: This retrospective study comprising 37 patients with sepsis and 12 healthy controls was conducted from February 2014 to July 2015. Blood samples (maximum of 7 samples per patient, 185 samples in total) were collected from the patients on days 1 (within 24 hours of the diagnosis), 2, 4, 6, 8, 11 and 15 and from the healthy controls. Adipocytokines (adiponectin, leptin, resistin, chemerin, visfatin, vaspin, CXCL-12/SDF-1, angiotensinogen), inflammatory cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12/IL-23p40, TNF-α, MCP-1), and PAI-1 were measured. The APACHE II score was evaluated on day 1, and the SOFA score and JAAM and ISTH overt disseminated intravascular coagulation (DIC) scores were assessed at the same time points at which blood was sampled.

Results: Hierarchical clustering analysis showed that the cluster formed by resistin, IL-6, IL-8, MCP-1, and IL-10 on days 1, 2 and 4 represented the cytokine network throughout the acute phase of sepsis. There was a significant association of each cytokine in this network with SOFA and JAAM DIC scores over the acute phase. In a Cox proportional hazards model focusing on the acute phase, each of these cytokines showed a significant relation with patient prognosis.

Conclusions: Adipocytokines and an inflammatory cytokine profile were assessed over time in patients with sepsis. We found that resistin was involved in an inflammatory cytokine network including IL-6, IL-8, IL-10 and MCP-1 in the acute phase of sepsis, and this network was associated with the severity and prognosis of sepsis.

Background

Sepsis is life-threatening acute inflammatory disease that remains the major cause of intensive care unit (ICU) deaths. Around the world, as many as one in four (and often more) deaths are due to sepsis every year [1]. In sepsis, damage-associated molecular patterns and pathogen-associated molecular patterns bind to pattern recognition receptors on immune cells such as monocytes, macrophages and dendritic cells. The activated immune cells produce both pro-inflammatory and anti-inflammatory cytokines. These cytokines regulate acute inflammatory responses, including immune, coagulation and neuroendocrine responses. The pro-inflammatory cytokines play an important role in inducing inflammation, and excessive amounts of these cytokines can lead to vascular endothelial damage, resulting in systemic inflammatory response syndrome. Uncontrolled inflammation in severe cases of sepsis causes disseminated intravascular coagulation (DIC) and multiple organ dysfunction syndrome, which eventually leads to death [2].

The term “cytokine” is a generic term for low-molecular-weight polypeptide or glycoproteins produced by immune cells. Interleukin (IL) and interferons are included in a narrow sense, and chemokines and the

tumor necrosis factor (TNF) family are included in a broader sense. Cytokine bind to multiple target cells and induce diverse effects such as inflammatory responses and cell differentiations [3, 4].

Adipose tissue is considered an energy storage organ. Recent novel studies on obesity and its secondary disease, metabolic syndrome, have shown adipose tissue to be an important endocrine organ in immunity that releases large numbers of bioactive mediators [5, 6]. Adipocytokines are defined as signaling proteinaceous mediators mainly produced or released by adipose tissue. More than 50 adipocytokines have been reported, and the number has been rising in recent years [7]. These adipocytokines include adiponectin and leptin, which are produced specifically by adipocytes, and resistin and visfatin, which are produced mainly by macrophages in adipose tissue. As the most abundant protein produced by adipose tissue, adipocytokines have anti-inflammatory properties and improve insulins sensitivity [8].

Adipocytokine levels change in sepsis, and several adipocytokines are considered useful biomarkers of severity and prognosis [9, 10]. Although previous reports have assessed individual adipocytokines at a few time points, a comprehensive adipocytokine profile and the cytokine network has not been assessed. We previously reported that cytokines (both pro- and anti-inflammatory) composed a network and were efficacious in elucidating the pathogenesis and prognosis in patients with sepsis [4, 11, 12]. Focusing on cytokine networks could help in accurately understanding the complicated pathology of inflammation.

Thus, the aim of this study was to evaluate 8 adipocytokines—adiponectin, leptin, resistin, chemerin, visfatin, vaspin, CSCL12/SDF1 and angiotensinogen—and classical cytokines at multiple time points and to assess the role of adipocytokines in sepsis from a network perspective.

Patients And Methods

Patients

This single-center, retrospective study was conducted at the Department of Traumatology and Acute Critical Care Medicine, Osaka University Graduate School of Medicine from February 2014 to July 2015. The inclusion criteria were patients aged > 18 years and sepsis as defined by the Sepsis-3 criteria [13]. Patients who were in cardiopulmonary arrest on admission were excluded. Healthy people with no previous history of chronic diseases were recruited as controls via public poster advertisements.

Blood samples

Blood sample were collected from the patients until discharged from ICU or death on days 1, 2, 6, 8, 11 and 15 (maximum of 6 time points per patients) and once from the healthy controls. Blood samples were stored at -30 °C until analyzed.

Analysis of 8 cytokines

Serum levels of IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12/IL-23p40, TNF-α and monocyte chemotactic protein 1 (MCP-1) were measured using cytometric bead array kits (BD Biosciences) with a FACS Canto II flow cytometer (BD Biosciences). The detectable range of each cytokine was 9.77 to 3000 pg/ml.

Analysis of 8 adipocytokines and endothelial damage marker

Serum adipocytokines (adiponectin, leptin, resistin, chemerin, visfatin, vaspin, C-X-C motif chemokine-12/stromal cell-derived factor-1 [CXCL-12/SDF-1] and angiotensinogen) were measured with an enzyme-linked immunosorbent assay kit (R&D Systems). To evaluate endothelial damage, plasma concentrations of plasminogen activator inhibitor-1 (PAI-1) were measured. Frozen samples were thawed, and subsequent measurement processes were conducted according to the manufacturer's protocol. Absorbance was analyzed using a microplate reader (SH-9000Lab; Corona Electric Co., Ltd). The minimum detectable level was less than 0.014 ng/ml for PAI-1, 0.032 ng/ml for chemerin, leptin, resistin and CXCL-12/SDF-1, 0.064 ng/ml for adiponectin and vaspin, 0.094 ng/ml for angiotensinogen and 0.500 ng/ml for visfatin.

