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Serum peptide profiling for potential biomarkers in early diagnosis of *Escherichia coli* bloodstream infection

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ABSTRACT

Background: Bacterial bloodstream infection (BSI) remains an important cause of morbidity and mortality, which is a widespread and uncontrolled inflammatory response. There are some cytokines for the auxiliary diagnosis, such as procalcitonin (PCT), C reactive protein (CRP), and interleukin 6 (IL-6), which are not sufficient. This study was aimed to explore a new method of diagnosing bacterial BSI and to find some new biomarkers that could differentiate bloodstream infected patients from healthy people.

Methods: An animal model was used to find relevant changes of peptides in the serum and was validated in clinical samples. Mice (25–27 g) were randomized to infection with *Escherichia coli* ATCC25922 or phosphate buffer saline. The serum samples were purified by weak cation exchange beads and the serum peptide profiling was established by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Statistical analysis and diagnostic modeling were conducted on BioExplorer. Amino acid sequences of the candidate peptides were identified by nano-liquid chromatography electrospray ionization–tandem mass spectrometry and relevant proteins were recognized on the Uniprot database. The identified proteins were confirmed via enzyme-linked immunosorbent assay on clinical samples.

Results: Five peptide peaks (*m/z* 1941, 2924.1, 3962.1, 4126.9 and 5514) were found as candidate biomarkers for *E. coli* infection, and the diagnostic model discriminated *E. coli* infected patients from healthy controls with an accuracy of 92.2%. Peptide peaks *m/z* 1941, 2924.1 and 4126.9 were identified as the fragments of Serotransferrin (TRF), Complement C3 and Serum amyloid A-1 protein (SAA1), respectively, but only C3 and SAA1 showed significant difference in clinical samples.

Conclusion: MALDI-TOF MS could be a new method to find the changes of serum peptides after infection, C3 and SAA1 could be new biomarkers in diagnosing BSI.

1. Introduction

Clinical bloodstream infections (BSI) are often induced by bacteria and cause high mortality or morbidity [1]. *Escherichia coli* is one of the most common Gram-negative pathogenic bacteria [2]. Blood culture has been used as the gold standard in clinical microbiology diagnosis, but is inadequate for the early diagnosis because of the long turnaround time and low positive rate [3,4]. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), one soft ionization mass spectrometry, has been widely used for microorganism identification [5], but still needs blood culture in advance. Up to now, MALDI-TOF MS has not been widely used to study serum BSI. For this reason, we planned to analyze the changes of serum peptide profiles of BSI and establish a diagnostic model to aid in diagnosis. However, because it was very difficult to determine the exact infection time for patients with BSI and collect blood samples at precise time points for all recruited patients, we decided to use an animal model to study the peptide alteration after infection at different time points.

2. Materials and methods

2.1. Bacterial strains

E. coli standard strain ATCC25922 was donated by the Department of Microbiology in the Chinese PLA General Hospital.

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2.2. Animals

Totally 90 specific pathogen-free male ICR mice (25–27 g) purchased from Beijing Weitonglihua Experimental Animal Center were used to establish an BSI model. The mice were kept in a specific pathogen-free facility and each only used for 1 experiment. All mice were adapted to the new environment for one week in advance and then maintained with humane care in accordance with the *Guide for the Care and Use of Laboratory Animals* approved by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

2.3. The mice BSI model

All experiments were implemented at the same time to eliminate the interference of the circadian rhythms of the mice. Mice were weighed and randomly assigned to weight-matched groups in advance. On the experiment day, the mice were separated into 2 groups treated with *E. coli* or phosphate buffer saline (PBS). Standard strain was activated on flat plates and cultured in the LB liquid nutrient medium for 12 h, and then 100 μl of the bacterial fluid was transferred into a new LB medium to culture for 4–6 h. The challenge dose of *E. coli* was similar to a previous study [6], as the mice were injected in a volume of 0.1 mL/10 g by the intravenous tail. Blood samples were collected from 10 infected individuals at 1, 3, 6, 12, 24, 48, 96, 128 h and PBS separately, followed by 20 min of centrifugation at 5000 g and serum of each sample was transferred into a new tube and stored at −80 °C.

2.4. Serum from clinical patients

Serum samples were obtained from 41 patients in PLA Hospital from May 1st to October 31st 2017, including 22 patients whose blood culture had been diagnosed with the *E. coli* infection and 19 healthy individuals. The infected patients including 14 men and 8 women were aged 29 to 87 years old (average = 60.37) and found with biliary disease (10), leukemia (4), urinary system disease (5) and liver abscess (3). The healthy individuals including 8 men and 11 women were aged 27 to 65 years old (average = 44.8) and received a normal physical examination. Fasting blood samples from the participants were collected in the morning and allowed to clot at 37 °C for 20 min. Serum was then separated by centrifugation at 3000 rpm for 15 min and then stored at −80 °C until further analysis.

