

Transferrin Receptor (TFRC): A Potential Biomarker for The Diagnosis and Prognosis of Sepsis

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Research Article

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Abstract

Objective

This study applies the data independent acquisition (DIA) technique combined with bioinformatics to identify differential proteins in sepsis patients and performed ELISA method to validate the candidate protein of clinical value, in an attempt to find new biomarkers for the diagnosis and prognosis of sepsis.

Methods

Blood samples from sepsis patients (Sepsis group, n = 50) and healthy individuals (NC group, n = 10) were collected from Affiliated Hospital of Southwest Medical University. Mass spectrometry analysis was designed for 22 sepsis samples (randomly selected) and 10 healthy controls by DIA method, and the obtained differential proteins were subjected to GO annotation, meta-analysis and survival analysis to identify the candidate biomarker protein. ELISA was applied to validate the protein expression in original cohorts. ROC curves based on ELISA data were plotted to discuss the diagnostic and prognostic performance of the candidate protein and several clinical indexes, including C-reactive protein (CRP), procalcitonin (PCT) and lactate (Lac).

Results

DIA data showed that there were 142 differential proteins in the Sepsis group versus the NC group, comprising 36 down-regulated and 106 up-regulated. GO annotation revealed that the differential proteins were significantly enriched in the biological functions involved in immune response, response to stress, inflammatory response, and cell activation. The top 11 proteins with the greatest difference were found according to the p-values in DIA (FUCO2, MGAT1, OAF, AACT, TFRC, CCL14, EXTL2, KLKB1, TETN, CRP, SAA1). Meta-analysis identified significant differential expression of TFRC in the NC versus Sepsis and in the Survival versus Non-survival groups based on GEO database. Survival analysis revealed that the low expression of TFRC indicated a higher survival rate in sepsis patients. ELISA found TFRC concentration in collected clinical samples were significant differential in the NC versus Sepsis and in the Survival versus Nonsurvival groups ($p < 0.05$). ROC curves gave an AUC of 0.790 for TFRC in distinguishing the normal individuals and sepsis patients, showing good diagnostic performance. Besides, the AUC for TFRC in distinguishing the survivors and deaths was 0.744, indicating good prognostic performance, which was superior to PCT, CRP and Lac.

Conclusion

This study identified TFRC through DIA, bioinformatics and ELISA analyses, which showed differential expression in sepsis patients as well as good diagnostic and prognostic value. TFRC is expected to be a

potential biomarker for sepsis.

Introduction

Sepsis has now been a great difficulty and challenge in the field of medicine. Despite the intensive studies from all fields and the management of a series of active "rescues" in the world, sepsis still suffers from a high morbidity and fatality[1]. There was research covering adult sepsis in 27 developed countries and reporting that, the annual incidence of sepsis over the last 10 years was around 437/100,000, and approximately 17% of them were succumbed to death. Besides, 270/100,000 had a severe disease with 26% deaths[2]. In China, a study in 2020 which involved the multi-center intensive care unit (ICU) from 44 hospitals uncovered that, the incidence and fatality of ICU sepsis were approximately 20.6% and 35.5%, respectively, and the fatality of those with severe disease was up to 50%[1]. Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection[3]. During the onset process, there exist a series of physiological and pathological processes, including pathogen invasion, release of cytokines, microcirculation dysfunction, and the imbalance between the pathogen and the human immune system. Currently, diagnosis of sepsis adopts the Sepsis3.0, where sepsis is defined when Infection + Δ Sequential Organ Failure Assessment (SOFA) ≥ 2 . In terms of the treatment for sepsis, the rescue and recovery of organ function are the focus. However, the prevention, treatment and prognosis of sepsis remain unsatisfactory, although great efforts have been made over the past two decades.

Biomarker is a sort of molecule of biological significance that can subjectively indicate the physiological, pathological and pharmacological conditions in patients[4], including DNA, RNA, proteins, and small metabolites, which are mainly applied in the diagnosis and staging of disease, as well as the prediction of therapeutic effect in certain population[5]. In clinic, patients having the same symptoms or vital signs but different biomarkers often have different prognoses and therapeutic outcomes[6]. In this context, biomarkers are increasingly important in disease diagnosis and prognosis, especially in precision medicine and individualized treatment[7]. Currently, C-reactive protein (CRP), serum procalcitonin (PCT) and lactate (Lac) are frequently used in infection determination and pathogen identification in sepsis, and PCT is the specific biomarker of bacterial infection[8]. Since the issue of the international diagnostic criteria for sepsis (Sepsis3.0) in 2016, PCT, CRP and Lac have less value in the diagnosis and prognosis of sepsis. Hence, searching for new markers that can predict the development and outcome of sepsis can be a new direction for the prevention and treatment.

