Metabolomics:
What is it?
How might it be useful in ALI?

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Conflict of interest disclosures

- None to declare
Overview/Objectives

- To understand the gravity ALI/ARDS
- To understand the principles behind metabol(n)omics
- To understand its potential utility in general.
- To gain some understanding how metabolomics may be used in ALI/ARDS:
  - As a biomarker or fingerprint of ALI/ARDS
  - As a potential diagnostic tool
  - As a marker showing response to therapy.
The burden and significance of ALI/ARDS

- SIRS in sepsis leads to ALI in ~30% of patients (Wenzel, NEJM, 2002)
- ~200,000 cases of ALI/ARDS annually in the US (2005) with a mortality rate of ~40% (Ware, NEJM, 2000)
  - onset of multiple organ dysfunction syndrome (MODS)- mortality ~62% (Jacobs, S., 1999)
- There are no acceptable predictive biomarkers of ALI/ARDS in sepsis, or ALI in general...
- Despite multiple novel therapeutic trials, overall outcomes have not changed.
- Clearly, we need to gain a better understanding of the disease and we need to better identify those who will respond to specific therapies.
- Sepsis-induced ALI/ARDS lends itself to biomarker discovery, but our knowledge and application of systems biology to this critical illness is still very new and evolving.
- As in septic shock (Kumar, A 2007), it may well be that early and appropriate diagnosis and treatment of ALI may save lives.
Metabol(n)omics

Why metabolomics?
➢ Can we use metabol(n)omics to:
   » identify patients at risk for ALI/ARDS?
   » establish a ‘fingerprint’ to better categorize patients into those who will respond to different types of therapies (like we see happening in cancer therapy)?
➢ Can we do this quickly to guide early and appropriate therapy?

➢ In general metabolomic fingerprinting of diseases in mice have been proven to be successful, however, human genetic variability makes this process much more complex than that seen in syngeneic strains of mice.
Metabolomics uses a systems biology approach to look at individual patients.

‘Omic’ type research:
- Genomics
- Transcriptomics
- Proteomics
- Metabolomics
What is Metabol(n)omics?

Cell
- DNA
  - mRNA
    - protein
      - metabolites

Technology
- Genomics
- Transcriptomics
- Proteomics
- Metabolomics

Systems Biology

Products
- Biomarkers

Diet
Why is ‘omics’ work so complex?

When you include lipids
the number of human metabolites increases to ~10,000 (and when you include all plant secondary metabolites the number is estimated at ~200,000)
Metabolic Pathways

Biochemical Pathways

http://www.expasy.ch
Metabolomics - definition

➢ The comprehensive and simultaneous systematic determination of metabolic levels in a whole organism in response to stimuli such as infection, diseases, diet, lifestyle, drugs, and genetic effects. (Lindon, et al. FEBS J, 2007, 274:1140)

➢ Unlike genomics and proteomics, quantitative metabolomics is in its infancy.

➢ There are several methods one can use to identify metabolomic profiles (molecules < 1kDa in size):
  » NMR Spectroscopy – little sample prep, quantitative, non-destructive
  » Mass Spectrometry – more samples prep required
    » Gas Chromatography-MS (GC-MS) – less sample, more sensitive
    » Liquid Chromatography-MS (LC-MS) – complex sample isolation
A modern shielded NMR instrument

600 MHz instrument in the Bio-NMR Centre
Explaining biofluid NMR spectroscopy

*NMR is a physical phenomenon* utilized to investigate molecular properties of matter by irradiating atomic nuclei in a magnetic field with radio waves.
Proton NMR spectrum ultrafiltered mouse serum

Weljie et al (2007) J. Proteome Res. 6, 3456
Gas Chromatography Mass Spectrometry (GC-MS)

- Provides access to different metabolites in the gas-phase or polar soluble metabolites that have been made volatile through chemical derivatization.
- GC-MS is complementary to NMR metabolomics, as it analyzes other groups of metabolites.