2.5. Serum peptide extraction with magnetic beads

With the previous established method, serum samples were fractionated by weak cation exchange magnetic beads (MB-WCX) to gather and enrich the low-molecular-weight proteins or peptides (1000–10,000 Da). Then serum samples were purified and isolated through three steps of binding, washing and elution. In brief, 10 μl of beads, 95 μl of MB-WCX binding solution (SPE-CB) and 10 μl of serum were mixed and incubated for 5 min. The tube of the incubated serum bound beads was then placed on a magnetic bead separation device (Bioyong Tech, Beijing, China) and the beads were collected on the tube wall for 1 min. The supernatant was then removed, while the residue was added and mixed thoroughly with 100 μl of a magnetic bead washing solution (SPE-CW). This washing process was carried out twice and the supernatant was removed after each wash. Another 10 μl of the magnetic bead eluting solution (SPE-CE) was added and the beads were collected from the tube wall in the separation device. This final sample was stored at −20 °C for further MS analyses. No proteolytic enzyme was used during the whole procedure.

2.6. Anchor-chip spotting and protein/peptide profiling by MALDI-TOF MS and data processing

After sample preparation, 1 μl of the final sample was pipetted onto a Clin-TOF II target plate (Bioyong Tech) and dried at room temperature. Then 1 μl of a matrix solution of α-cyano-4-hydroxycinnamic acid (8 mg/ml in 0.1% TFA/50% acetonitrile) was added onto the same spot of each sample. For each sample, profile spectra were recorded via MALDI-TOF MS on a Clin-TOF II instrument (Bioyong Tech) and acquired from an average of 100 laser shots with mass m/z of 1000–10,000 defined. Quality was controlled before the MS analyses using 3 peptides with molecular weights of 1533.8, 2465.7 and 5733.5 Da as external standards and the average molecular weight deviation was within 100 ppm. Each standard was re-calibrated after testing every 24 samples. For data processing, all spectra obtained from the serum samples were analyzed by BioExplorer™ 3.0 (Bioyong Tech). Each spectrum was normalized, baseline-corrected and smoothed by default parameters. To align the spectra, a mass shift of no more than 0.1% was determined. The peak intensities between the two groups were compared via the Wilcoxon test. The best diagnostic model for distinguishing between the infected group and the control group was established using the KNN algorithm.

2.7. Identification of amino acid sequences of the candidate peptides by nano-LC/ESI–MS/MS

Amino acid sequences of the candidate peptides were identified by nano-liquid chromatography electrospray ionization–tandem mass spectrometry (nano-LC/ESI–MS/MS). After serum peptide extraction using MB-WCX beads, each peptide sample was desalted by an Strata X column (Phenomenex), vacuum-dried and then resuspended in 200 μl of buffer A (2% ACN, 0.1% FA). After centrifugation at 20,000 g for 10 min, the supernatant was recovered to form a peptide solution with a final concentration of ~0.5 μg/μl. Then 10 μl of the supernatant was loaded on an LC-20AD nano-HPLC device (Shimadzu, Kyoto, Japan) by the auto-sampler onto a 2 cm C18 trap column. Then the peptides were eluted onto a 10 cm analytical C18 column (inner diameter 75 μm) packed in-house. The samples were loaded at 8 μl/min for 4 min, then treated first by a 44 min gradient at 300 nL/min starting from 2 to 35% B (98% ACN, 0.1% FA) and then by a 2 min linear gradient to 80%, maintained at 80% B for 4 min, and finally returned to 5% in 1 min. The peptides were treated by nano-ESI and then MS/MS in a Q EXACTIVE instrument (Thermo Fisher Scientific, San Jose, CA) coupled online to HPLC. Intact peptides were detected in the Orbitrap at a resolution of 70000. Peptides were selected for MS/MS using a high-energy collision dissociation mode with a normalized collision energy setting of 27.0; ion fragments were detected in the Orbitrap at a resolution of 17500. A data-dependent procedure that alternated between one MS scan and 15 MS/MS scans was applied for the 15 most abundant precursor ions above a threshold ion count of 20,000 in the MS scan followed by a dynamic exclusion duration of 15 s. The electrospray voltage applied was 1.6 kV. The spectra generated by Orbitrap were optimized through automatic gain control (AGC). The AGC target was 3e6 for full MS and 1e5 for MS2.

2.8. Reproducibility of the experiment

To assess the precision and reproducibility of our proteomic data, we collected sera from ten infected mice and ran six within-run assays and six between-run assays on the same Clin-TOF II instrument to determine the deviation. The mean coefficient of variation (CV) was 13.6% (11.4–15.8%) in the within-run assays and 16.8% (10.5–23.1%) in the between-run assays. The CV of the relative intensity of each peak was less than 20%, which confirmed the high repeatability of our serum peptide profiling system.