DIA (data independent acquisition) is a label-free quantitative technique that provides high repeatability, high detection sensitivity, high quantitative accuracy, and allows for data informatization[9]. It has been the most noteworthy technique in recent years. It allows to conduct protein identification and quantitative analysis in multiple samples or in samples of different batches at the same time, which is available for large-scale clinical studies into plasma/serum protein biomarkers. In 2015, international proteomics researchers Ruedi et al. [10] used DIA to uncover the influence of genetic and environmental factors on individuals, which is helpful for the discovery and evaluation of clinical biomarkers. By now, DIA has been

used in the research of biomarkers in multiple fields, such as tumor[11, 12], lung disease[13], and obesity[14].

In this study, DIA method was applied to screen out differential proteins in serum samples from sepsis patients and normal controls, and a public database was consulted for verification. Besides, the candidate protein was further validated by ELISA test. This study attempts to find new biomarkers for diagnosis and prognosis of sepsis.

Materials And Methods

Data acquisition

Peripheral blood samples were collected from Affiliated Hospital of Southwest Medical University from January 2019 to December 2019, including 50 sepsis patients hospitalized in emergency intensive care unit (EICU), Department of Emergency Medicine, and 10 healthy volunteers. Inclusion criteria: (1) Sepsis patients admitted to EICU; (2) Sepsis was diagnosed according to the Sepsis3.0, the diagnostic criteria jointly issued by the American Society of Critical Care Medicine (SCCM) and the European Society Intensive Care Medicine (ESICM) in 2016 (Infection+SOFA score ≥ 2); (3) Patients aged ≥ 14 and ≤ 70 years old; (4) Patients or their legal representatives were willing to participate the study and signed the informed consent. Exclusion criteria: (1) A history of organ failure; (2) A history of immune system disease; (3) A history of hematologic disease; (4) Patients who refused to be involved in the study. The study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (ethics number: ky2018029), with the written informed consent from the participants or their family. Registration number: ChiCTR1900021261. Clinical test data were collected.

DIA mass spectrometry

DIA was completed with the samples randomly selected from the sepsis cohort ($n = 22$) and the normal cohort ($n=10$). Liquid Chromatography-Mass Spectrometry (LC-MS) (Q-Exactive HFX, Thermo Scientific) was applied to conduct mass spectrometry for protein enzymatic peptides of the samples. A spectra database was constructed by traditional data dependent acquisition (DDA). The Ratio values and corresponding p-values that indicate the changes in protein expression abundance of the 32 samples were identified and quantitatively analyzed by using mProphet algorithm.

Functional enrichment and screening of target proteins

All data were firstly subjected to log treatment, and then mapped into a box plot to identify the homogeneity of the samples. Principal component analysis (PCA) was implemented to exclude outliers from the two cohorts. Proteins with differential expression between the normal and sepsis cohorts were screened out assisted by the online tool iDEP91 (<http://bioinformatics.sdstate.edu/idep/>)[15, 16]. Protein meeting fold change (FC) ≥ 2.0 and false discovery rate (FDR) < 0.05 were selected, and then analyzed in enrichment analysis for main biological functional pathways.

Expression of target protein at transcription level

To know more about the transcription level of target protein in different groups, Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) was consulted to obtain seven sepsis-related data sets: GSE28750[17], GSE54514[18], GSE63042[19], GSE67652[20], GSE69528[21], GSE65682[22] and GSE95233[23]. All the datasets were documented with human peripheral blood samples with a sample size ≥ 20 . The GSE65682 dataset contains 28-day prognostic data for 479 patients with sepsis, which were here used for survival analysis of the candidate target. Data from the 7 data sets were normalized (\log_2 treatment). Four groups were generated: sepsis group (Sepsis) and normal group (NC), sepsis survival group (Survival) and death group (Nonsurvival). A comprehensive meta-analysis was designed for single genes which were from different datasets but in the same group, based on R language.

Enzyme-linked immunosorbent assay (ELISA)

All samples from the Sepsis group ($n = 50$) and the NC group ($n = 10$) were tested by ELISA. Double antibody sandwich method was applied to quantitatively analyze the proteins of each sample. Briefly, purified specific antibodies were seeded into a micropore plate to immobilize, followed by an addition of tested protein samples and horseradish peroxidase (HRP) -conjugated testing antibodies in turn. An antibody-antigen-enzyme labeled antibody complex was formed. Substrate TMB (3,3',5',5'-Tetramethyl benzidine) was added for color development after washing. The darkness of the color is positively correlated with the amount of protein in the sample. The optical density (OD) at 450 nm was read on a microplate reader, and the protein content was calculated by a standard curve. The obtained data were loaded to GraphPad Prism8.0 software. Between-group differences were statistically analyzed by independent sample t -test, and considered significant when $p < 0.05$.

