NMR-based metabolomics of bacterial infections studied in a mouse-model

V. Hoerr[1], G. Andonegui[2], L. Zbytnuik[3], P. Kubes[3], B. W. Winston[3], and H. J. Vogel[1]

[1]Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada, [2]Department of Critical Care Medicine, University of Calgary, Calgary, Alberta, Canada, [3]Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada

Introduction: The clinical diagnosis of bacterial infections traditionally requires the recovery of microorganisms in patients' blood, urine or specimen. The conventional culture methods are time-consuming, labour-intensive and show a high rate of failure, but are still routinely employed. In recent years, metabolomic studies applied magnetic resonance spectroscopy to define metabolic profiles of biological fluids. Combining metabolomic profiling and multivariate data analysis, different bacterial groups could be automatically identified in growing cultures [1] and allowed for the distinction between bacterial and viral meningitis by analyzing cerebrospinal fluid samples [2]. Here we demonstrate in mouse-models of *Streptococcus pneumoniae* and *Staphylococcus aureus* infection that NMR-based metabolomics can be a powerful tool to distinguish between two bacterial strains which are both anaerobic and gram-positive, regarding metabolic information of bacteria and host and using statistical pattern recognition techniques.

Experiments: *NMR-based metabolic profiling:* For NMR profiling, one-dimensional nuclear overhauser effect spectroscopy (NOESY) spectra were acquired on a 600 MHz Bruker Avance NMR spectrometer with a 5 mm TXI probe head equipped with z-gradient. The metabolomic profiles were created using targeted profiling in Chenomx NMR Suite 4.6. After normalizing the data to minimize the diurnal variation, the profiles were used as input in the software package SIMCA-P for statistical modelling. *Mouse-models*: The experiments were performed using C57BL/6 wild-type mice. 9 male mice were not treated and served as control. *Staphylococcus aureus:* 7 male mice were infected with *S. aureus* strain Xen 29 possessing a stable copy of the modified Photorhabdus luminescens lux ABCDE operon at a single integration site on the bacterial chromosome. 100 microliters of the bacterial suspension (1 x 10^8 CFU) were injected subcutaneously [3]. After 4 h and 24 h of infection, lesion development was monitored by bioluminescence and 300 µl of serum were collected. *Streptococcus pneumoniae*: 6 male mice were infected with *S. pneumoniae* strain SPN 15814 by transorally injecting 50 microliters of bacteria suspended in PBS (2 x 10^8 CFU) into the lungs [4]. After 24 h of infection serum samples were collected, pooled and included in the NMR study.

Results: *Metabolites:* 45 metabolites were identified and quantified in the mouse serum samples by NMR-based metabolomic profiling. The ^{1}H NMR spectra for infected mice were distinctly different form those for control subjects. In *Staphylococcus aureus* infected mice resonances attributed to malonate, ethanol, 2-hydroxybutyrate and the amino acids (phenylalanine, tryptophan, ornithine, lysine threonine, proline, histidine, alanine, creatine, leucine and isoleucine) were elevated while the significiant energy metabolites (2-oxoglutarate, glucose, citrate, succinate) were depressed. In contrast serum of *Streptococcus pneumoniae* infected mice showed significantly higher concentrations of leucine, creatine and taurine compared to the control. *Model generation:* For statistical analysis a two component PLS-DA model was applied to all samples. It produced scores plots with excellent separation between control and bacterial infected classes (Fig. 1). The corresponding cross-validation accuracy was 86 (Fig. 1A) and 67 (Fig. 1B) percent respectively.

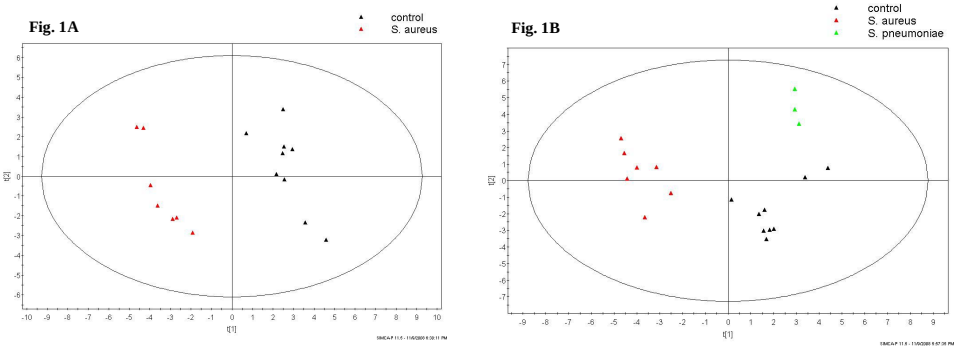
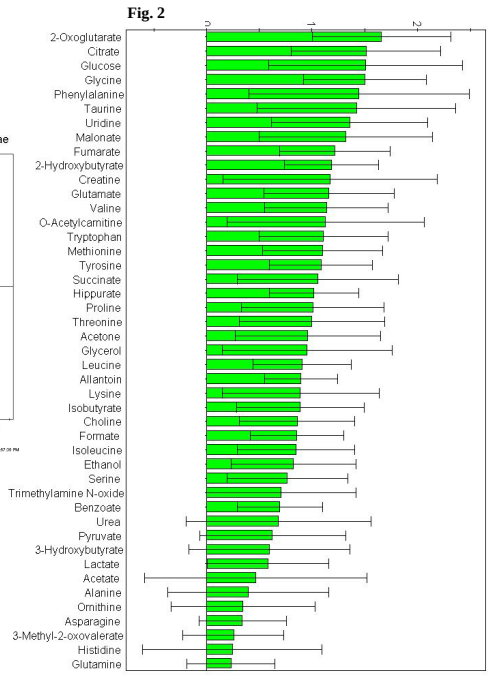


Fig. 1: Partial least squares discriminant analysis (PLS-DA) scores plots, representing ^{1}H NMR spectral data from control subject and mice with **(A)** *S. aureus* infection and **(B)** *S. aureus* and *S. pneumoniae* infection and showing the separation between samples along the type of infection. Two components encapsulate 97 % **(A)** and 83 % **(B)** of the interclass variation. **Fig. 2**: The variable importance plot (VIP) shows the relative contribution that each metabolite makes to the difference between the classes of the PLS-plot in Fig. 1(B).



Conclusion: Metabolomic technologies allow for the investigation of a large number of metabolites in small volumes of body fluids. In this pilot study we have shown by investigating serum samples of mice**,** that NMR-based metabolomic analysis can be a powerful diagnostic tool for distinguishing between different bacterial infections.

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References:

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