Metabolomics of urinary tract infection : a multiplatform approach

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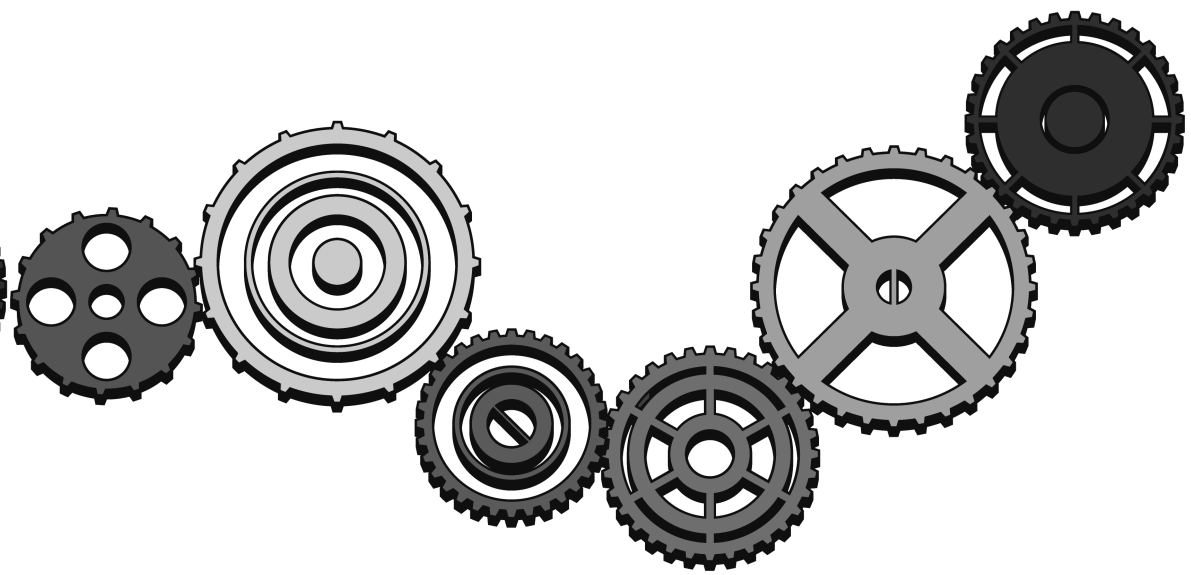
General discussion and conclusion



Metabolomics is a technology driven discipline but unlike genomics or even proteomics there is not a single analytical platform which enables a comprehensive overview of the entire metabolic space. One can always find an argument in favor of (or against) a particular analytical solution but, regardless of the argumentation, any analytical technology provides only a slice of the metabolome. Thus, with this thesis one particular clinical problem has been studied, namely urinary tract infection, using several analytical approaches: this included well-established methods such as NMR and LC-MS, as well as a novel GC-APCI-MS platform. Each platform was used for the investigation of the urinary metabolite profile of a carefully matched selection of UTI patients and controls.

In chapter two we used a non-targeted profiling by NMR showing the number of metabolites such as acetate, lactate and trimethylamine which are correlated with number of bacteria (CFU/ml). However, more interesting results using the NMR technique were obtained when the longitudinal axis of the study design was used rather than the simpler case/control approach. A few compounds such as p-aminohippuric acid and scyllo-inositol were proposed as the markers of the recovery process. Those compounds could reflect the renal plasma flow and glomerular filtration efficiency and thus of the kidneys' functionality.

The NMR study in chapter two has once more demonstrated that an exploratory approach has some advantages of over the targeted. A non-targeted study gives the opportunity to discover the unknown associations in the data and uses, to the full extend, the multivariate nature on NMR and mass spectrometric data sets. For instance, the exploratory study performed with LC-MS (chapter 3) demonstrated a new and unexpected finding: using a combination of unsupervised and supervised multivariate modeling we discovered a fibrinogen alpha 1-chain peptide as potential morbidity marker. The release of proteolytic fragments of fibrinogen has earlier been reported in response to an infection or involved in cases of kidney disease is not exciting itself and is not new [1]. Recently, antimicrobial activity has also been attributed to the peptide released from fibrinogen [2]. The novelty of the findings reported here lies in the structure of the fibrinogen that carries a glycosylation which changes our understanding of the fibrinogen structure that was considered glycosylation free in this part of its chain. Certainly this observation needs further validation in a larger and more heterogeneous cohort of samples; nevertheless the increased release of the glycosylated fibrinogen alpha chain in febrile UTI patients has shown a reasonably good predictive value.