ROC

To assess the diagnostic and prognostic value of the candidate biomarker, receiver operating characteristic (ROC) curve was drawn by MedCalc 15.2 software, and the area under the ROC curve (AUC) was calculated. Favorable diagnostic performance was defined when $AUC > 0.7$ in the NC group versus Sepsis group, and excellent prognostic performance was defined when $AUC > 0.7$ in the Survival group versus Death group of sepsis. Besides, the diagnostic and prognostic performance of the candidate were compared with those of several clinical indexes, including CRP, PCT and Lac.

Results

DIA data

Two hemolysis samples were excluded. In box plots and PCA analysis by iDEP91, there was good homogeneity in NC and Sepsis samples and the between-group discrimination is good as well, with no outliers (Fig. 1A-B). There were 142 differential proteins in the Sepsis group versus NC group, composed of 36 down-regulated proteins and 106 up-regulated proteins (Fig. 1C-D).

Screening of candidate biomarkers

Based on the differential proteins, Gene Ontology (GO) annotation was operated to find the most enriched GO terms, which were immune response, response to stress, inflammatory response, cell activation, etc. (Fig. 2A). The top 10 proteins with the greatest differential expression were filtered according to corresponding p-values in DIA analysis, including FUCO2, MGAT1, OAF, AACT, TFRC, CCL14, EXTL2, KLKB1, TETN, CRP, and SAA1 (Fig. 2B). All the differential expressions in the NC versus Sepsis were statistically significant ($p < 0.05$).

Transcriptional expression of candidate biomarker

A meta-analysis was conducted based on the transcription data of candidate protein (TFRC) documented in GEO database. It demonstrated a significant increase of TFRC in the Sepsis group versus the NC group (Fig. 3A). Besides, TFRC expression was even higher in the Nonsurvival group versus the Survival group in sepsis patients, with statistically significant difference (Fig. 3B). Further survival analysis for TFRC was carried out using the GSE65682 data. It was found that patients poorly expressing TFRC had a higher survival rate ($p = 0.00034$) (Fig. 4A).

ELISA verification

There were 10 samples in the NC group, 48 in the Sepsis group (excluding 2 hemolysis samples), 14 in the Nonsurvival group and 34 in the Survival group. ELISA analysis identified a significant increase of TFRC expression in the Sepsis group versus the NC group (156.83 ± 84.71 nmol/L versus 87.99 ± 47.89 nmol/L), and the difference between the two groups was of statistical significance ($p < 0.05$) (Fig. 4B). In the sepsis cohort, the expression of TFRC in the Survival group was 130.97 ± 40.45 nmol/L, much lower than 219.63 ± 125.59 nmol/L in the Non-survival group, with a statistically significant difference ($p < 0.05$) (Fig. 4C).

ROC curve

ROC curves were designed to assess the diagnostic and prognostic value of TFRC and clinical indexes including PCT, CRP and Lac. In the Sepsis group versus the NC group, the TFRC was detected with AUC = 0.790, specificity = 90.0%, and sensitivity = 65.1%; PCT was with AUC = 0.905, specificity = 100.0%, and sensitivity = 81.4%; Lac was with AUC = 0.494, specificity = 70.0%, and sensitivity = 58.1%; CRP was with AUC = 0.791, specificity = 100.0%, and sensitivity = 79.1% (Fig. 5A). In the Survival group versus the Nonsurvival group, the TFRC was detected with AUC = 0.744, specificity = 94.1%, and sensitivity = 57.1%; PCT was with AUC = 0.547, specificity = 55.9%, and sensitivity = 64.3%; Lac was with AUC = 0.540, specificity = 82.4%, and sensitivity = 42.9%; CRP was with AUC = 0.595, specificity = 61.8%, and sensitivity = 64.3% (Fig. 5B). It demonstrated that the TFRC performed well in diagnosis and prognosis of sepsis. Though the diagnostic performance was inferior to PCT, the prognostic performance was superior to PCT, CRP and Lac.

