Using Metabolomics for H1N1 Pneumonia Diagnosis and Prognosis

Mohammad Mehdi Banoei
**Metabolomics**

- Metabolomics = high-throughput analysis of metabolome.
- Metabolome = the total metabolite pool.
- All low molecular weight (MW < 1000 Da) organic molecules in a sample such as a Plasma, Serum, Urine, CSF, Saliva, and etc.

- Metabolites:

  - Peptides
  - Sugars
  - Organic acids
  - Aldehydes
  - Amino acids
  - Steroids
  - Drugs (xenobiotics)
  - Oligonucleotides
  - Nucleosides
  - Ketones
  - Amines
  - Lipids
  - Alkaloids
Metabolomics?

**What is possible**

**What appears to be happening**

**What makes it happen**

**What is happening**

*DNA*

*RNA*

*Proteins*

*Metabolites*

Material

Information

Phenotype
<table>
<thead>
<tr>
<th>Analytical platforms</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Type of Metabolites Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NMR</strong></td>
<td>Robustness and reproducibility</td>
<td>Metabolite overlapping</td>
<td>Sugar</td>
</tr>
<tr>
<td></td>
<td>No Sample preparation</td>
<td>Lower sensitivity</td>
<td>Amines</td>
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<td></td>
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<td></td>
<td>Volatile liquid</td>
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<td></td>
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<td>Large Metabolites</td>
</tr>
<tr>
<td><strong>GC-MS</strong></td>
<td>Excellent sensitivity</td>
<td>Need to derivatize</td>
<td>Volatile compounds</td>
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<td></td>
<td>Comprehensive Database</td>
<td>Destructive to the samples</td>
<td>Carbohydrates</td>
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<td>Esters</td>
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<tr>
<td><strong>LC-MS</strong></td>
<td>Excellent sensitivity</td>
<td>Lower reproducibility than GC</td>
<td>Medium to high lipophilicity,</td>
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<tr>
<td></td>
<td>High Resolution</td>
<td>Destructive to the samples</td>
<td>Fatty Acids</td>
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</tbody>
</table>
Multivariate Data Analysis (Statistical Methods)

- Simplifying large data sets for human consideration
  - Clustering and Principal Components.

- Pattern Recognition:
  - Classifying unknowns into previously defined groups.

- Principal Component Analysis (PCA)
  - unsupervised

- Orthogonal Partial Least Square- Discriminant Analysis (OPLS-DA)
  - supervised
Question?

- Can we use plasma metabolomics to determine the patients at highest risk of dying from H1N1 pneumonia on presentation to the ICU?

- Diagnosis and prognosis
Results
H1N1 Samples

Examined plasma from 21 patients with H1N1 pneumonia.

- List of blood plasma samples with corresponding to sample number, age and sex.

<table>
<thead>
<tr>
<th>H1N1 Sample</th>
<th>Death†</th>
<th>Survivor†</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2 (62-73)</td>
<td>4 (57-73)</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>5 (28-74)</td>
<td>10 (26-69)</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

† Numbers in brackets indicate the distribution of age for each group.
Results (PCA Analysis)

- 54 metabolites were detected by NMR (all known)
- 273 features were detected by GC-MS (110 Known)

The unsupervised PCA analysis shows the general separation (clustering) and finds the outlier.
The supervised OPLS-DA analysis shows the best possible prediction and interpretation of discrimination between nonsurvivors and survivors.

20 metabolites NMR-based for OPLS-DA analysis (All known)

63 features GC-MS based for OPLS-DS analysis (30 Known & 33 unknown)
Results (Coefficient Plot)

**A (NMR)**

- 2- Hydroxyisopropanol
- 2- Oxoglutamate
- Methionine
- Dimethylamine
- Fumarate
- Lactate
- Acetate
- Tyrosine
- Phenylalanine
- 3-Hydroxybu
- Adipate
- Creatinine
- Proline
- Ornithine
- Acetoacetate
- 2-Hydroxybutyrate
- Arginine
- 2- Aminobutyrate
- Mannose

**B (GC-MS)**

- Glucopyronnoside
- Isocaproic acid
- Decanoic acid
- Dodecane
- Butanoic acid
- Glycerol
- Fructose
- Octadecane
- Siloxane
- Theronic acid
- Hepatadecane
- Mannitol
- Phosphoric acid
- Galactose
- Valine
- Tridecane
- Glyceric acid
- Glucose
- Isoleucine
- Pyroglutamic acid
- Hydroxylamine
- Alanine
- Heneicosan
- Pyruvic acid
- Octadecadienoic acid
- Methionine

Coefficient plot refers to scaled and centered metabolites’ data, with confidence interval derived from jack-knifing.
Top biological pathways (detected by MetaboAnalyst and IPA based on NMR and GC-MS data)