Both NMR and GC-MS: Quantitative!
GC-MS Made Rediculously Easy

http://www.shimadzu.com/products/lab/ms/swf/retention.html
What is the experience to date with metabolomics as it applies to critical care.

- Mouse work
- Human work
Can we identify specific infections in mice? NMR-based metabolomics of bacterial infections studied in a mouse-model

- Hypothesis:
  - Using mouse-models of *Streptococcus pneumoniae* and *Staphylococcus aureus* infections, NMR-based metabolomics can be a powerful tool to distinguish between two bacterial strains which are both aerobic and gram-positive, regarding metabolic information of bacteria and host and using statistical pattern recognition techniques.
Methodology

- NMR-based metabolic 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.

- The profiles were then used as input in the software package SIMCA-P for statistical modelling.
  - Multivariate analysis was done using:
    - PCA (unsupervised) - principal component analysis or
    - OPLS-DA (supervised) – orthogonal partial-least-squares discriminate analysis
Mouse-models:

- The experiments were performed using C57BL/6 wild-type mice.
  - 9 male mice - untreated controls.
- *Staphylococcus aureus*: 7 male mice were infected with *S. aureus* strain Xen 29.
  - 100 µl of the bacterial suspension (1 x 10^8 CFU) were injected subcutaneously. After 4 h and 24 h of infection, 300 µl of serum were collected.
- *Streptococcus pneumoniae*: 6 male mice were infected with *S. pneumoniae* strain SPN 15814.
  - Intratracheal (IT) injection of 50 µl of bacteria suspended in PBS (2 x 10^8 CFU) into the lungs. 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 one-dimensional NOESY spectra for infected mice were distinctly different from those of control mice.
  - *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 significant energy metabolites (2-oxoglutarate, glucose, citrate, succinate) were depressed.
  - *Streptococcus pneumoniae* infected mice showed significantly higher concentrations of leucine, creatine and taurine compared to the controls.
Model generation:

For statistical analysis a two component OPLS-DA model was applied to all samples. It produced scores plots with excellent separation between control and bacterial infected...
Score plot of 3 component OPLS-DA analysis of Control, S. aureus and S. pneumoniae separation.
From mouse models to humans?
Can we separate patient profiles from serum samples taken from 4 distinct patient groups?

- 4 groups of patients:
  - Control volunteers (C), N = 12
  - ICU post-op non-infected controls (O), N = 9
  - Patients with pneumonia admitted to a general ward (N), N = 10
  - Patients with pneumonia and septic shock admitted to the ICU (S), N = 10

- Samples drawn within 12 h of admission.
  - Clinical data and APACHE II scoring.
The PLS-DA model distinguishing patients with septic shock (S), pneumonia (N), patients that were post-operative uninfected ICU controls (O), and the control groups (C).
The PLS-DA loadings plot highlight the metabolites important in the model differentiating between the various patient groups.
Can we do this type of work on sepsis on a large scale minimizing sample material? A preliminary pediatric study.

- Analysis of 40 pediatric ICU patients:
  - 20 control
  - 20 septic shock
    - 10 Gm +
    - 10 Gm -

- Samples from Dr. Hector Wong, Cincinnati.

- Samples analyzed by $^1$H-NMR and by CG-MS.
Methods

- NMR-based metabolic profiling:
  - 200 µl of serum was filtered through a 3 Kd filter and pH 7.0 buffered sample (diluted to 650 µl) was analyzed.
  - 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. Relative values for metabolites were determined.
  - The profiles were then used as input in the software package SIMCA-P for statistical modelling. A totally unbiased (unsupervised) principle component analysis as well as a supervised PLS-DA analysis were done.
Unsupervised PCA analysis Shock vs Control
Supervised OPLS-DA Shock vs Control
Shock vs controls - metabolite concentrations
Can we identify Gm+ vs Gm- sepsis: Supervised analysis
Gm+ vs Gm- comparisons - metabolite concentrations
We have begun to look at ICU-related diseases but first examined ICU controls. What is the appropriate control to use?