Discussion

TFRC (Transferrin receptor/CD71) has two subtypes: TFR1 and TFR2. TFR1 commonly expresses on the surface of common cells, while TFR2 specifically expresses in liver cells. TFRC has balancing act on iron metabolism, as a mediator of iron transport through endocytosis by binding to transferrin. Besides, it has biological activities in multiple biological processes, such as production of free radicals, hemoglobin synthesis, oxygen transport, DNA synthesis, production and release of neurotransmitters, and the regulation of steroid hormones[24]. The expression of TFRC is regulated by hypoxia-inducible factors and the iron regulatory protein-iron response element system. Presence of both hypoxia and iron deficiency will augment TFRC expression, which yet can be regulated by some microRNAs (miRNAs)[24]. Currently, there have been many studies into the role of TFRC in tumors. For instance, TFRC over expresses in tumor cells due to excessive proliferation and increased iron requirement. In addition, TFRC can facilitate tyrosine phosphorylation to inhibit apoptosis, and also can promote tumor cell growth by activating JNK signaling pathway in multiple cancers, such as breast cancer, liver cancer, ovarian cancer, etc.[25–27] It suggests that the high expression of TFRC in tumor may indicate adverse outcomes of patients.

Recent studies have found that TFRC can regulate immune function, while the specific mechanism is not clear. TFRC missense mutation (Y20H) does not affect peripheral lymphocytes but leads to impairment of the function of peripheral T cells and a decrease of memory B cells. In the meantime, the ability to produce antibody and the function of immunoglobulin class conversion can also be impaired, manifested as serious immunodeficiency[28]. In a mRNA sequencing study for preterm and term infants in Korean population, TFRC was found to be the core factor involved in T cell activation and closely related to the occurrence of preterm birth, indicating that the TFRC could be a predictor of preterm birth[29]. In addition, TFRC deficiency is regarded as a syndrome of immunodeficiency, manifested as recurrent severe lung infections. In this case, there are TFRC mutations, and the proliferation, function and transformation of T cells and B lymphocytes are affected[30]. A study reported the clinical manifestations and immunological characteristics of 8 patients with TFRC mutations and found T cell function impairment in all patients[31].

Due to the certain morbidity and high fatality[32], sepsis has always been a hot topic in Emergency Medicine, Critical Care Medicine, Infectious Disease and even Surgery. However, there are certain limitations in the prevention and understanding of sepsis because of the complex pathological and physiological processes. It might be possible to reduce the morbidity and fatality of sepsis if more positive and effect managements are provided, in case of an early diagnosis via cytokines or biomarkers, awareness of disease severity and a prediction of possible outcomes. This study combined bioinformatics and DIA analysis to identify TFRC as a potential biomarker of sepsis. According to literature, we found that TFRC has the function of immunoregulation, yet the underlying mechanism remains to be elucidated, and its role in sepsis is rarely reported. We further performed ELISA and found that the expression of TFRC in sepsis cohort was significantly increased relative to that in healthy individuals, and it was much higher in patients who died. Besides, the ROC curves for the TFRC along with several clinical indexes (PCT, CRP and Lac) identified that, the TFRC had diagnostic and prognostic performance to a certain extent. It was superior to PCT in prognostic judgement with both higher specificity and sensitivity.

Conclusions

To sum up, this study identifies TFRC as a potential diagnostic and prognostic indicator of sepsis. Further study will be carried out to discuss the mechanism of TFRC acting on sepsis, and more experiments from the cellular and animal levels will be designed in a larger sample cohort.

Declarations

Ethics approval and consent to participate

In accordance with the guidelines of the Declaration of Helsinki of 1975, the study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (ethics number: ky2018029), with the written informed consent from the participants or their family. Registration number: ChiCTR1900021261. Clinical test data were collected.

Consent for publication

Not applicable

Availability of data and materials

The datasets of DIA and ELISE used in the current study are available from the corresponding author on reasonable request, from Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) or can be downloaded from GEO (GEO accession: GSE28750, GSE54514, GSE63042, GSE67652, GSE69528, GSE65682, GSE95233)

Competing interests

None

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Authors' contributions

Lin fang Li and Yao Liu wrote the main manuscript text and prepared all the figures. Mu hu Chen and Ying chun Hu supported the research in the manuscript. They modified the manuscript and the plan of the research. All authors read and approved the final manuscript.