Clinical severities and outcome

The Acute Physiology and Chronic Health Evaluation (APACHE) II score was evaluated at enrollment of the patients with sepsis. The APACHE II score is widely used to evaluate the severity of critically ill patients based on 12 physiological variables and to predict outcome [14]. The Sequential Organ Failure Assessment (SOFA) score was evaluated at the same time points as blood sampling. The SOFA score consists of six variables related to the cardiovascular, respiratory, hepatic, renal, neurologic and coagulation systems. This score is useful in assessing organ dysfunction and prognosis [15].

The severity of disseminated intravascular coagulation (DIC) was also assessed at the same time points as blood sampling according to two definitions of DIC: the Japanese Association for Acute Medicine (JAAM) DIC score [16] and the International Society of Thrombosis and Haemostasis (ISTH) overt DIC score [17]. The JAAM score is sensitive for detecting septic DIC, whereas the ISTH overt DIC score is specific for diagnosing DIC [18]. Both DIC scores correlate with each other. Patient outcomes were assessed within 28 days after admission. Sepsis was divided into two phases based on a previous report: the acute phase (days 1–4) and the later phase (days 5–14) [19].

Statistical analysis

The 8 inflammatory or anti-inflammatory cytokines, PAI-1 and the 8 adipocytokines were transformed to common logarithm values to normalize data distribution before analyses. The Dunnett test was used to evaluate the difference of each value between the patients and healthy controls. The patients were divided into two groups during the acute phase (days 1, 2, 4): septic shock or non-shock on admission and 28-day survivor or non-survivor. Wilcoxon rank tests were used to evaluate the differences between patients with and without shock in the acute phase and between 28-day survivors and non-survivors. The Dunnett test was also used to evaluate differences between septic patients, non-shock patients and the

healthy controls and between the 28-day survivors, non-survivors and healthy controls. Correlations between the 8 pro-inflammatory or anti-inflammatory cytokines, PAI-1 and the 8 adipocytokines were evaluated by hierarchical clustering analysis based on Spearman correlation coefficients.

Network analysis was performed with Cytoscape® software (www.cytoscape.org) version 3.8.0 [20]. The mediators showing significant differences between two groups (healthy controls vs. patients/septic shock vs. non-shock/28-day survivors vs. non-survivors) were included in the network analysis. The network was visualized based on significant Spearman correlation coefficients among pro-inflammatory or anti- inflammatory cytokines, PAI-1 and adipocytokines. Two types of combined scores were defined: daily and common scores. Daily combined score was calculated with all of the cytokines composing the network (day 1: IL-1β, IL-6, IL-8, IL-10, MCP-1, leptin, chemerin, adiponectin and resistin; day 2: IL-6, IL-8, IL-10, MCP-1, adiponectin and resistin; day 4: IL-6, IL-8, IL-10, MCP-1, resistin and visfatin). Common combined score was calculated with IL-6, IL-8, IL-10, MCP-1 and resistin, which composed the network throughout day 1 to day 4. To calculate the combined scores, patients were divided into two groups based on the 75th and 25th percentile of each mediator's level. The patients with mediator levels showing a decreased compared to healthy controls (i.e., day 1: leptin, chemerin and adiponectin; day 2: adiponectin) and below the 25th percentile were assigned the value "1", and those with mediator levels above the 75th percentile were assigned the value "0". The patients with mediator levels showing an increase compared to controls (i.e., day 1: IL-1β, IL-6, IL-8, IL-10, MCP-1, and resistin; day 2: IL-6, IL-8, IL-10, MCP-1 and resistin; day 4: IL-6, IL-8, IL-10, MCP-1, resistin and visfatin) and above the 75th percentile were assigned the value "1", and those with mediator levels below the 25th percentile were assigned the value "0". The combined scores were calculated by adding the values of each mediator (e.g., the daily combined score for day 1 contains total values of 9 mediators: IL-1β, IL-6, IL-8, IL-10, MCP-1, leptin, chemerin, adiponectin and resistin. Minimum and maximum values of each mediator were 0 and 9, respectively). The contribution rate (CR) of each mediator and the combined scores to SOFA or DIC scores were evaluated on the basis of Spearman correlation coefficients and are shown by radial network diagrams (Circos® software version 0.69.9 [21]; http://circos.ca/).

Cox proportional hazards analysis with time-dependent covariates was performed for the 5 pro-inflammatory or anti-inflammatory cytokines, PAI-1 and the 5 adipocytokines composing the network in the acute phase, and for daily and common combined scores to assess the association of each value with 28-day mortality. The hazard ratio is provided as Q1 to Q3 to allow comparison of the strength of the association between the cytokines. To investigate new predictive factors of sepsis, receiver operating characteristic (ROC) analysis was performed.

Statistical analyses were conducted with JMP Pro 14.0 (SAS Institute Inc., Cary, NC, USA) and R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). The level of statistical significance was set to $p < 0.05$.

Results

Patient characteristics

We enrolled 37 patients with sepsis (10 women, 27 men) and 12 healthy controls in this study (Table 1). In total, 185 blood samples from the patients and 12 blood samples from healthy controls were analyzed. At enrollment, 24 patients were in septic shock as defined by Sepsis 3 criteria [13]. The mortality rate in the patients was 21.6%. The median APACHE II and SOFA scores were 20.0 (12.5–28.0) and 8.0 (5.0-11.5), and the median ISTH and JAAM DIC scores were 3.0 (2.0–4.0) and 5.0 (2.0–5.0), respectively. The sites of infection and comorbidities are shown in Table 1. The sepsis patients were older than the controls, but the difference in sex was not significant.