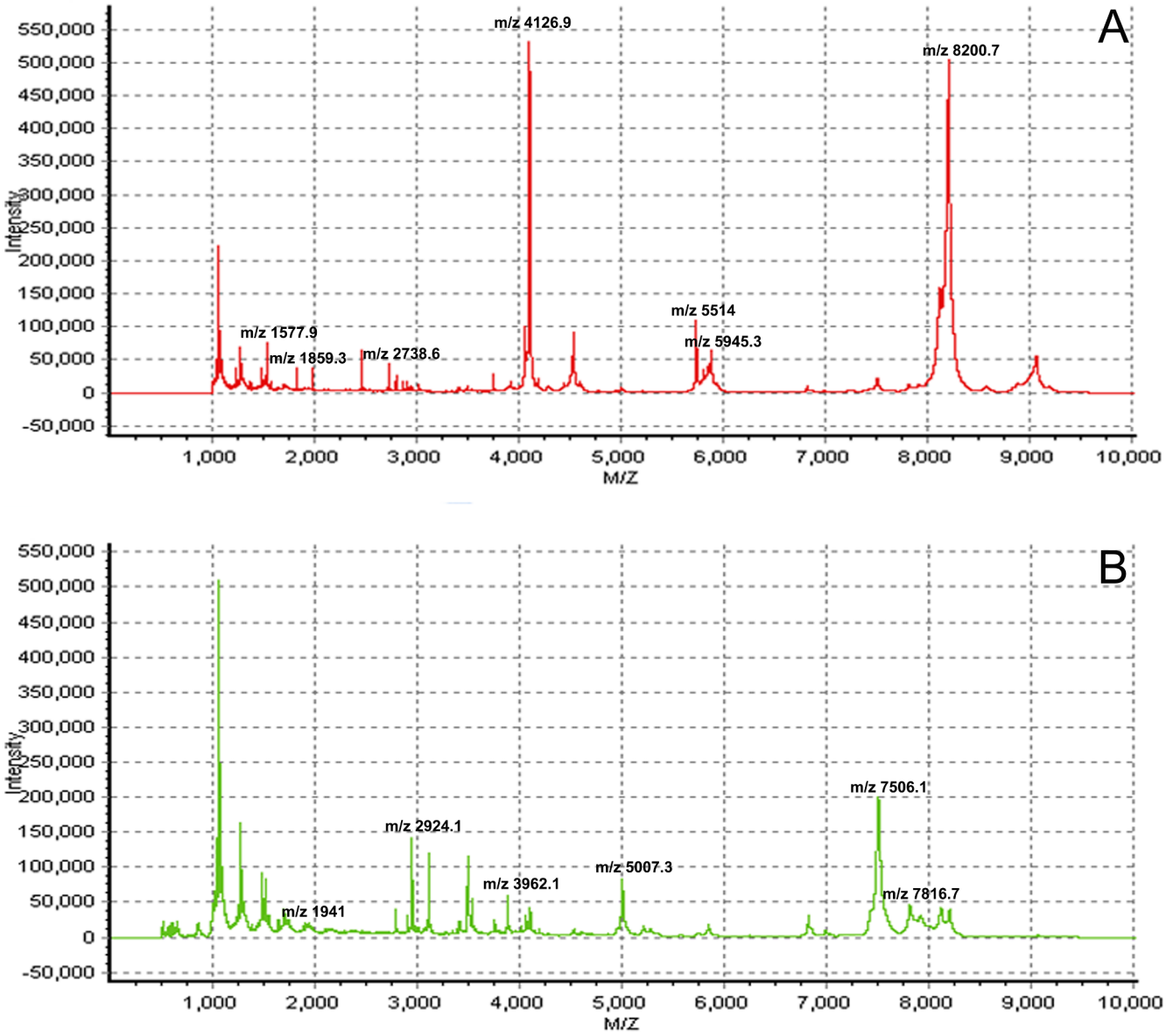

Fig. 1. Serum peptide mass spectra of (A) the infected group and (B) the control group in the mass range from 1 to 10 kDa created by Clin-TOF II instrument. x-axis, molecular mass to charge ratio (*m/z*); y-axis, relative intensity.

2.9. Enzyme-linked immunosorbent assay (ELISA)

To validate the potential biomarkers, the concentrations of serotransferrin (TRF), complement 3 (C3), and serum amyloid A-1 (SAA1) in clinical serum were measured using ELISA (JiangLai, Biological) according to the manufacturer's instructions. After development with a chromogen-substrate solution, the reaction was terminated by adding 50 µl of a stop solution. Optical densities were read at 450 nm, and the concentrations were auto-calculated according to the standard curve.

2.10. Statistical analysis

Statistical analysis was performed on GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA) at the significance level of $P < 0.05$. All data were shown as mean ± standard deviation (SD). Multiple group comparison was examined via repeated measures analysis of variance and the least significant difference test.

3. Results

3.1. The signs of infected mice

The infected mice had significantly abnormal behaviors of tremor retardation irritability piloerection, tail stiffness, and weight loss at 6 h after the infection, which were not found in the control group.

3.2. Detection of serum peptide mass spectra of infected and control samples

After normalization, baseline-correction and smoothing by default parameters, 95 serum peptide peaks were detected in mass (*m/z*) within the range of 1000–10,000 Da (Fig. 1) between the infected group and the control group.