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Figures

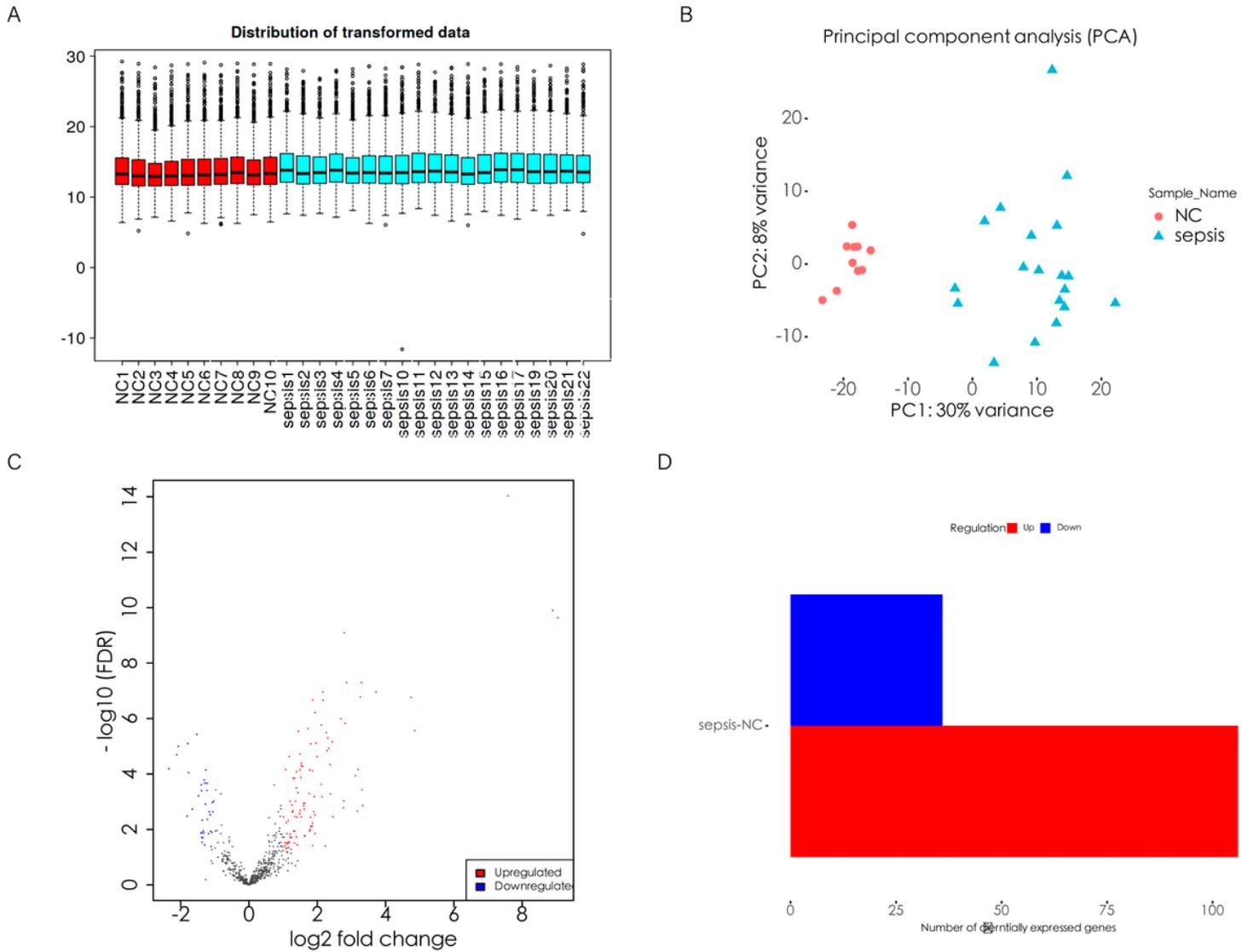


Figure 1

A. Box plot shows the normalized DIA data of each sample. The proteins are distributed at the same level, which is comparable; B. PCA analysis shows good discrimination of the two cohorts, and there are no outliers; C. Volcano plot shows the differential proteins screened by t-test (up-regulated in red and down-regulated in green). The X-axis is the log₂ fold change, and the Y-axis is the -log₁₀; D. There are 142 differential proteins in the Sepsis group versus the NC group, including 36 down-regulated and 106 up-regulated. PCA, principal component analysis; NC, normal control.