Changes in adipocytokines, cytokines, PAI-1 and SOFA/DIC scores

Adiponectin levels were significantly decreased compared with those of the control on day 2 and gradually rose thereafter. Compared with those of the controls, serum leptin levels of the patients were significantly lower on days 1, 2 and 4, and visfatin levels were increased on day 2. There were no significant differences between patients and controls for the other adipocytokines. Compared with those of the controls, serum levels of IL-8 of the patients were significantly increased throughout the study period, serum levels of IL-1β and MCP-1 were higher on day 1, and serum levels of IL-6 (days 1, 2, 4, 6, 8), IL-10 (days 1, 2) and PAI-1 (days 1, 2) were significantly increased. There were no significant differences in the other inflammatory cytokines. SOFA and JAAM and ISTH DIC scores showed similar trends (Fig. 1).

Adipocytokine, cytokine and PAI-1 levels in septic shock vs. non-shock and 28-day survivors vs. non-survivors

The 8 adipocytokines, 8 pro-inflammatory or anti-inflammatory cytokines and PAI-1 analyzed in Fig. 1 were also evaluated for differences between control, septic shock patients and non-shock patients in Fig. 2A, and between control, 28-day survivors and non-survivors during the acute phase of sepsis in Fig. 2B. Serum adiponectin levels were significantly lower in septic patients without shock than those with shock on days 1, 2 and 4. There were significant differences in adiponectin levels between control and non-shock patients on each day. Resistin levels in the patients with shock (days 1, 2) and in the non-survivors (days 1, 2, 4) increased significantly compared with those in the patients without shock, the survivors or the controls. The levels of chemerin in the patients with shock on day 2 were significantly lower than those in the patients without shock. Serum visfatin levels in the patients with or without shock significantly increased compared to control on day 2 as did those in the survivors (days 1, 2) and non-survivor (days 2, 4), but the levels of the non-survivors on day 4 were significantly higher than those of the survivors. Serum leptin levels showed significant decreases in the patients with shock (days 2, 4) or without shock (days 1, 2, 4) in comparison with the control and in the survivors (days 1, 2, 4) and non-survivor (days 1, 4). The levels of IL-1β (day 1), IL-6 (day 1), IL-8 (days 1, 4), IL-10 (day 1), MCP-1 (day 1) and PAI-1 (day 1) in the patients with shock were significantly increased compared to those in the patients without shock. IL-6 (days 1, 2, 4), IL-8 (days 1, 2), IL-10 (day 1) and PAI-1 (day 1) in the patients

with shock showed significant increases compared to the control as did IL-1β (day 1), IL-6 and IL-8 (days 1, 2, 4), IL-10 (days 1, 2), MCP-1 (day 1) and PAI-1 (days 1, 2) in the patients without shock.

The levels of IL-1β (day 1), IL-6 (days 1, 2, 4), IL-8 (days 1, 2, 4), IL-10 (days 1, 2, 4), MCP-1 (days 1, 2) and PAI-1 (days 1, 2, 4) in the non-survivors increased significantly compared with those of the 28-day survivors. IL-6 (days 1, 2, 4), IL-8 (days 1, 2, 4), IL-10 (days 1, 2, 4) and PAI-1 (days 1, 2) in the survivors were significantly increased compared to the control as were IL-1β (day 1), IL-6, IL-8 and IL-10 (days 1, 2, 4) and MCP-1 and PAI-1 (days 1, 2) in the non-survivors (Fig. 2). There were no significant differences in serum angiotensinogen, CXCL-12/SDF-1 and vaspin levels between the patients with or without shock or the survivors and non-survivors (Supplemental Fig. S1).

Hierarchical clustering and network analyses of adipocytokines, cytokines and PAI-1

Hierarchical clustering analysis based on Spearman's correlation coefficients between the 8 adipocytokines, 8 cytokines and PAI-1 was performed during the study period. Different main clusters were seen on days 1 (PAI-1, resistin, IL-1β, IL-6, IL-8, MCP-1, IL-10), 2 (PAI-1, resistin, IL-1β, TNF-α, IL-6, IL-8, MCP-1, IL-10) and 4 (PAI-1, resistin, IL-10, TNF-α, IL-6, IL-8) (Fig. 3A).

Network visualization showed that 4 pro-inflammatory cytokines (IL-1β, IL-6, IL-8, MCP-1), 1 anti-inflammatory cytokine (IL-10), PAI-1 and 4 adipocytokines (adiponectin, leptin, resistin, chemerin) were connected on day 1. Three pro-inflammatory cytokines (IL-6, IL-8, MCP-1), 1 anti-inflammatory cytokine (IL-10), PAI-1 and 2 adipocytokines (adiponectin, resistin) were connected on day 2, and 3 pro-inflammatory cytokines (IL-6, IL-8, MCP-1), 1 anti-inflammatory cytokine (IL-10), PAI-1 and 2 adipocytokines (visfatin, resistin) were connected on day 4. The common network composed of IL-6, IL-8, IL-10, MCP-1, PAI-1 and resistin was seen over the entire acute phase (Fig. 3B).

