Summary

The recent advances in mass spectrometry (MS) technologies have made MS-based approaches a promising tool for biomarker discovery in human samples. Blood represents the sample of choice for clinicians as it is easily accessible and repetitive sampling is possible. However, many challenges are faced with MS analysis of blood proteomics. The blood proteome is believed to be the most complex biological sample as it contains not only classical blood proteins but also proteins derived from cells. It is characterized by a very wide dynamic range spanning over 10 orders of magnitude. Moreover, few proteins represent more than 95% of the total protein content of blood. In order to overcome these challenges, many technologies have been developed in order to access the low abundant proteins that are most likely to be disease markers. High abundant protein removal and excessive fractionation seemed to be imperative to reach these goals. Proteins carrying posttranslational modifications (PTMs) are known to play a pivotal role in many diseases and among them cancer. Targeting these PTMs by different enrichment methods will lead to the increase of the blood proteome coverage as well as the identification of potentially modified biomarkers.

Therefore, we have developed a comprehensive PTMomics strategy for blood proteome characterization. The strategy included the enrichment of phosphopeptides, formerly sialylated N-linked glycopeptides, cysteine-containing peptides and non-modified peptides combined with a 2D offline fractionation prior to LC-MS/MS analysis. The strategy was then applied to two independent studies.

First, we have sought to compare the plasma proteome and PTMome of patients with benign pelvic mass to patients with ovarian cancer. In this study, we have identified 7 formerly SA N-linked glycopeptides that were able to clearly distinguish the patients with benign and malignant pelvic masses. These peptides could serve as potential biomarker to predict ovarian cancer.

On other hand, we applied the developed strategy to characterize serum from gastric cancer patients. Many cancer-related mechanisms such as glycolysis, PPP, vesicle transportation and proteolysis were identified to be upregulated in late stage gastric cancer.