3.3. Comparison between groups

Among the 95 peaks, 17 peptide peaks were significantly different between groups ($P < 0.01$), including 7 peaks lower and 10 peaks higher in the infected group than the control group (Fig. 2), relevant

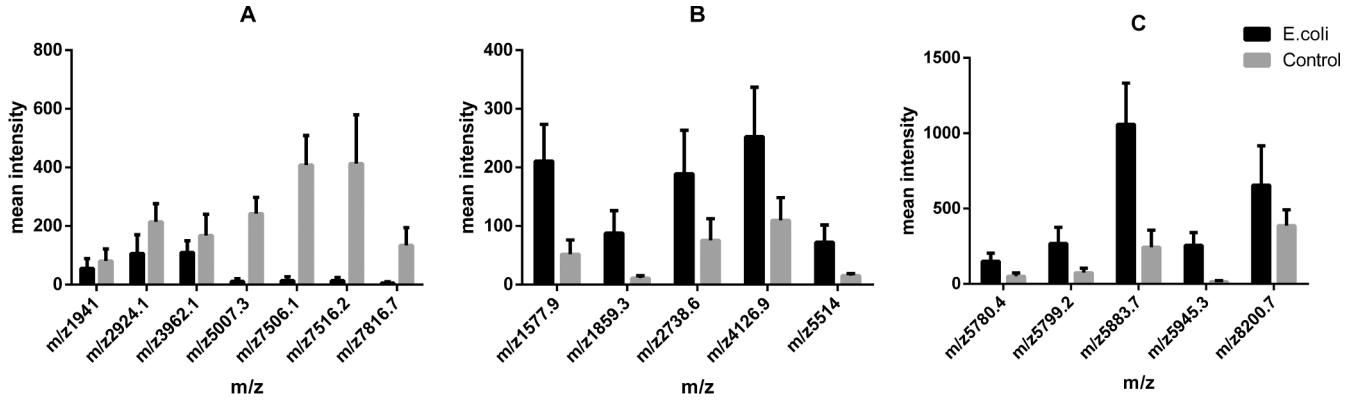

Fig. 2. Different serum peptide peak expressions between the infected group and the control group. A represents the peptide peaks lower in the infected group than the control group including 7 peptides. B and C represents the peptide peaks higher in the infected group than the control group including 10 peptides. All the results are expressed as mean ± SD. x-axis, molecular mass to charge ratio (*m/z*); y-axis, mean intensity.

Table 1
Statistical information of different peaks between groups.

m/z ratio	PTTA(t)	Ave-1	Ave-2	SD-1	SD-2
1941	< 1e−6	55. 3	60. 5	33. 8	41. 1
2924. 1	1.43E−05	106	214. 5	50. 2	61. 7
3962. 1	< 1e−6	109. 3	127. 8	80. 4	122. 4
4126. 9	< 1e−6	264. 9	109. 6	114. 4	79
5514	1.52E−06	72. 2	55	69. 6	30. 8

Note: Group 1: infection; Group2: control; Ave: average peak intensity, SD: standard deviation.

peptide peaks were shown in the mass spectrum (Fig. 1).

3.4. Establishment of the peptide diagnostic panel

To improve the diagnostic accuracy of *E. coli* infection, a diagnostic panel with these 17 candidate peptides was established using the K-nearest neighbor (KNN) analysis. The best panel of peptides was selected through the stepwise method (entry criterion: $P < 0.05$ and exclusion criterion: $P > 0.10$). Among these peptides, 5 peaks at *m/z* 1941, 2924.1, 3962.1, 4126.9 and 5514 can be combined to establish a KNN diagnostic model to discriminate the discovery cohort with the highest accuracy of 92.2%, sensitivity of 95% and specificity of 70%. Among these 5 peptides, the peaks *m/z* 1941, 2924.1 and 3962.1 were less intense and peaks *m/z* 4126.9 and 5514 were more intense in the infected group. The corresponding *P*-values were shown in (Table 1).

3.5. Identification of peptide biomarkers by nano-LC/ESI–MS/MS

With the nano-LC/ESI–MS/MS and Uniprot search, we were able to identify the amino acid sequences of 3 peptides. Peaks *m/z* 1941, 2924.1 and 4126. 9 were identified as the fragments of TRF, C3 and SAA1, respectively. The sequences of the three peptides were shown in (Table 2).

3.6. ELISA

We further validated the serum expressions of TRF, C3 and SAA1 from 41 clinical samples using ELISA. The mean expressions of TRF, C3 and SAA1 in the infected group versus the control group were 1.656 ± 0.5872 vs. 2.032 ± 0.0673, 70.33 ± 34.06 vs. 221.4 ± 116.3, and 29.67 ± 24.74 vs. 5.873 ± 3.954 μg/ml, respectively. Comparison of the serum expressions of the above 3 proteins showed significant difference only in C3 and SAA1 ($P < 0.05$, Fig. 3). The corresponding receiving operating characteristic (ROC) curves were shown in (Fig. 4). These three combined biomarkers better discriminated the infected group from the control group compared with the sole biomarkers.