Spearman's correlations between pro- or anti-inflammatory cytokines, PAI-1, adipocytokines and combined scores and SOFA/DIC scores

The radial network diagrams allow visualization of the CR of correlation coefficients to SOFA and ISTH DIC scores with the 8 inflammatory or anti-inflammatory cytokines, PAI-1, 8 adipocytokines, and daily and common combined scores. The CRs ranged from 0–11%. The ratios of $CR < 4$, $4 \leqq CR < 8$ and $8 \leqq CR < 12$ were defined as low, moderate and high, respectively. High CRs to SOFA scores were seen on day 1 (IL-8, IL-10, PAI-1, chemerin, resistin and daily combined scores), day2 (IL-6, IL-8, IL-10, PAI-1, resistin, daily and combined scores) and day 4 (IL-6, IL-8, IL-10, resistin, daily and common combined scores). High CRs to ISTH DIC scores were seen on day 1 (IL-8, PAI-1, resistin, daily and common combined scores), day 2 (daily score) and day 4 (IL-6, IL-8, IL-10, resistin, daily and common combined scores). IL-8, IL-10, resistin and daily combined scores had high CRs to correlations with SOFA scores from day 1 through day 4. High CRs of correlations between ISTH DIC score and daily combined scores were seen over the acute phase.

Cox proportional hazards analysis with time-dependent covariates for survival

Cox proportional hazards analysis with time-dependent covariates for survival were conducted to assess the relation of L-1β, IL-6, IL-8, IL-10, MCP-1, PAI-1, adiponectin, leptin, resistin, chemerin and visfatin, which composed the networks in the acute phase, and combined scores with prognosis. IL-6, IL-8, IL-10, MCP-1, PAI-1, resistin, visfatin, daily combined score and common combined score showed significant correlations with patient prognosis (Table 2).

ROC analysis of 5 adipocytokines, combined scores and SOFA scores

To explore potential biomarkers of prognosis, ROC analysis was conducted with 5 adipocytokines composing the network in the acute phase (adiponectin, leptin, resistin, chemerin, visfatin), combined score and SOFA score on day 1. Evaluation with the SOFA score is essential in the diagnosis of sepsis [13] and is an important prognostic marker in clinical practice. The area under the ROC curve (AUC) was analyzed for each adipocytokine and the combined scores. The AUCs of adiponectin, leptin, resistin, chemerin and visfatin on day 1 were 0.656, 0.594, 0.777, 0.607 and 0.665, respectively. The AUCs of the daily combined score on day 1 (IL-1β, IL-6, IL-8, IL-10, MCP-1, PAI-1, adiponectin, leptin, resistin, chemerin) and common combined score were 0.926 and 0.940, respectively. That of SOFA on day 1 was 0.665. (Fig. 4)

Discussion

This is the first study, to our knowledge, to identify an association between adipocytokines and the pathogenesis of sepsis (inflammation, coagulation, severity and prognosis) from the perspective of a cytokine network. Adipocytokines, especially resistin, were suggested to play a critical role in forming a strong network with other inflammatory cytokines throughout the acute phase of sepsis and were associated with organ dysfunction, DIC and prognosis.

Resistin was originally discovered in 2001 in mice as a hormone released from adipocytes and was associated with obesity and insulin resistance [22]. In vivo and in vitro assays showed that resistin was released from adipocytes by lipopolysaccharide administration in mice and might have displayed pro-inflammatory characteristics [23]. In humans, however, resistin seems to be mainly secreted by macrophages rather than adipocytes [24]. Patients with sepsis displayed greater elevation in serum resistin levels than did control or critically ill patients without sepsis, and its concentrations were closely related to severity and prognosis [10]. In the present study, resistin was significantly increased in the hyper-acute phase (day 1) of sepsis in the patients with shock and in the non-survivors compared with the controls (Fig. 2). Hierarchical clustering analysis and network analysis revealed that resistin formed a strong network with IL-1β, IL-6, IL-8 and MCP-1 (Fig. 3A, B), thus suggesting that resistin might participate in the pro-inflammatory response by forming a network with the other pro-inflammatory cytokines.

Chemerin represents adipocytokines mainly expressed in adipocytes [25]. Initially, it was shown to be a pro-inflammatory molecule, but later studies revealed the existence of anti-inflammatory chemerin cleavage products [30, 31]. Circulating chemerin was increased in septic patients and was associated with mortality [28]. Serum chemerin levels were significantly lower in the patients with shock on day 1 compared to those without shock (Fig. 2). Hierarchical clustering analysis and network analysis revealed that chemerin composed a cytokine network that correlated negatively with IL-6, IL-8 and MCP-1 (Fig. 3B), suggesting that chemerin might be consumed while working as an anti-inflammatory cytokine.

Adiponectin is a well-known 30-kDa peptide hormone exclusively produced by adipocytes that is involved in glucose and lipid metabolism and insulin resistance [29]. Circulating adiponectin levels in patients with obesity and diabetes mellitus are reduced compared with healthy subjects [30]. Given its important anti-inflammatory and vasculoprotective effects, adiponectin was shown to play a protective role in mouse models of sepsis [35, 36]. Only limited data on adiponectin levels in critical illness and sepsis are available. A preliminary study reported that critical illness was associated with lower adiponectin concentration [33]. A study assessing circulating levels of adiponectin in 170 critically ill ICU patients with or without sepsis showed them to be comparable with healthy controls, but a low adiponectin level at ICU admission was an independent positive predicative marker for prognosis [34]. A recent study showed a protective role of adiponectin by producing exosomes from vascular endothelium interacting with activated T-cadherin [35], which might partly explain adiponectin-related organ protection. Adiponectin levels in the sepsis patients without shock were significantly lower than those in the patients with shock and tended to be lower in the 28-day survivors versus non-survivors (Fig. 2), suggesting that adiponectin might play a defensive role through its consumption in the acute phase of sepsis. However, a network of adiponectin with other pro- and anti-inflammatory cytokines was not shown (Fig. 3A, B), indicating that the protective effect of adiponectin against sepsis might work differently than that of pro- and anti-inflammatory cytokines.