NMR and LC-MS are the most frequently used techniques in metabolomics; both can rely on broad range of data processing software solutions (commercial and open source), spectral libraries and data bases. However, a quest for novel analytical solutions is still ongoing since none of the existing methods matches chemical diversity and wide concentration range of the metabolites comprising human metabolome. At first glance GC-MS hardly can be presented as a novel method, this method was used for the investigation of metabolic profiles in body fluids long before the term metabolomics was introduced [3]. The modernization of such a technique flows into a variety of platforms which find applications in any type of metabolic profiling. Nonetheless, GC-MS under atmospheric pressure (GC-PCI-MS) is certainly a novel (for metabolomics) method.

Indeed, for GC-APCI-MS an extensive evaluation of the analytical performance is still needed. As indicated in the chapters 4 and 5, the combination of gas chromatography and mass spectrometry under atmospheric pressure is not entirely a new concept but rather a re-introduction of an old idea in a modern, user-friendly form. The value of this method for metabolomics was recognized soon after its reintroduction [4] and it allows the combination

of mass spectrometrer as detector with an addition, simple but nonetheless powerful detector - FID. Chapter 4 describes the detailed analytical evaluation of this idea and demonstrates the applicability of this platform to the study of the metabolite profile in biological samples. The dual detector set-up may be very convenient for the analysis of complex biological samples such as for example body fluids. On the one hand, GC-APCI-TOF-MS offers high-quality data that are essential for the structural assignment of the metabolites. Using the APCI source, in fact, the protonated molecule ($[M+H]^+$) is the dominant feature in the spectra, that provides the possibility for structural characterization of unknown compounds using the combination of the accurate mass, the isotopic pattern and the MS^n experiments. On the other hand, in explorative studies, the relative abundance of the observed compounds in the metabolic space covers a wide dynamic range which the mass spectrometer cannot cover. In this respect, FID is one of the few detectors capable of handling such wide concentration ranges and it can provide more reliable quantitative data.

Despite the growing number of studies which demonstrate its value, GC-APCI-MS is not yet considered as a routine platform. There are a few reasons for this but the lack of a spectral library certainly limits the practical value of GC-APCI-MS for metabolomics studies. The development of such library, as described in the chapter 5 of this thesis, might facilitate the identification of metabolites in body fluids. The spectral library presented here includes the majority of the compounds most commonly encountered in the analysis of body fluids, such as amino acids, sugars, organic acids and so on. The web-based library is fully searchable and publicly available. As any web-based application, it is planned to continuously update our database and the first practical applications are included in the chapters 5 and 6.

Finally, the application of GC-APCI-MS platform proved to be successful for the explorative study of the urinary metabolites in the context of UTI (Chapter 6). In this work, uremic solutes such as myo-inositol and citrate were found to play a role in the characterization of UTI patients as well as the altered concentration of two isomers of hydroxyhippuric acid, which is known as a potent human erythrocytic Ca^{+2} - ATPase inhibitor in end-stage renal disease. This might reflect the link between the tissue invasive process in febrile UTI, such as pyelonephritis, and functionality of the kidney.

The joint effort of these technologies has allowed a comprehensive overview of the complex sequence of the physiological events unfolding the process of an infection. The analytes detected with different techniques reflect the various aspects of the UTI such as the effect of the bacterial growth, the affected renal functionality and also the production of aberrant peptides.

In a longer and speculative perspective, this type of markers could potentially be used in routine clinical chemistry and for therapy decision making.

FUTURE PERSPECTIVES

The multiplatform approach, here reported in the UTI context, demonstrate the value of metabolomics as general profiling tool in a clinical environment, providing a better insight into altered metabolic phenotype and disease pathogenesis.

Despite the explosive growth of the field of metabolomics, 'clinical metabolomics' is still in its infancy, but with the analytical technologies and the computing power evolving rapidly it is to be expected that it will not take long before metabolomics will be fully integrated

into clinical and epidemiological studies. But can we foresee which analytical solution will become 'the standard' of metabolomics? If a question "What is the best analytical technique for metabolomics?" is going to be asked, no straightforward answer will be possible. The chance to find a molecule (or a set of molecules) with high clinical impact depends on the ability to efficiently combine the right technology with a meaningful study design. As shown for UTI, each analytical platform uncovers a slice of the biology of a certain status and the key of the success lies in the formulation of the proper biological question. From that point of view, one should choose the proper collection of samples and instrument according to the phenomenon of interest.

This thesis has shown the complexity of the UTI problem demonstrating how the metabolic patterns measured with different analytical instrumentation can reflect different physiological processes: bacterial infection (NMR), the host response (LC-MS) and the physiological status (GC-APCI-MS). Each of this aspect is a valuable point in the extensive understanding of the UTI as infectious disease and could be useful during the assessment of the disease severity or the decision making.

All this was possible due to the flexibility in the selection of the analytical instrumentation and an appropriate study design. From this experience, it is clear that with regard to generating markers with a potentially high clinical impact, the key of success does not lay in the chosen platform itself but what makes those markers suitable for practical application is the right biological question, a study design that describes adequately the phenomenon of interest, and the last but not the least practical considerations such as the number of samples and the platform robustness.

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