**Targeted Metabolomics for Subgroup Discrimination in Breast Cancer**

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**Methods and Materials**

Tissue and plasma samples from ~80 breast cancer patients were collected at the Institut Curie in Paris. The frozen tissues (tumour or healthy surrounding tissue) were homogenized in ice-cold ethanol/phosphate buffer. Plasma samples were centrifuged in the presence of a stabilizing agent (BHT) to prevent oxidation. A multi-parametric (LC-MS/MS, FIA-MS) targeted, mass-spectrometry-based analytical platform was used to quantify hundreds of metabolites from different biochemical classes (see table below). MRM detection was performed using an API 4000™ and a 4000 QTrap® tandem mass spectrometer (AB Sciex), quantitative data were obtained using Analyst 1.4.2 Software (AB Sciex) and finally exported for statistical analysis.

**Results: tissue**

**MALIGNANT vs HEALTHY TISSUE:**

The metabolic profile of the tumour tissue was compared with the non-tumour tissue for each patient. A general significant increase (Wilcoxon T-test, p < 0.05) in all metabolite classes was observed.

This increase was particularly evident for amino acids (see figure 1).

**Conclusion**

The main purpose of this metabolic disease characterization was to obtain a tumour tissue-specific metabolite profile that will serve as the basis for the identification of cancer-related biomarkers in plasma, especially under the aspect of cancer recurrence after surgery and treatment. Our preliminary analysis shows that we can find promising discriminators for different types of breast cancer amongst acylcarnitines, phosphatidylincholines and other metabolic classes.

**Acknowledgments:**

We would like to thank the BIOCRATES Contract Research Team and Methods Development Team which made this study possible.

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**Introduction**

Breast cancer (BC) is the most common cancer in Western women, affecting 0.6 million people annually. It is known to be a heterogeneous disease difficult to classify and therefore difficult to target with the most effective therapy. (The) BC subtype discrimination is made by looking at the morphology, molecular profile and response to therapy. However, the identification of specific groups is still controversial.

The multi-platform EU project COBRED was launched in 2005 in order to advance the methodological repertoire for cancer diagnosis and follow-up. The aim of this project is to discover suitable biomarkers for breast and colon cancer diagnosis using a multiplex–omics technology approach. Part of the project comprises the metabolic characterization of plasma and tissue samples from 80 BC patients.

Metabolomics is a non-invasive technology, which enables simultaneous measuring of hundreds of macro- and micromolecules, as well as functional monitoring of multiple pivotal cellular pathways, and allows to draw a dynamic portrait of the metabolic status of living system in different states. Through this approach, we were able to identify some metabolites which specifically change in certain disease condition, even at this preliminary stage. Following histo-pathological and physiological parameters, we were able to find some difference at the metabolic level, which may serve to improve BC diagnosis and therapy.

**BC SUBTYPES:**

Cancer patients were divided in subgroups according to the receptors expression (PR, ER, Her2+) to assess changes associated with:

- TN (with no receptors)
- Her2+ (with the Her2 over-expressing receptors)
- Others (no match the previous criteria)

The general outcome of subgroup division’s analysis is given in figure 3 and the most significant metabolite differences are shown in figure 4.

**BC and MENOPAUSAL STATUS:**

Statistically significant changes after tumour removal were assessed in respect to the menopausal status. Groups were divided into NO (no menopause), PreM (in the process of transition to menopause) and YES (menopause) (see figure 5).

**BC and FAMILY HISTORY OF BC:**

Statistically significant changes after tumour removal were evaluated in respect to the presence of other cases of BC in the patient’s family. Groups were divided in YES (other family case of BC) and NO (first case of BC in the family) (see figure 6).

**Results: plasma**

Normalized plasma metabolite levels were compared in samples taken immediately before surgery (1), and one month after surgery but before therapy (2). Statistically significant changes were detected by the Wilcoxon paired test.

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