Resistin has been shown to induce the nuclear translocation of NF-kB transcription factors in macrophages and lead to the increased expression of several pro-inflammatory cytokines including IL-1, IL-6, IL-12 and TNF-α in vitro (in both mice and humans) and an enhanced inflammatory response [36]. Also, resistin induced aggravation of an inflammatory condition in vessel walls of rabbits by stimulating monocytes to infiltrate and activate endothelial cells and vascular smooth cells, leading to vascular endothelial damage [37]. The serum resistin levels in the sepsis patients with shock or the non-survivors were elevated compared to the healthy control (Figs. 1, 2), and resistin composed a network with IL-6, IL-8 and MCP-1 over the acute phase of sepsis (Fig. 3A, B). This suggested that resistin, which participates in the inflammatory response, might form a network with pro-inflammatory cytokines leading to vascular endothelial damage throughout the acute phase of sepsis.

Recent clinical and experimental studies have described the concurrent presence of both pro- and anti-inflammatory cytokines, and both types of cytokines were simultaneously activated and balanced in the acute phase of sepsis [42, 43]. IL-10, an anti-inflammatory cytokine, formed a network with pro-inflammatory cytokines including IL-6, IL-8, MCP-1 and resistin (Fig. 3), suggesting that this anti-

inflammatory cytokine might be elevated due to negative feedback from a pro-inflammatory cytokine storm.

Pro-inflammatory cytokines are strongly associated with the progression of coagulopathy in sepsis. Excessive pro-inflammatory cytokines activate monocytes and promote the expression of tissue factor [40], leading to disordered coagulation and microthrombus formation [2], which could contribute to microcirculatory impairment and result in multiple organ failure that leads to death [36]. Pro-inflammatory cytokines such as IL-6, IL-8 and MCP-1 are associated with SOFA and prognosis in the pathogenesis of sepsis [7]. Both resistin and the common score composed of resistin, inflammatory cytokines (IL-6, IL-8, MCP-1, IL-10) and endothelial damage marker (PAI-1) were significantly related to the SOFA and the ISTH DIC scores in the acute phase (Fig. 3C) and showed strong associations with prognosis (Table 2). This indicates that the cytokine network including resistin could cause inflammation and endothelial dysfunction, resulting in the development of organ failure while also exerting a positive feedback mechanism against pro-inflammation.

To explore clinically useful markers of prognosis on day 1, we evaluated AUCs of adiponectin, leptin, resistin, chemerin and visfatin, which composed the cytokine networks in the acute phase of sepsis (Fig. 4). Adiponectin, resistin, chemerin and visfatin had AUCs > 0.6, and that of resistin was 0.777, higher than that of SOFA alone (0.665), and thus, it could be a useful prognostic marker. Both the daily and common combined scores had AUCs > 0.9, indicating that the combination of cytokines in the cytokine network could be an even more useful marker of prognosis.

Our study has some limitations. This study included a small number of patients and was conducted in a single institution. Further large-scale studies will hopefully establish the importance of cytokine networks in the pathogenesis of sepsis and their usefulness as a prognostic marker, which may lead to novel treatments for sepsis.

Conclusion

Adipocytokines and classical cytokine profiles were assessed over time in patients with sepsis. We found that resistin was involved in an inflammatory cytokine network that included IL-6, IL-8, IL-10 and MCP-1 in the acute phase of sepsis and that this network was associated with the severity and prognosis of sepsis.

Abbreviations

APACHE
Acute Physiology and Chronic Health Evaluation; AUC:Area under the ROC curve; CR:Contribution rates; CXCL-12/SDF-1:C-X-C motif chemokine-12/stromal cell-derived factor-1; DIC:Disseminated intravascular coagulation; ICU:Intensive care unit; IL-1β:Interleukin-1 beta; IL-4:Interleukin-4; IL-6:Interleukin 6; IL-8:Interleukin 8; IL-10:Interleukin-10; IL-12/IL-23p40:Interleukin-12/23p4; ISTH:International Society of Thrombosis and Hemostasis; JAAM:Japanese Association for Acute Medicine; MCP-1:Monocyte

chemotactic protein; PAI-1:Plasminogen activator inhibitor-1; ROC:Receiver operating characteristic; SOFA:Sequential Organ Failure Assessment; TNF-α:Tumor necrosis factor alpha

Declarations

Ethics approval and consent to participate

This study followed the principles of the Declaration of Helsinki and was approved by the institutional review board of Osaka University Hospital (Permit Number: 16109). Informed consent was obtained from the patients or their relatives and the healthy volunteers for the collection of all blood samples.

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors of this manuscript have no conflicts of interest to disclose.

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Authors' contributions

TE conceived and designed this study, acquired data, analyzed and wrote the manuscript. Hisatake M helped with designing the study and data interpretation and conducted the literature review. TM, Hiroshi M, KS and SK contributed to acquiring data. TH helped with designing the study. TT, HO, and TS conducted the literature review. All authors have read and understood journal's policies and believe that neither the manuscript nor the study violates any of these. All authors meet the authorship criteria detailed in the submission guidelines, and all authors agree with the contents of the manuscript.

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Tables

Due to technical limitations, table 1-2 is only available as a download in the Supplemental Files section.

