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Whole Blood Gene Expression Profiling in Response to Treatment for Septic Shock: A Case Report

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Abbreviations:

EGDT: early goal-directed therapy; PMX-DHP: polymyxin B direct hemoperfusion; APACHE-II score: High Acute Physiology and Chronic Health Evaluation II score; SOFA score: Sequential Organ Failure Assessment scores; SIRS: systemic inflammatory response syndrome; ICU: intensive Care Unit; WBC: white blood cell; IL: interleukin; CRP: C-reactive protein; P/F ratio: PaO2/FiO2 ratio; TNF-α: tumor necrosis factor-alpha; NF-κB: nuclear factor -κB

1. Abstract

Septic shock develops as a result of the rapid progression of sepsis due to the massive release of proinflammatory mediators, and is characterized by severe hypotension and tissue hypoxia. While early goal-directed therapy (EGDT) and polymyxin B direct hemoperfusion (PMX-DHP) have shown effectiveness in the treatment of septic shock, no biomarkers are available to accurately assess the therapeutic responses in such patients. Here, we performed the first whole blood gene expression profiling in a successfully treated case of septic shock. A 74-year-old woman was admitted to the intensive care unit and diagnosed with septic shock and dangerously low blood pressure (50/38 mmHg). High Acute Physiology and Chronic Health Evaluation (APACHE)-II and Sequential Organ Failure Assessment (SOFA) scores (40 and 14, respectively) indicated a significant probability of a fatal outcome. PMX-DHP was performed for 3.5 h, which increased blood pressure to 90/46 mmHg. Comparative gene expression analysis of the patient's whole blood samples identified 867 and 1,467 genes that were upregulated and downregulated, respectively, after PMX-DHP; the former genes were found to be involved in oxidative stress, whereas the latter were related to neutrophil defensins, tumor necrosis factor-α/nuclear factor-κB, interleukin-8, and -6 signaling cascades, and pyruvate metabolism. As the identified differentially expressed genes have been recognized for their association with sepsis, these results suggest that whole blood gene expression profiling may aid in developing a panel of biomarkers for evaluating therapeutic responses in patients with septic shock and in the selection of an appropriate treatment.

2. Introduction

Sepsis is a systemic inflammatory response syndrome (SIRS) caused by bacterial infection; it is one of the most prevalent diseases in the Intensive Care Unit (ICU), and is associated with a high mortality risk (up to 50%) [1]. Sepsis can develop into its most life-threatening form, septic shock, manifested by low blood pressure, ultimately causing global tissue hypoxia and multi-organ failure [2].

Sepsis is diagnosed on the basis of the following SIRS criteria: fever, elevated respiratory and heart rates, abnormal white blood cell (WBC) counts, and bacterial culture. Early goal-directed therapy (EGDT) using vasopressors, artificial ventilation, and antibiotics is recommended to balance oxygen delivery and prevent irreversible organ damage [2].

In addition, direct hemoperfusion (DHP) with polymyxin B (PMX) immobilized in a polystyrene fiber column is performed to remove the bacterial endotoxin, the principal factor in septic shock [3], which causes hyperactivation of the innate immune response associated with abnormal expression of more than 3,000 genes [4]. However, there are no known targets that determine therapeutic effectiveness during the course of this treatment. We therefore performed the first whole blood genome-wide expression profiling in a patient with septic shock who was successfully treated with PMX-DHP to determine whether there are any in vivo changes before and after shock withdrawal following an apparent rise in blood pressure. We also determined whether we could identify any targets for potential treatment based on changes in gene expression.

3. Case Presentation

A 74-year-old woman was admitted to the ICU where she was diagnosed with septic shock based on the following test results: blood pressure 50/38 mmHg, heart rate 140/min, body temperature 38.2°C, respiratory rate 50/min, total leukocyte count 9290/μl, and serum C-reactive protein (CRP) 19.1 mg/dl, which fulfilled three of the four SIRS diagnostic criteria. Urine cultures were positive for Escherichia coli, and plasma endotoxin and serum procalcitonin levels were 14.7 pg/ml (normal: ≤1.0) and ≥100 ng/ml (normal: ≤0.05), respectively, indicating bacteremia and suggesting that the septic shock was caused by urinary tract infection. The Acute Physiology and Chronic Health Evaluation (APACHE)-II score was 40 and the Sequential Organ Failure Assessment (SOFA) score was 14, indicating an extremely high mortality risk (over 80%). As the blood pressure was very low and the heart rate high, we performed EGDT, including administration of dopamine (15 × g/kg/min), noradrenalin (0.07 μg/kg/min), biapenem (0.3 g), γ-globulin (5 g), gabexate (80 mg/h), urinastatin (15 × 104 units), and sivelestat (9 mg/h), together with massive parenteral fluid infusion and mechanical ventilatory support. However, the blood pressure did not rise; therefore, we applied PMX-DHP for 3.5 h starting at about 1 h after the patient's admission to the ICU. As a result, the blood pressure rose to 90/46 mmHg at 5 h after admission, followed by an increase of the PaO2/FiO2 (P/F) ratio, an indicator of oxygenation, from 166 to 271, suggesting achievement of the early goal. However, on the following day, the WBC count and serum CRP level were higher than normal (11,860/μl and 28.9 mg/dl, respectively). The patient was subjected to continuous hemodiafiltration with a polymethyl methacrylate membrane for 4 days and was administered the broad-spectrum antibiotic meropenem (0.5 g twice a day) and vasopressors (dopamine and noradrenaline) for 5 days. After extubation on day 5, the patient was moved from the ICU to the high-care unit on day 6. She was then treated according to standard guidelines and discharged on day 12 after admission.