4. Discussion

Bacterial bloodstream infection (BSI) is a common infectious disease. As reported, the incidence of BSI outside China was 2% in 1990s and 4.7% in 2007, and the death rate was 23–45% [1,2]. According to Chinese reports, the incidence of BSI in some first-class hospitals rose from 103 cases in 2006 to 312 cases in 2009, showing a year-to-year uptrend [3,4]. Meanwhile, the costs of diagnosis and treatment also increased. The annual costs of medical treatment were up to 16.7 billion dollars in the United States and 6.7 billion dollars in Europe. Even with such huge costs, BSI still killed about 400,000 people each year [7,8]. Therefore, the diagnosis and treatment of BSI need much clinical attention. Since the primary disease variety and clinical manifestations vary widely, clinicians need to make appropriate diagnosis based on the early symptoms and laboratory tests, which highlight the clinical significance of early laboratory tests [9,10]. The current clinical diagnostic methods of BSI are limited and inefficient. Pathogenic culture, as the gold standard for the diagnosis, is limited in early diagnosis due to its large time-consumption and low positive rate (nearly 25–30%) [1,11]. Some infection indicators such as procalcitonin (PCT), C reactive protein (CRP), and interleukin 6 (IL-6) have low diagnostic values due to the low sensitivity and specificity [12–14]. Therefore, a novel, sensitive and efficient method for auxiliary BSI diagnosis is urgently needed.

Table 2
Identified peptides sequence of three differential peaks.

m/z	Amino sequences	Protein name
1941	FSSPLGKDLLFKDSAFGL	Serotransferrin (TRF)
2924. 1	SVQLMERRMDKAGQYLWENGNLLR	Complement C3 (C3)
4126. 9	FFSFVHEAFQGAGDMAAEKISDGREAPNYYRPPGLPD	Serum amyloid A-1 (SAA1)

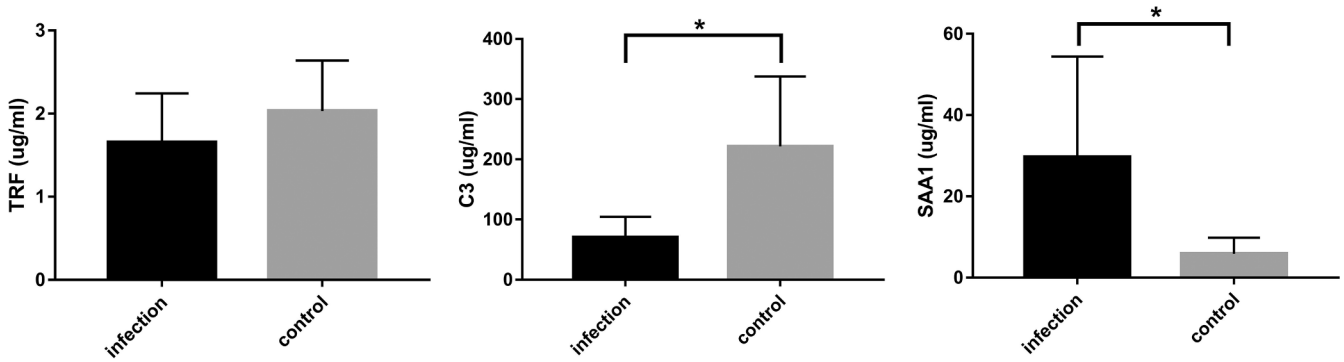

Fig. 3. Serum TRF, C3 and SAA1 levels were compared between the infected group and control group using ELISA. Results are expressed as mean ± SD. $^{*}P < 0.05$ (Unpaired T test).

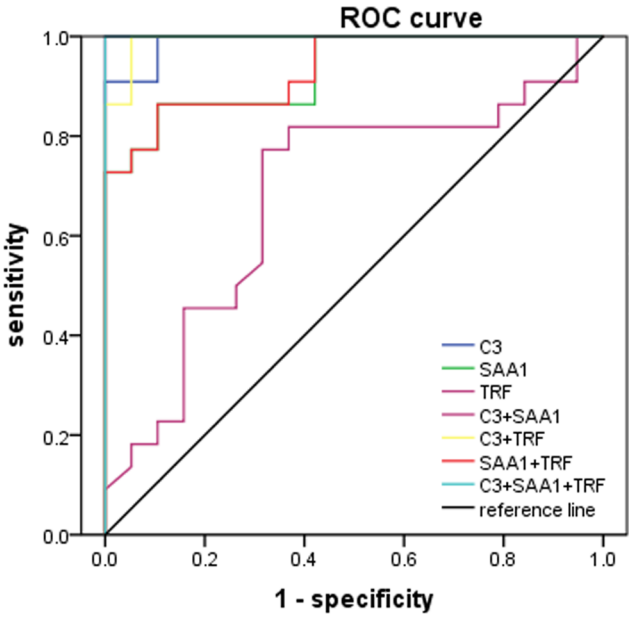

Fig. 4. ROC curves of TRF, C3 and SAA1 for the diagnosis between infected group and control group. The largest AUC for these three combined biomarkers was 0.99.