Figures

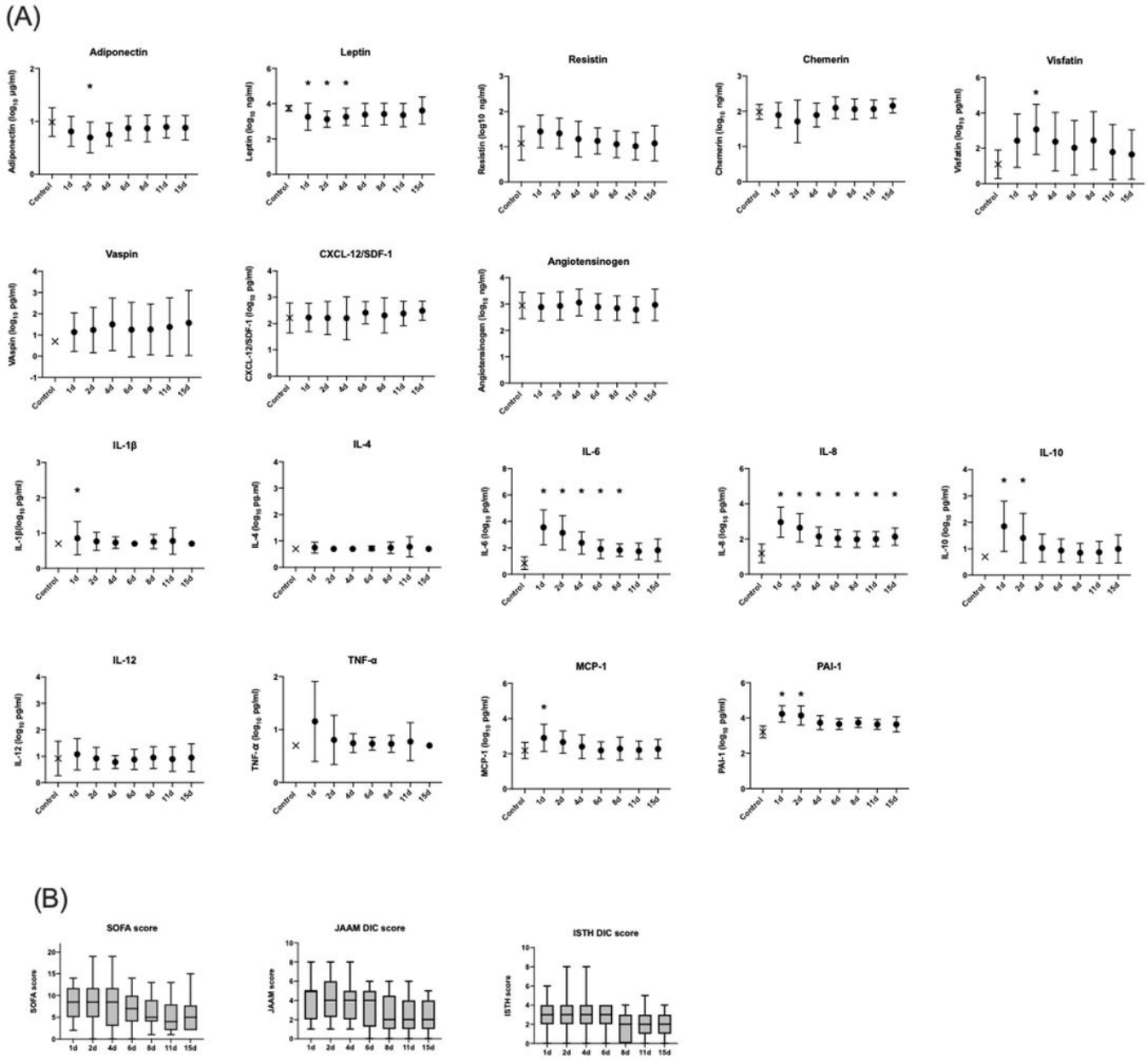


Figure 1

Change in the levels of 9 adipocytokines, inflammatory cytokines, PAI-1 and severity scores. (A) The cytokines and PAI-1 were transformed to common logarithm values to normalize data distribution. All data are expressed as mean ±SD. Asterisks indicate a statistical difference in cytokines and PAI-1 between control and septic patients on each day ($p<0.05$). (B) Changes in the SOFA score. The boxes indicate the lower and upper quartiles, the central line is the median, and the ends of the whiskers represent the maximum and minimum values. CXCL-12/SDF-1 C-X-C motif chemokine-12/stromal cell-derived factor-1, IL interleukin, TNF-α tumor necrosis factor-α, MCP-1 monocyte chemotactic protein-1, PAI-1 plasminogen activator inihibitor-1, SOFA Sequential Organ Failure Assessment, JAAM Japanese Association for Acute Medicine, ISTH International Society of Thrombosis and Haemostasis

