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Lipid mediators of inflammation as novel plasma biomarkers to identify patients with bacteremia

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Summary *Objectives:* Rapid diagnostic tests for bacteremia are important for early treatment to improve clinical outcome. We sought to identify plasma biomarkers that can identify patients with bacteremia using an untargeted global metabolomic analysis.
Methods: Plasma metabolomic profiles were analyzed for 145 adult patients with (cases) and without (controls) bacteremia using ultra-high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF-MS). All metabolites were compared between cases and controls using a 2-tier filtering approach, and each metabolite underwent receiver operating characteristic (ROC) curve analysis. Individual metabolites that distinguish between cases and controls were characterized. Subgroup analysis was performed to identify metabolites with prognostic significance.
Results: After 2-tier filtering, 128 molecular features were identified to be potential biomarkers that could distinguish cases from controls. Five metabolites had an area under the ROC curve (AUC) of >0.8 in ROC curve analysis, including a sphingolipid, an acylcarnitine, a

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fatty acid ester, and 2 glycerophosphocholines. These metabolites could distinguish cases from controls in the unsupervised hierarchical clustering analysis. Subgroup analysis of bacteremic patients showed that the level of trans-2,3,4-trimethoxycinnamate was lower in fatal than non-fatal cases.

Conclusions: Plasma lipid mediators of inflammation can distinguish bacteremia cases from non-bacteremia controls. These biomarkers may be used as targets for rapid test in clinical practice.

Introduction

Bacteremia is a major cause of morbidity and mortality.[1–3] Delayed treatment of bacteremia has been associated with a longer length of hospital stay and an increase in mortality.[4] Clinical criteria cannot reliably distinguish patients with or without bacteremia.[5] Procalcitonin and C-reactive proteins have been the most promising biomarker for bacterial infection, but studies have shown that neither of these can reliably predict bacteremia.[6] Differentiating bacteremia from other causes of fever such as tumor fever and autoimmune diseases is also clinically relevant. The identification of reliable markers of bacteremia for appropriate triage of such patients is therefore important.

Recently, the metabolite profile of micro-organisms has been employed in rapid identification of bacterial or fungal species, typing of outbreak strains and identification of microbial virulence factors using mass spectrometry.[7–10] Studies have also been conducted to examine the metabolomic profile of patients' blood during infection, including pneumonia,[11,12] urinary tract infection,[13] meningitis,[14] and sepsis.[15] Specific metabolites in serum or plasma have been associated with specific organisms causing human infections, including *Plasmodium* species,[16] *Mycobacterium tuberculosis*[17] and dengue virus.[18] These metabolites can be related to host metabolites from inflammatory response or from microbial metabolites. In this study, we used an untargeted metabolomic approach to examine and compare the plasma metabolomic profiles of patients with or without bacteremia using ultra-high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF-MS).

Patients and methods

Patients and clinical data analysis

This study was conducted in a university-affiliated teaching hospital with 1650 beds. Hospitalized adult patients 18 years or above with blood culture collected within 2 days of hospital admission were included. Consecutive patients with positive cultures growing bacteria were considered as bacteremia cases, while 50 patients with negative blood cultures (no growth after 5 days of incubation) were randomly selected as non-bacteremia controls. Archived plasma samples collected at admission were retrieved. Clinical information was entered into a pre-designed database. Patients were excluded if they were aged <18 years, their blood cultures were collected ≥ 3 days after hospital admission, their clinical records were not accessible for review, or if archived plasma samples were not available. The indication of a blood culture was considered appropriate if it fulfills the recommendation by Chandrasekar et al.[19] (See Supplementary Methods). Comparison of the demographics and outcomes were performed by Fisher's exact test and Mann Whitney U test for categorical variables and continuous variables, respectively. A P value of <0.05 was considered to be statistically significant. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority of Hong Kong.

Bacterial identification

The BACTEC 9240 blood culture system (Becton Dickinson, MD, USA) was used for processing of blood specimens as described previously.[1] Blood culture bottles were incubated for 5 days as recommended.[20] Bacterial isolates were identified using the VITEK GP, GN and ANC identification cards (bioMérieux Vitek Inc., Hazelwood, MO, USA).

Untargeted metabolomic profiling of plasma samples using ultra-high-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry (UHPLC-ESI-Q-TOF-MS)

Detailed methodology of the plasma sample analysis using UHPLC-ESI-Q-TOF-MS are presented in the supplementary methods section. UHPLC-ESI-Q-TOF-MS analysis was performed using Agilent 1290 Infinity UHPLC (Agilent Technologies, Germany) coupled with Agilent 6540 UHD Accurate-Mass QTOF system (Agilent Technologies, USA) accompanied with a MassHunter Workstation software for QTOF (version B.03.01 for Data Acquisition, Agilent Technologies, USA) as we described previously with modifications.[8,21] Multivariate analysis was applied to the liquid chromatography-mass spectrometry (LC-MS) data as described previously.[8] Only entries with at least 50% frequency present in either one of bacteremia and non-bacteremia groups were included for further statistical analysis to reduce noise. Molecular features (MFs) were further filtered using volcano plot based on $P < 0.01$. MFs were sequentially filtered using fold-change (FC) analysis. Stepwise reduction of the data dimensionality was followed by multivariate analyses principal component analysis (PCA) and partial-least squares discriminant analysis (PLS-DA). PCA was performed for unsupervised pattern recognition technique enabling data dimensionality reduction,