We found 17 peptide peaks with significant differences between the infected group and the control group. Then nano-LC/ESI–MS/MS successfully identified the amino acid sequences of 3 peptides (*m/z* 1941, 2924.1, 4126.9). Moreover, seven peptide peaks including peaks *m/z* 1941 and *m/z* 2924.1, declined in the infected group compared with the control group, suggesting these peptides are likely to combat bacterial infections and consume some of their own substances. Peptide peak *m/z* 1941 was identified as the fragment of TRF, a single-chain polypeptide protein with molecular weight of 75–90 KD (average 80 KD) and a beta 1-glycoprotein that can bind to iron in the serum [15]. TRF synthesized by liver cells and macrophages of lymphoid tissues has the main function of iron ion transportation in the body and is required for the liver and brain development. Iron ion is critical in maintaining cell membrane potential and in cell proliferation and redox reactions, including energy metabolism and intracellular electron transfer. TRF plays an important role in both hematopoiesis and bacterial immune function [16]. In our study, the TRF content declined after BSI, which may be attributed to three reasons. (1) In the reticuloendothelial system phagocytic clearing antigen, TRF is consumed by opsonization and may be degraded by granulocytes and cathepsin. (2) When the blood iron is largely captured in bacterial growth and reproduction, the TRF level decreases with the increasing serum iron consumption. Iron ion plays an important role in the biological oxidation, and is transported by most fungi and bacteria through iron ligands in vivo and then is absorbed by hemoglobin, heme and transproteins. (3) Some substances in the blood combine with TRF, which affects the detection of TRF. The increased iron ion release is beneficial to the bacteria growth [16]. Our MADLI-TOF MS and ELISA showed the TRF concentration in the infected group was lower than the control group, but not significantly. We speculated that it may be caused by the small sample size and will validate in large cohorts.

Peptide peak *m/z* 2924.1 was identified as the fragment of C3. C3 locates at the junction of the two activation pathways of the complement system, playing a pivotal role in the activation of this system, and is also a key molecule for the pathway activation [17]. Some gram-negative bacteria, yeast polysaccharide, glucose, and condensation of Ig (e.g. IgG4, IgA, and IgE) can be used to directly activate the alternative pathway. During bacterial infection, before the body produces the corresponding specific antibody, the complement bypass, which is important in anti-infection immunity, removes the pathogenic microorganisms [18]. Wang Q et al. found that C3 was higher in the infection group at 16 h after the infection comparing with the control group in the endotoxemia mice model [19], In our study we also found that C3 was significantly increased at 3 h after the *E. coli* injection in the mouse model and dropped sharply at 48 h (data not shown), which was consistent with the previous finding. However, the alteration of C3 might be different in human patients with severe diseases. In a recent case report [20], a 2-year-old boy with circulatory failure owing to streptococcal toxic shock syndrome showed that C3 was lower in the sepsis shock, indicating that C3 might be consumed in the severe phrase of infection. In our study, the enrolled inpatients were also accompanied with severe diseases and the immunity was reduced, so the concentration of C3 in patient group was lower than that of the normal control group in the ELISA test results.

The other ten peptide peaks were more expressed in the infected group than the control group. Among them, peptide peak *m/z* 4126.9 was identified as the fragment of SAA1, which is a member of the multigene encoding polymorphic protein family and is the precursor of tissue amyloid protein A, an acute phase reactive protein [21]. During the acute phase inflammation or infection, the SAA1 level increases rapidly from 48 to 72 h, and then decreases rapidly. The SAA1 level is upregulated after bacterial infection, viral infection, atherosclerosis, coronary heart disease, acute graft rejection, cancer and other diseases [22]. SAA1 is more sensitive than CRP to some diseases (e.g. viral

infection, graft rejection, coronary heart disease), which provides a better reference for clinical practice. SAA1 is the protein mainly regulated by cytokines (e.g. IL-1, IL-6 or tumor necrosis factor alpha) and would be produced in inflammation, injury, infection, tumor or some other situations [23]. In our study, SAA1 was up-regulated in the infected group compared with the control group, which is consistent with the previous study.

We found clinical serum TRF and C3 levels were lower and SAA1 level was higher in the infected group, as verified by ELISA in 41 specimens. Furthermore, the combination of these three biomarkers had a better discriminating power than the sole biomarkers, indicating this combination can be a good panel for BSI diagnosis.

This study has two limitations. (1) This study involved an animal experiment and some clinical samples tested using ELISA, which were still insufficient. Nevertheless, we will further validate in a large cohort of clinical samples. (2) Some of the peptides were not successfully identified, which may be due to some chemical modifications that interfered with the detection, including methylation and phosphorylation [24–26]. In the future, we will take further measures for successful identification.