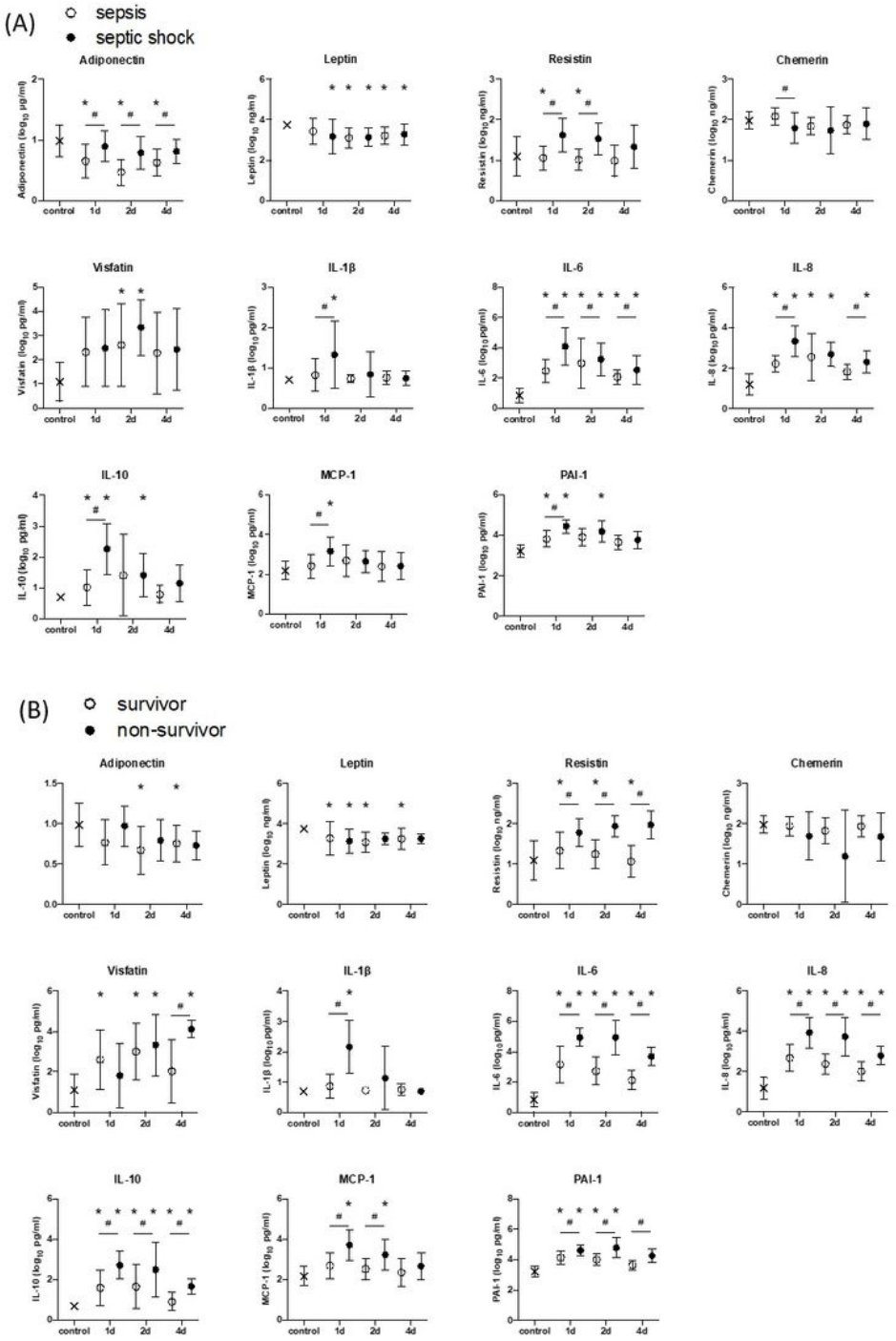


Figure 2

Levels of cytokines and PAI-1 in septic shock vs. non-shock patients and survivors vs. non-survivors. (A) Levels of mediators in septic shock and non-shock patients on Day 1 (septic shock patients, n=24; non-shock patients, n=12), Day 2 (n=22; n=10, respectively) and Day 4 (n=24; n=12, respectively). (B) Levels of mediators in 28-day survivors and non-survivors on Day 1 (n=24; n=12, respectively), Day 2 (n=26; n=6, respectively) and Day 4 (n=19; n=5, respectively). All data are expressed as the mean±SD. Asterisks

indicate a statistical difference between control and septic patients on each day ($p<0.05$), and # indicates a statistical difference between shock and non-shock or survivor and non-survivor on each day ($p<0.05$). IL interleukin, MCP-1 monocyte chemotactic protein-1, PAI-1 plasminogen activator inihibitor-1