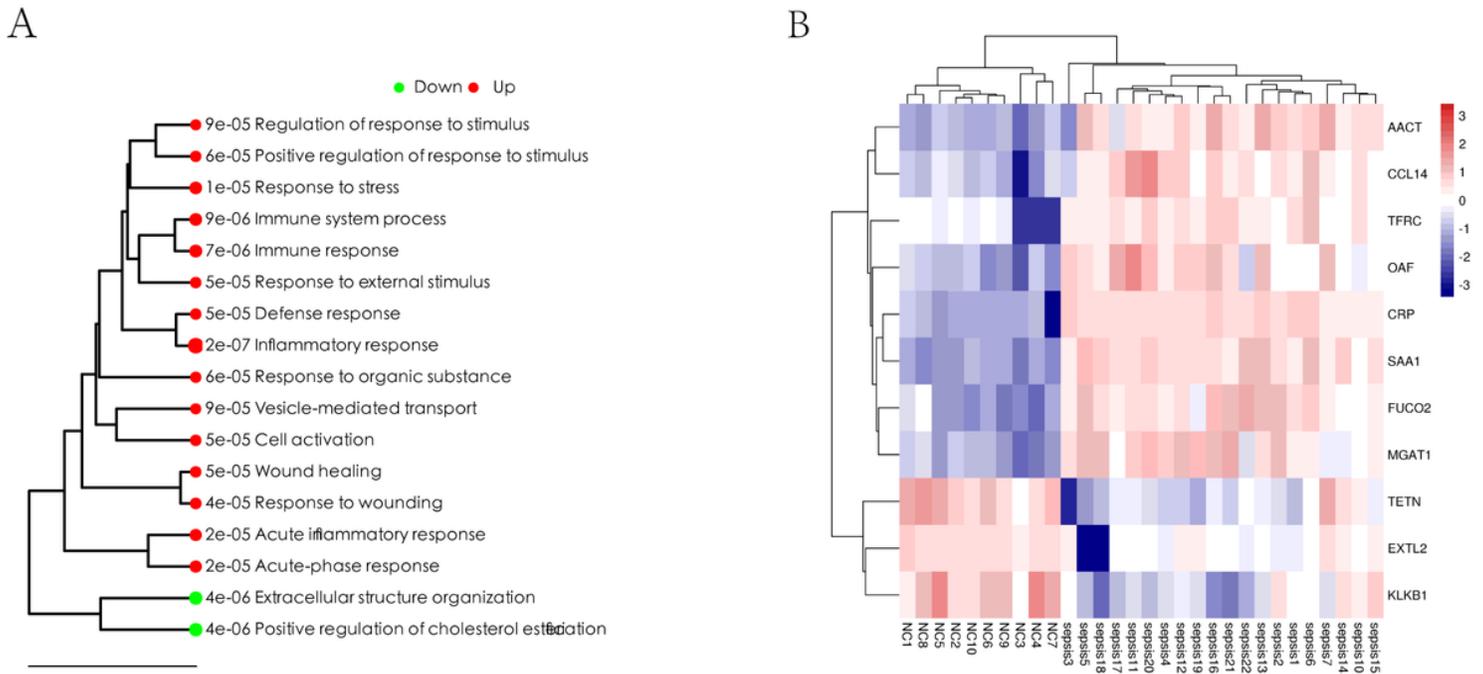
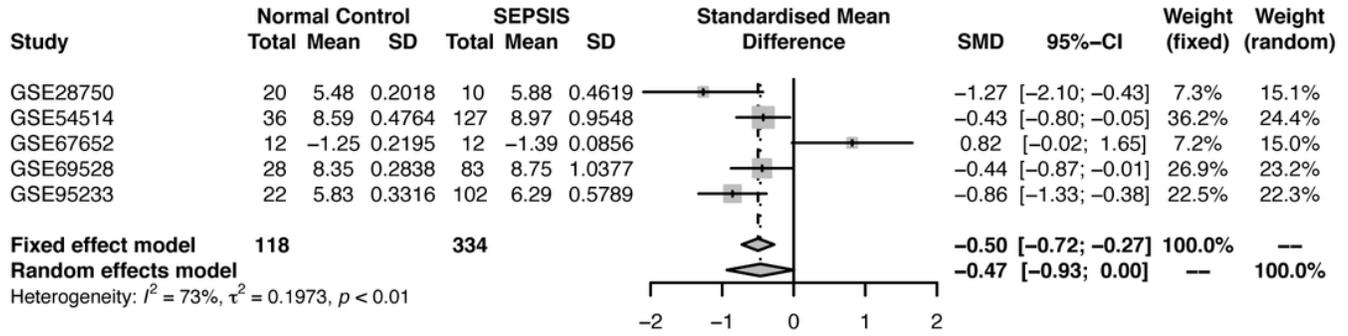


Figure 2

A. GO annotation for differential proteins (up-regulated in red and down-regulated in blue). The size of the dots indicates the number of enriched proteins in the GO term; B. Heatmap shows the top 11 proteins with the greatest differences (FUCO2, MGAT1, OAF, AACT, TFRC, CCL14, EXTL2, KLKB1, TETN, CRP, SAA1). Red refers to high expression and blue refers for low expression.