5. Conclusion

The change of peptides may reflect not only protein content alteration or abnormal expression in body fluids, but also the overall protein quantity and dynamic response of some diseases. MALDI-TOF MS is one useful tool for separation and analysis of complex mixtures of proteins, and WCX magnetic beads could capture more peptides in the serum, especially in the low- molecular-weight range [27,28]. The captured proteins were then analyzed by MALDI-TOF MS to generate a spectral map that approximately depicted the molecular weight (m/z) and relative concentration (intensity) of each protein (ion). Our study was only preliminary and aimed to explore the feasibility of this method in BSI diagnosis. MALDI-TOF MS combined with WCX magnetic beads is expected to be a new method for BSI diagnosis, but should be further validated by large comprehensive clinical cohorts.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.04.010.

References

[1] C. Mahende, B. Ngasala, J. Lusingu, A. Butichi, P. Lushino, M. Lemnge, B. Mmbando, Z. Premji, Bloodstream bacterial infection among outpatient children with acute febrile illness in north-eastern Tanzania, BMC Res Notes 8 (2015) 289–296.

[2] J.A. Otter, T.J. Galletly, F. Davies, J. Hitchcock, M.J. Gilchrist, E. Dyakova, S. Mookerjee, A.H. Holmes, E.T. Brannigan, Planning to halve Gram-negative bloodstream infection: getting to grips with healthcare-associated Escherichia coli bloodstream infection sources, J. Hospital Infect. (2018), https://doi.org/10.1016/j.jhin.2018.07.033.

[3] Y. Xie, B. Tu, X. Zhang, J. Bi, L. Shi, P. Zhao, W. Chen, S. Liu, D. Xu, E. Qin, Investigation on outcomes and bacterial distributions of liver cirrhosis patients with gram-negative bacterial bloodstream infection, Oncotarget 9 (2018) 3980–3995.

[4] L. Tian, Z. Sun, Z. Zhang, Antimicrobial resistance of pathogens causing nosocomial bloodstream infection in Hubei Province, China, from 2014 to 2016: a multicenter retrospective study, BMC Public Health 18 (1) (2018) 1121–1128.

[5] A.K. Chang, M.D. Foca, Z. Jin, R. Vasudev, M. Laird, S. Schwartz, M. Qureshi, M. Kolb, A. Levinson, M. Bhatia, A. Kung, J. Garvin, D. George, P. Della-Latta, S. Whittier, L. Saiman, P. Satwani, Bacterial bloodstream infections in pediatric allogeneic hematopoietic stem cell recipients before and after implementation of a central line-associated bloodstream infection protocol: a single-center experience, Am. J. Infect. Control 44 (2016) 1650–1655.

[6] J. Duan, Y. Xie, J. Yang, Y. Luo, Y. Guo, C. Wang, Variation of circulating inflammatory mediators in Staphylococcus aureus and Escherichia coli bloodstream infection, Med. Sci. Monit. 22 (2016) 161–171.

[7] S.M. Mendis, S. Vasoo, B.D. Johnston, S.B. Porter, S.A. Cunningham, S.R. Menon, C.B. Teng, P.P. De, R. Patel, J.R. Johnson, R. Banerjee, Clinical and molecular correlates of escherichia coli bloodstream infection from two geographically diverse centers in Rochester, Minnesota, and Singapore, Antimicrob. Agents Chemother. 62 (2018) e00937–e1018.

[8] V.V. Lorenzoni, T.V. Dalmolin, L.N. Franco, A.L. Barth, R. Horner, Bloodstream infection by mcr-1-harboring Escherichia coli in a cancer patient in southern Brazil, Brazil. J. Infect. Diseases: an official publication of the Brazilian Society of Infectious Diseases 22 (2018) 356–357.

[9] S.B. Doernberg, T.P. Lodise, J.T. Thaden, J.M. Munita, S.E. Cosgrove, C.A. Arias, H.W. Boucher, G.R. Corey, F.D. Lowy, B. Murray, L.G. Miller, T.L. Holland, Gram-Positive Committee of the Antibacterial Resistance Leadership Group, Gram-positive bacterial infections: research priorities, accomplishments, and future directions of the antibacterial resistance leadership group, Clin. Infect. Diseases: an official publication of the Infectious Diseases Society of America 64 (2017) S24–S29.

[10] X. Wang, Z.Y. Li, L. Zeng, A.Q. Zhang, W. Pan, W. Gu, J.X. Jiang, Neutrophil CD64 expression as a diagnostic marker for sepsis in adult patients: a meta-analysis, Crit. Care 19 (2015) 245–253.

[11] M. Lesinska, M. Hartleb, K. Gutkowski, E. Nowakowska-Dulawa, Procalcitonin and macrophage inflammatory protein-1 beta (MIP-1beta) in serum and peritoneal fluid of patients with decompensated cirrhosis and spontaneous bacterial peritonitis, Adv. Med. Sci. 59 (2014) 52–56.

[12] J. Crouzet, J.F. Faucher, M. Toubin, B. Hoen, J.M. Estavoyer, Serum C-reactive protein (CRP) and procalcitonin (PCT) levels and kinetics in patients with leptospirosis, Eur. J. Clin. Microbiol. Infect. Diseases: official publication of the European Society of Clinical Microbiology 30 (2011) 299–302.

