Phenotyping of multiple biofluids for liquid biomarkers for diagnostics and personalized medicine

By Allan Stensballe; PhD. (ATV)
Phenotyping for enhanced diagnostics and personalized medicine – A focus on autoimmune diseases & Inflammation

- More than 80 diverse autoimmune diseases affect 6.4% percent of women vs. 2.7% of men.
- Researchers don’t know exactly what causes many autoimmune diseases.
- Genetics, diet, infections, and exposure to chemicals might be involved.
- Many subtypes remain excessively difficult to validate and no single test can diagnose most autoimmune diseases.
- Low grade inflammation is central in most pathologies.
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Personalized and precision medicine are key a focus in the future.
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Personalized Rheumatic Medicine through Dose Reduction Reduces the Cost of Biological Treatment – a retrospective intervention analysis

Accumulated savings above 2mill AUD within 10 years
Improved and truly translational biomarkers are needed

- Enabling technologies including all Omics technologies paves the way to understanding disease etiology.
- Key to insight remains the ability to find good answers to relevant clinical questions
- Why responders and non-responders?

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The transition at birth and the preterm problem

Preterm infants are susceptible to sepsis, which in turn may lead to neurodevelopmental disorders.

Milk and microbiota effects on immunity, gut and brain development is poorly understood.
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Preterm: Inflammation

Normal

Protected environment

Blood-brain barrier

Lung/gut barrier

Lung

Liver

Gut

Brain

Oxygen

Milk

Microbes
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Challenged environment:
- Oxygen variability
- Milk mal-digestion
- Bacterial dys-colonization

Protected environment:
- Normal lung/gut barrier
- Normal blood-brain barrier

Milk:
- Oxygen
- Milk
- Microbes

Lung:
- Lung/gut barrier

Liver:
- Blood

Gut:
- Normal

Brain:
- Preterm:
  - Inflammation

NEOMICS
Tik Muk
Azra Karamehmedovic
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Preterm: Inflammation
- RDS, BPD, CLD
- PNALD
- FI, NEC
- LOS, DIC
- ND, PVL, CP, IVH, WMD

Microbes
- Milk
- Oxygen

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Sepsis-induced changes in cerebrospinal fluid affect proteome of the developing hippocampus - correlation to systemic changes.

- How systemic infection affects brain development and functions is poorly understood.
- Good biomarkers for early diagnosis and treatment for sepsis-induced brain injury are lacking.
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Preterm infant (GA<32 wk)

CSF Control  CSF Sepsis

Plasma Control  Plasma Sepsis

**Formula-fed 5d preterm pigs (90% GA)**

CSF Control  CSF Infection

*in vitro* stimulation

Primary hippocampal slices derived from preterm pigs

Collect culture medium and tissue

**Brain Inflammation**

Lung/gut barrier

Blood-brain barrier

Normal

Blood

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Omics analysis of Dried whole blood samples – Whole blood analysis

- Dried blood spots offer many advantages as a sample format including ease and safety of transport and handling.
- Dried blood spots are a potentially rich source of protein biomarkers, an area that has been overlooked.
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Finger puncture whole blood

FASP extraction

5% SDC

S-TRAP

Single column setup (25cm ionoptiks) and Bruker timsTOF PRO PASEF

Data analysis
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Finger puncture whole blood → FASP extraction → 5% SDC → S-TRAP → Single column setup (25cm Ionoptiks) and Bruker timsTOF PRO PASEF → Data analysis → PEAKS’X

Deep proteome coverage reducing missing values for diagnostics and prognostics
Dynamic molecular changes during the first week of human life follow a robust developmental trajectory

Systems biology can unravel complex biology but has not been extensively applied to human newborns, a group highly vulnerable to a wide range of diseases.

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Improved phenotyping of disease subtypes by Immunome array

- Elevated levels of autoantibodies are present before the clinical diagnosis of autoimmune diseases

Antibodies (also called immunoglobulins) are large Y-shaped proteins. They are found in the blood or other body fluids of vertebrates. Antibodies are the key element in the adaptive immune system.

Proportion of Patients with Positive Antibody Tests Relative to the Time of Diagnosis or Appearance of the First Clinical Manifestation of Systemic Lupus Erythematosus

- Systemic Lupus Erythematosus Up to 28yr
- Rheumatoid Arthritis – Up to 16yr
- Multiple Sclerosis - Up to 14yr
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- *State-of-the-art* personalized protein array technology allows phenotyping of autoantibodies.

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Digging deeper into the autoantibody-ome in biofluids

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- IMMUNOME Discovery Array is a protein array which utilizes the patented Sengenics KREX™ functional proteomics technology.
- The array contains human proteins from biologically significant protein families including kinases, signalling molecules, cytokines, interleukins, chemokines and cancer antigens.
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Glucocorticoid Responsive mechanisms in plasma from Polymyalgia Rheumatica patients

Polymyalgia rheumatica is a relatively common inflammatory rheumatic disease.

There are no validated international guidelines available for the diagnosis and treatment of PMR; however, diagnostic and classification criteria are currently being developed.

A quantitative proteome study design to compare with DMARD naïve RA patients and matched controls

- 9 patients included in the study;
- Serum samples before versus after 3 months treatment
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Translational Biomarker Research Unit, Aalborg University, Denmark

- Svend Birkelund
- Tue Bjerg Bennike
- Thomas B. G. Poulsen
- Joakim Bastrup
- Ditte Kristensen
- Kenneth Kastanjegaard
- Michael Kruse Meyer

Sangild Lab
- Tik Muk
- Ping ping Jiang
- Ninh Duc Nguyen

Other Important People for the presented work
- Malene Møller Jørgensen
- Rikke Bæk
- Rikke Gry Nielsen (vet)
- Jonathan Blackburn - Sengenics

Danish National Hospital
- Claus H. Nielsen
- Dres Damgaard

Funding sources

- LUNDBECK FOUNDATION