Since PMX-DHP proved to be successful in the present case of septic shock, we compared the whole blood expression profiles before and after PMX-DHP using microarray analysis. The identified differentially expressed genes were analyzed for involvement in metabolic and signaling pathways and functionally classified with a focus on immune, inflammatory, and defense responses relevant to sepsis [5].

In total, 867 and 1,647 genes were upregulated and downregulated, respectively, in the patient's blood after PMX-DHP (Figure 1 and Table 1). The upregulated genes are functionally involved in oxidative stress, whereas the downregulated genes are related to neutrophil defensins, the tumor necrosis factor-alpha(TNF-α)/nuclear factor (NF)-κB cascade, interleukin (IL)-8 and IL-6 signaling, and pyruvate metabolism (Table 2).

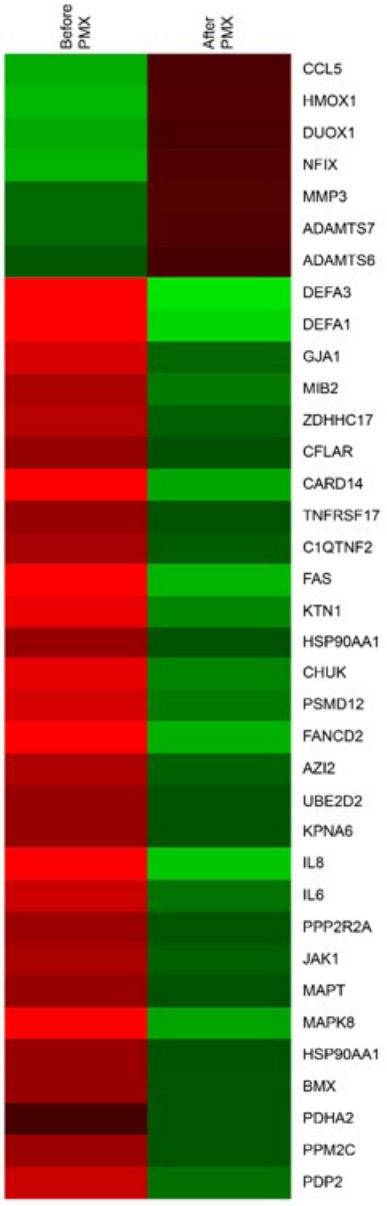

Figure 1: Heat map showing the differential expression of functional genes after polymyxin B direct hemoperfusion. Genes with a ≥1.5-fold change between blood samples taken before and after PMX-DHP were considered differentially expressed ($P < 0.05$).

Table 1: Signaling pathways differentially regulated after PMX-DHP (Polymyxin B Direct Hemoperfusion).

MAPP name	P-value
Upregulated pathways	
Hs_Electron_Transport_Chain	0.001
Hs_Oxidative_Stress	0.033
Hs_Matrix_Metalloproteinases	0.05
Downregulated pathways	
Hs_Ribosomal_Proteins	0.001
Hs_Cholesterol_Biosynthesis	0.001
Hs_Electron_Transport_Chain	0.004
Hs_Sterol_biosynthesis	0.006
Hs_Terpenoid_biosynthesis	0.031
Hs_Proteasome_Degradation	0.004
Hs_Translation_Factors	0.007
Hs_TNF-alpha-NF-kB_NetPath_9	0.002
Hs_mRNA_processing_Reactome	0.019
Hs_G13_Signaling_Pathway	0.027

[a] Pathway analysis was performed using Gen MAPP (Gladstone Institute, San Francisco, CA, USA).

Table 2: Functional analysis of genes exhibiting differential expression after PMX-DHP (Polymyxin B Direct Hemoperfusion).