[13] P. Kopyra, A. Seremak-Mrozikiewicz, K. Drews, Usefulness of PCT, IL-6, CRP measurement in the prediction of intraamniotic infection and newborn status in pregnant women with premature rupture of membranes, Ginekologia polska 81 (2010) 336–341.

[14] K.S. Massaro, S.F. Costa, C. Leone, D.A. Chamone, Procalcitonin (PCT) and C-reactive protein (CRP) as severe systemic infection markers in febrile neutropenic adults, BMC Infect. Dis. 7 (2007) 137–144.

[15] M.C. Carlsson, P. Bengtson, H. Cucak, H. Leffler, Galectin-3 guides intracellular trafficking of some human serotransferrin glycoforms, J. Biolog. Chem. 288 (2013) 28398–28408.

[16] M. Golizeh, K. Lee, S. Ilchenko, A. Osme, J. Bena, R.G. Sadygov, S.R. Kashyap, T. Kasumov, Increased serotransferrin and ceruloplasmin turnover in diet-controlled patients with type 2 diabetes, Free Radical Biol. Med. 113 (2017) 461–469.

[17] Y.M. Kim, K.H. Park, H. Park, H.N. Yoo, S.Y. Kook, S.J. Jeon, Complement C3a, but not C5a, levels in amniotic fluid are associated with intra-amniotic infection and/or inflammation and preterm delivery in women with cervical insufficiency or an asymptomatic short cervix (< /= 25 mm), J. Korean Med. Sci. 33 (2018).

[18] S.J. Rotz, N. Luebbering, B.P. Dixon, E. Gavriilaki, R.A. Brodsky, C.E. Dandoy, S. Jodele, S.M. Davies, In vitro evidence of complement activation in transplantation-associated thrombotic microangiopathy, Blood Adv. 1 (2017) 1632–1634.

[19] Q. Wang, J.J. Wang, J.E. Fischer, P.O. Hasselgren, Mucosal production of complement C3 and serum amyloid A is differentially regulated in different parts of the gastrointestinal tract during endotoxemia in mice, J. Gastrointest. Surg. 2 (1998) 537–546.

[20] W. Keenswijk, J. Vande Walle, A 2-year-old boy with circulatory failure owing to streptococcal toxic shock syndrome: case report, Paediatr. Int. Child Health 38 (2018) 223–226.

[21] N. Xie, Z. Li, R. Zuo, S. Qi, T. Zhu, L. Liu, L. Wan, J. Yuan, Serum SAA1 and APOE are novel indicators for human cytomegalovirus infection, Sci. Rep. 7 (2017) 13407–13413.

[22] M. De Buck, M. Gouwy, N. Berghmans, G. Opdenakker, P. Proost, S. Struyf, J. Van Damme, COOH-terminal SAA1 peptides fail to induce chemokines but synergize with CXCL8 and CCL3 to recruit leukocytes via FPR2, Blood 131 (2018) 439–449.

[23] Y.L. Ko, L.A. Hsu, S. Wu, M.S. Teng, H.H. Chou, CRP and SAA1 Haplotypes are associated with both C-reactive protein and Serum amyloid A levels: role of suppression effects, Mediators Inflamm. 2016 (2016) 5830361–5830372.

[24] Q. Meng, S. Ge, W. Yan, R. Li, J. Dou, H. Wang, B. Wang, Q. Ma, Y. Zhou, M. Song,

X. Yu, H. Wang, X. Yang, F. Liu, M.A. Alzain, Y. Yan, L. Zhang, L. Wu, F. Zhao, Y. He, X. Guo, F. Chen, W. Xu, M. Garcia, D. Menon, Y. Wang, Y. Mu, W. Wang, Screening for potential serum-based proteomic biomarkers for human type 2 diabetes mellitus using MALDI-TOF MS, Proteomics, Clinic. Applicat. (2017) 11, https://doi.org/10.1002/prca.201600079.

[25] Y. Kitamura, R. Usami, S. Ichihara, H. Kida, M. Satoh, H. Tomimoto, M. Murata, S. Oikawa, Plasma protein profiling for potential biomarkers in the early diagnosis of Alzheimer's disease, Neurol. Res. 39 (2017) 231–238.

[26] J. Yang, X. Xiong, S. Liu, J. Zhu, M. Luo, L. Liu, L. Zhao, Y. Qin, T. Song, C. Huang, Identification of novel serum peptides biomarkers for female breast cancer patients in Western China, Proteomics 16 (2016) 925–934.

[27] S.H. Yeh, W.C. Chang, H. Chuang, H.C. Huang, R.T. Liu, K.D. Yang, Differentiation of type 2 diabetes mellitus with different complications by proteomic analysis of plasma low abundance proteins, J. Diabetes Metab. Disord. 15 (2015) 24–30.

[28] P. Dixon, P. Davies, W. Hollingworth, M. Stoddart, A. MacGowan, A systematic review of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry compared to routine microbiological methods for the time taken to identify microbial organisms from positive blood cultures, Eur. J. Clin. Microbiol. Infect. Diseases : official publication of the European Society of Clinical Microbiology 34 (2015) 863–876.