<table>
<thead>
<tr>
<th>NMR (MetaboAnalyst)</th>
<th>NMR (IPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis and degradation of ketone bodies</td>
<td>Tyrosine degradation I</td>
</tr>
<tr>
<td>Arginine and Ornithine metabolism</td>
<td>Superpathway of Cirtulline metabolism</td>
</tr>
<tr>
<td>Arginine and Proline metabolism</td>
<td>Arginine degradation</td>
</tr>
<tr>
<td>Phenylalanine metabolism</td>
<td>Arginine biosynthesis</td>
</tr>
<tr>
<td>Citrate cycle (TCA cycle)</td>
<td>tRNA charging</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GC-MS (MetaboAnalyst)</th>
<th>GC-MS (IPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-Alanine metabolism</td>
<td>tRNA charging</td>
</tr>
<tr>
<td>Galactose metabolism</td>
<td>Alanine degradation</td>
</tr>
<tr>
<td>Alanine, Asparate and glutamate metabolism</td>
<td>Alanine biosynthesis</td>
</tr>
<tr>
<td>Glycerolipid metabolism</td>
<td>T cysteine degradation</td>
</tr>
<tr>
<td>Pyruvate metabolism and glucolysis or glucogensis</td>
<td>Superpathway of Methionine</td>
</tr>
</tbody>
</table>
Summary

• Using blood plasma samples, we created an OPLS-DA model that separate two cohorts of patient with H1N1 pneumonia, identifying those patients at high risk for death.

• Both GC-MS and NMR showed similar results adding strength to our findings
Summary

• The GC-MS data showed very good sensitivity and was quite specific in separating survivors and non-survivors at 90 days from a sample drawn within the first 24 hours of admission.

• Using a combination of NMR- and GC-MS-based platforms will help to enhance our understanding of the disease process especially when we examine pathways involved in the disease.
Acknowledgement

Dr. Brent W. Winston
Dr. Hans J. Vogel
Dr. Aalim M. Weljie
Dr. Anand Kumar
Dr. Chip Doig

Josee Wong
Farshad Farshidfar
Rustem Shaykhutdinov
Beata Mickiewicz

Granting Agencies:
University Research Granting Council (URGC),
Faculty of Medicine/ AHS, Team Grant,
AIHS/ Alberta Sepsis Network
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<tr>
<td>NMR Spectroscopy</td>
<td>Non-destructive to the sample</td>
<td>Less sensitive than other techniques but can detect nM with cryoprobes</td>
<td>Sugars, Amines, Volatile liquids, Large Metabolites</td>
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<tr>
<td></td>
<td>Quantitative</td>
<td>Requires large sample volumes</td>
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<td></td>
<td>Simple sample preparation</td>
<td>Very reproducible</td>
<td></td>
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<tr>
<td>UHPLC-MS</td>
<td>Short separation time</td>
<td>Destructive to the sample</td>
<td>Medium-to-high lipophilicity, Nucleosides and nucleotides, Lysophosphatidylcholines, Acylcarnitines, Acylglycines, Polar metabolites, Amino and organic acids, Non-polar metabolites (Fatty acids, Steroids, Eicosanoids, Carbohydrates, Esters, Phosphorylated metabolites, Cholesterol, Volatile compounds)</td>
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<tr>
<td></td>
<td>High resolution</td>
<td>Reproducibility of retention times between different systems is difficult</td>
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<td></td>
<td>High mass accuracy</td>
<td>Quality of CID mass spectra are dependent on intensity of molecular ion</td>
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<td>Simple sample preparation</td>
<td>Ion suppression can occur with co-eluting compounds</td>
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<td>Very sensitive</td>
<td>Can analyze a wider range of metabolites than GC-MS</td>
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<td>GC-MS</td>
<td>Reproducible retention times</td>
<td>Extensive sample preparation</td>
<td>Non-polar metabolites, Fatty acids, Steroids, Eicosanoids, Carbohydrates, Esters, Phosphorylated metabolites, Cholesterol, Volatile compounds</td>
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<td></td>
<td>Comprehensive databases for metabolite identification</td>
<td>Samples must be volatile so require derivitization</td>
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<td>Greater sensitivity for free fatty acids than LC-MS</td>
<td>Possible variation introduced due to sample preparation</td>
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<td>Does not suffer from ion suppression</td>
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NMR and GC-MS Spectrum
Summary of pathways analysis with MetaboAnalyst based on NMR data.
Summary of pathways analysis with MetaboAnalyst based on GC-MS data.

Biological Pathways

1. Beta-Alanine metabolism
2. Galactose metabolism
3. Glycerolipid metabolism
4. Alanine, Asparate and Glutamate metabolism
5. Pyruvate metabolism