- If we can see different metabolomic profiles in our controls we will likely see differences in those with disease.
- Three control groups:
  - Normal controls – healthy walk-ins
  - ICU controls:
    - Elective post-op non-infected (e.g. posterior fossa mass removal or spinal surgery for spinal stenosis)
    - Elective post-op CV-ICU patients who underwent coronary artery bypass surgery off pump.
- Plasma analyzed by $^1$H-NMR and GC-MS
$^1$H-NMR PCA analysis data
$^1$H-NMR metabolomic abundance and distribution
GC-MS OPLS-DA analysis model
($R^2_Y=0.89$, $Q^2 = 0.513$)
Histogram of relative abundance of metabolites of GC-MS
GC-MS OPLS-DA of just the ICU controls (CV vs ICU)

R² = 0.978
Q² = 0.351
p value 0.56
Metabolomics in ALI/ARDS – what is the published experience to date

- 5 papers, 3 with data
  - one is a mouse study
  - Very few patients in the 2 human studies – 19 and 20 patients and controls
- Look at the 2 human studies
Stringer, K.A., et al., 2010, AJP Lung Cell Mol Physiol

Pilot study

- Patients with ALI/ARDS by ERS/ATS criteria
- Study set up to examine effects of GM-CSF vs placebo
- Substudy compared plasma $^1$H-NMR profile of pts (13) 3-7 days from onset of ALI/ARDS vs healthy, med free, non-smoking volunteers ≥ 21 years (6).

- $^1$H-NMR 40 metabolites quantified:
  - Glutathione’
  - Adenosine
  - Phosphatidylinerine
  - Sphingomyelin

- Shows that sepsis-induced ALI caused measurable changes in biologically relevant metabolites.
Pilot study to examine plasma $^1$H-NMR as a means to monitor metabolism following albumin administration in ALI patients.

Substudy of a larger double blind trial.

» Mech vent hypoprotinemic pts who meet ERS/ATS criteria for ALI, randomized to receive furosemide by continuous infusion + 100 ml Alb 25% q8h x 9 doses (n=6) vs 100 ml 0.9% saline x 9 doses (n=6). 8 healthy controls.

» Plasma isolated at 1, 4, and 7 doses

» $^1$H-NMR metabolomics performed, PCA analysis of data
There is separation of the alb and placebo groups over time.

**Figure 3.** Principal component analysis of samples according to day of treatment. All samples were plotted in panels A–C. (A) On day 1, there was no separation of albumin-treated (red) or placebo-treated (blue) samples from the baseline samples (green) taken before initiation of treatment. The samples of other days were color coded cyan. (B) On day 2, samples from albumin-treated patients were clearly separated from those from placebo-treated patients. (C) On day 3, samples from albumin-treated patients were separated from those from placebo-treated patients. Data were analyzed with variance scaling. PC1, principal component 1; PC2, principal component 2; PC3, principal component 3.
Study concluded

- ¹H-NMR may be useful to evaluate the effects due to albumin therapy on plasma metabolites in critically ill patients with ALI.
- Metabolomic profiling may permit more focused trials in ALI patients, particularly monitoring treatment effects.
Summary

- For metabolomics analysis you first need access to human samples – although serum is preferred by some, plasma and urine can also be used.
- Metabolomics by: NMR, GC-MS or LC-MS.
- There is promise in using metabolomics in ALI/ARDS but very few studies.
- Based on few studies, we are encouraged to continue studies and are hopeful that metabolomics will allow us:
  » To help diagnose etiology and severity of disease
  » To help define those that will respond to specific therapy
  » To help to identify those at risk of disease progression
  » To help identify those that are responding to therapy
- We are still in a very early stage of these types of studies.
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There is an opportunity to shape the course of translational science and knowledge translation.