A



B

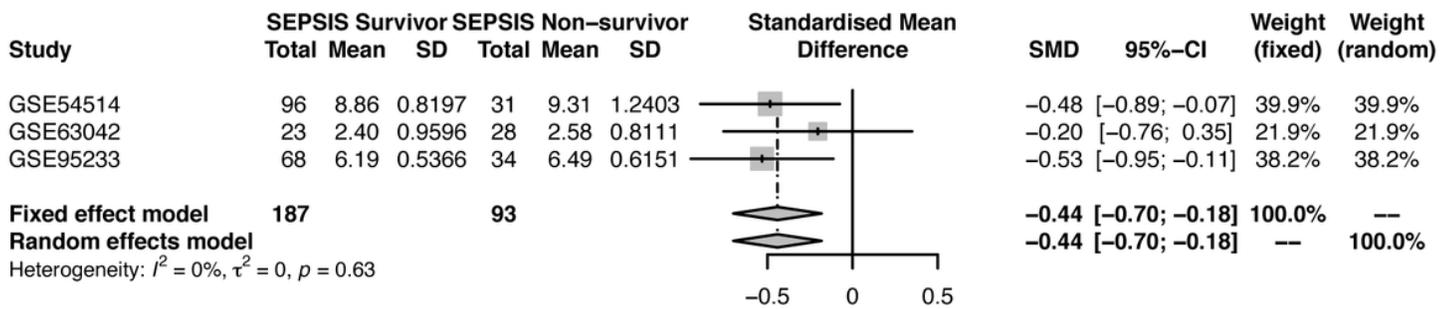


Figure 3

A. Meta-analysis for TFRC based on GSE28750, GSE54514, GSE67652, GSE69528 and GSE95233 data. TFRC shows a low expression in the NC group versus the Sepsis group in all datasets (heterogeneity, $p < 0.01$; random effect model; 95% CI, -0.93 - 0.00); B. Meta-analysis for TFRC based on GSE54514, GSE63042 and GSE95233 data. TFRC shows a low expression in the Survival group versus the Nonsurvival group in all datasets (heterogeneity, $p = 0.63$; fixed effect model; 95% CI, -0.70 - -0.18). NC, normal control; 95% CI, 95% confidence interval.

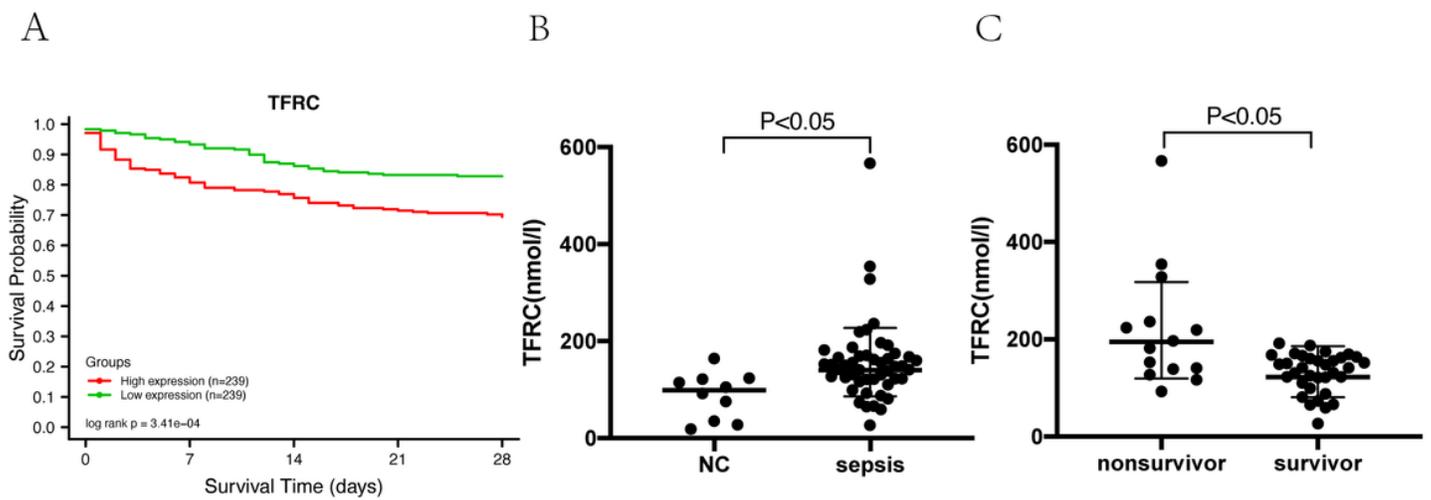


Figure 4

A. Survival analysis for TFRC based on GSE65682 data. Patients with low TFRC expression had better prognosis than patients with high expression; B-C. ELISA analysis showed that the expression of TFRC was significantly higher in the Sepsis group (B, versus NC group, $p < 0.05$) and the Nonsurvival group (C, versus Survival group, $p < 0.05$). NC, normal control.