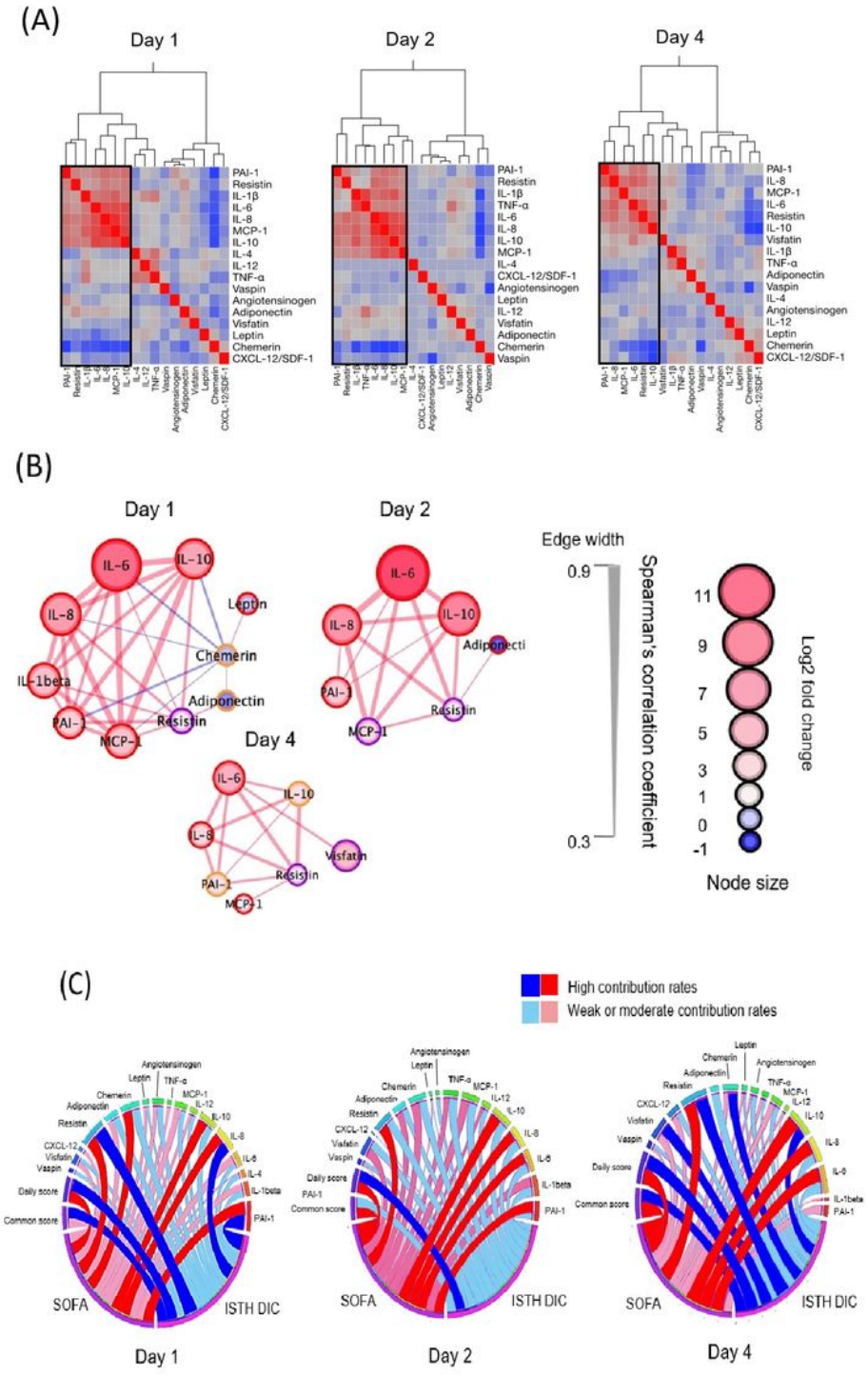
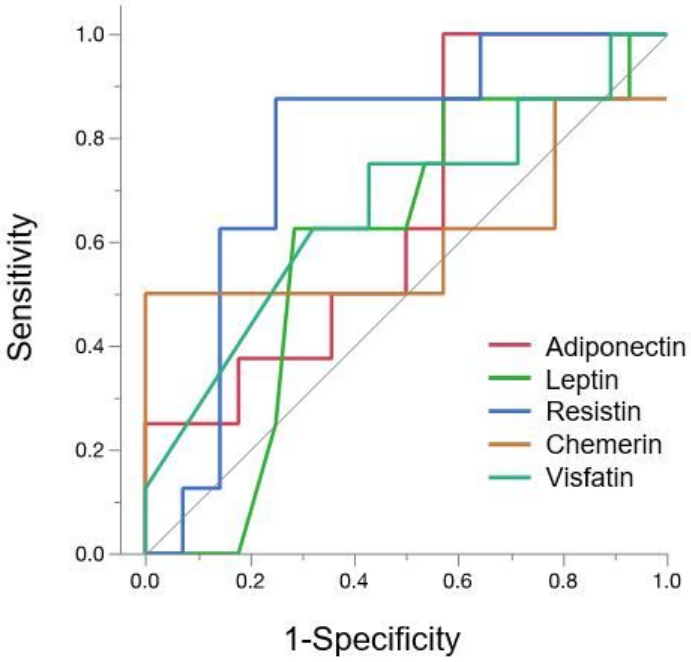
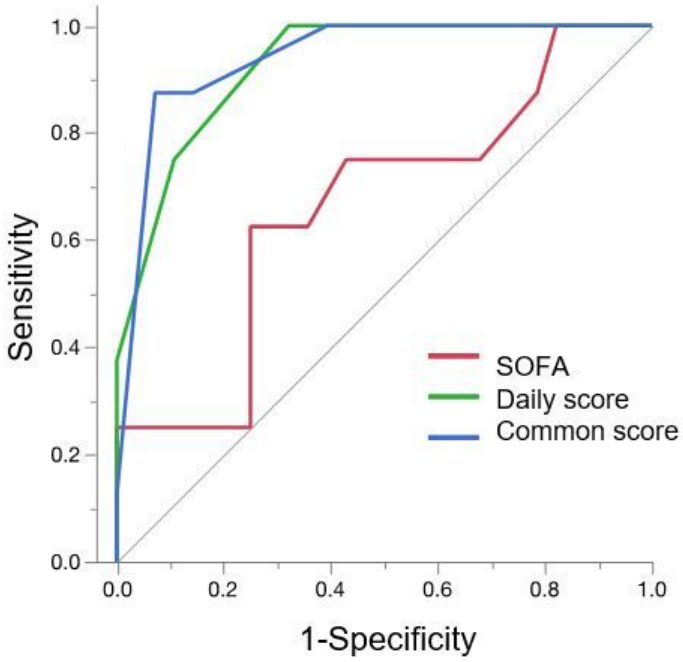


Figure 3

Hierarchical clustering and network visualization. The cytokines and PAI-1 values were transformed to common logarithm values to normalize the data distribution. Hierarchical clustering of Spearman's

correlation coefficients between cytokines and PAI-1. The outlined boxes show the common cytokine network in the acute phase of sepsis on days 1, 2 and 4. (B) This network allows visualization of the significant correlations in (A). The size of each node was determined based on the log2 fold change (i.e., average cytokine levels in sepsis patients/average cytokine levels in controls). Node colors depict increased cytokines (red color) and decrease cytokines (blue color) compared to those of the controls. Red, orange and purple colors of enclosing lines in each node indicate significant differences (increase or decrease) in the cytokines and PAI-1 levels between septic patients and controls, between septic shock patients or 28 day non-survivors and controls, and between shock patients and non-shock patients or survivors and non-survivors, respectively. (C) Radial network diagrams allow the visualization of the contribution of cytokines and the combined scores to SOFA and ISTH DIC scores. Red and blue colors indicate higher contributions than those of light red and blue colors. CXCL-12/SDF- C-X-C motif chemokine-12/stromal cell-derived factor-1, IL interleukin, TNF-α tumor necrosis factor-α, MCP-1 monocyte chemotactic protein-1, PAI-1 plasminogen activator inihibitor-1, SOFA Sequential Organ Failure Assessment, JAAM Japanese Association for Acute Medicine, ISTH International Society of Thrombosis and Haemostasis





	AUC
Adiponectin	0.656
Leptin	0.594
Resistin	0.777
Chemerin	0.607
Visfatin	0.665

	AUC
SOFA	0.665
Daily score	0.926
Common score	0.940

Figure 4

ROC analysis using adipocytokines, SOFA score and combined scores. The levels of the 6 adipocytokines, which were transformed to common logarithm values, and the combined scores were used for analysis. The AUC was calculated to evaluate the prognostic accuracy of each marker on day 1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Table1fin.xlsx
- Table2fin.xlsx