Gene symbol	Description	Fold change
Oxidative stress		
CCL5	Small inducible cytokine A5 precursor	1.6
HMOX1	Heme oxygenase 1	1.66
DUOX1	Dual oxidase 1 precursor	1.59
NFIX	Nuclear factor 1 X-type	1.63
Neutrophil defensins		
DEFA3	Neutrophil defensin 3 precursor	0.36
DEFA1	Neutrophil defensin 1 precursor	0.38
Pyruvate metabolism		
PDHA2	Pyruvate dehydrogenase E1 component alpha subunit	0.65
PPM2C	Pyruvate dehydrogenase [lipoamide]-phosphatase 1	0.58
PDP2	Pyruvate dehydrogenase [lipoamide]-phosphatase 2	0.65
IL-8		
IL8	IL8 precursor	0.41
TNF-a/NF-kB cascade		
GJA1	Gap junction alpha-1 protein	0.61
MIB2	E3 ubiquitin-protein ligase MIB2	0.56
ZDHHC17	Palmitoyltransferase ZDHHC17	0.62
CFLAR	CASP8 and FADD-like apoptosis regulator precursor	0.67
CARD14	Caspase recruitment domain-containing protein 14	0.47
TNFRSF17	Tumor necrosis factor receptor superfamily member 17	0.66
C1QTNF2	Complement C1q tumor necrosis factor-related protein 2 precursor	0.64
FAS	Tumor necrosis factor receptor superfamily member 6 precursor	0.44
KTN1	Kinectin	0.53
HSP90AA1	Heat shock protein HSP 90-alpha	0.56
CHUK	SPFH domain-containing protein 1 precursor	0.53
PSMD12	26S proteasome non-ATPase regulatory subunit 12	0.56
FANCD2	Fanconi anemia group D2 protein	0.45
AZI2	5-azacytidine induced 2	0.62
UBE2D2	Ubiquitin-conjugating enzyme E2D 2 isoform 2	0.66
KPNA6	Importin alpha-7 subunit (Karyopherin alpha-6)	0.66
IL-6 signaling		
IL-6	IL-6 precursor	0.58
PPP2R2A	Dedicator of cytokinesis protein 5	0.65
JAK1	Tyrosine-protein kinase JAK1	0.63
MAPT	Microtubule-associated protein tau	0.66
MAPK8	Mitogen-activated protein kinase 8	0.47
HSP90AA1	Heat shock protein HSP 90-alpha	0.56
BMX	Cytoplasmic tyrosine-protein kinase BMX	0.66

[a] Genes were classified according to Gene Ontology annotation (http://www.geneontology.org/)

4. Discussion

Currently used laboratory test parameters such as CRP, procalcitonin, and presepsin levels [6,7] do not necessarily indicate the pathophysiological state of sepsis and may fail in the timely selection of suitable treatment; therefore, there is a need for new biomarkers associated with various phases of sepsis that accurately reflect the heterogeneity of the disease in various age groups [8-10].

In our case, the patient was successfully treated with EGDT and PMX-DHP. PMX binds endotoxin, an outer membrane component of gram-negative bacteria, which is mainly responsible for septic shock [3]. Endotoxin (lipopolysaccharide) induces a rapid

innate immune response by activating Toll-like cell surface receptors, which results in the massive release of proinflammatory cytokines (the so-called cytokine storm) and nitric oxide, a powerful vasodilator considered one of the main causes of hypotension in septic shock [4]. PMX-DHP reduces circulating endotoxin levels, thereby improving hemodynamics and oxygenation in patients with septic shock due to gram-negative bacteria, although beneficial effects were also observed in fulminant infections caused by gram-positive microbes [11].

Endotoxin was shown to deregulate the transcription of 3,700 genes as early as 2 h after exposure [4]; thus, its removal should theoretically restore normal gene expression. However, no studies on transcriptional responses to PMX-DHP have been performed. Our comparative analysis revealed differential expression of six functional groups of immunity-related genes after PMX-DHP application. All the downregulated genes have previously been implicated in the pathophysiology of sepsis [4,12,13]. These results are consistent with the reduction of endotoxin levels due to PMX-DHP and the subsequent inhibition of biological cascade leading to sepsis [3,14]. However, PMX-DHP treatment increased the expression of genes related to matrix metalloproteinases and oxidative stress, also known to be associated with septicemia [4, 5], which may reflect the complex roles of these factors in combating severe infection.

Our patient was an elderly woman, and it is known that the effectiveness of septic shock treatment and its mortality rates depend on age, among other factors. Thus, a decrease in the IL-8 level by PMX-DHP was shown to predict the survival of pediatric patients [8], whereas no association between IL-8 secretion and sepsis outcome was observed in adult patients [10]. These results suggest that IL-8 alone (and probably other cytokines) may not be a suitable indicator of the therapeutic responses in sepsis, and that a panel of biomarkers adjusted to different age groups should be developed.

Our analysis was limited to only one patient and two time points, and we used the ≥1.5-fold cut-off criterion, which may have been too lenient; in addition, the changes in gene expression were not confirmed by PCR. Nevertheless, as the first attempt at comprehensive gene expression profiling after successful treatment for sepsis, this study suggests that whole blood expression analysis in patients with septic shock may lead to the discovery of signature molecules related to improvement of the disease status. Our findings also provide clues for further expression studies on candidate biomarkers for selection of suitable personalized treatment in septic shock. Similar genome-wide transcriptional profiling for other EGDT measures are worth performing to determine the optimal therapy and reduce the high mortality rates associated with fulminant infections.

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6. Conflicts of interest

The authors declare no conflict of interest.

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