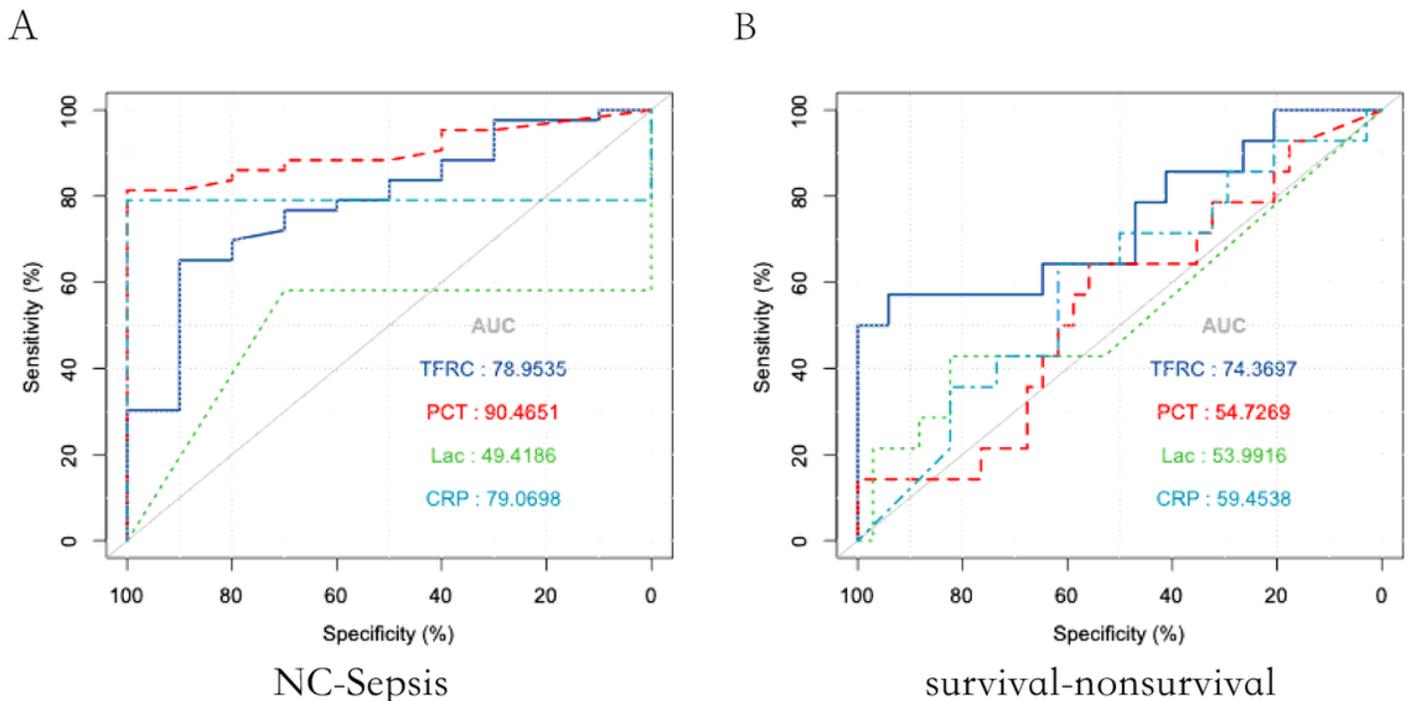


Figure 5

A. ROC curves of TFRC, PCT, CRP and Lac in the NC and Sepsis groups (AUC: 0.790, 0.905, 0.791, 0.494, respectively), suggesting that TFRC has diagnostic performance for sepsis; B. ROC curves of TFRC, PCT, CRP and Lac in the Survival and Non-survival groups of sepsis (AUC: 0.744, 0.547, 0.595, 0.540, respectively), suggesting that TFRC has prognostic value for sepsis, superior to PCT, CRP and Lac.

Supplementary Files

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- [rawdata.pdf](#)