Metabolomics, peptidomics and glycoproteomics studies on human schistosomiasis mansoni

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7 GENERAL DISCUSSION

The application of dedicated mass spectrometry (MS) and nuclear magnetic resonance (NMR) technologies for biomarker discovery and diagnostic purposes has increased substantially in the last decade. In the studies presented in this thesis, we have used these technologies to identify parasite or host-derived products (biomarkers) related to infection and morbidity associated with schistosomiasis and to better understand the host-parasite interaction.

This research was part of the EU 6th Framework Programme: "Multi-disciplinary studies of human schistosomiasis in Uganda, Kenya and Mali: New perspectives on morbidity, immunity, treatment and control" (MUSTSchistUKEMA). In these countries schistosomiasis is an important poverty-related health problem. The overall aim of the EU Programme was to increase the knowledge about the dynamics of infection and morbidity and how this is affected and modulated by treatment with praziquantel. Specific attention was paid to the effect of praziquantel on the host immune responses, the improvement of the use of existing drug in reduction of morbidity and on finding non-invasive and reliable ways of detecting morbidity and infection. Non-invasively detectable biomarkers have increasingly been recognized for their potential, not only as biologic precursors that provide early warnings of disease and disease status but also as biochemical monitors of patient responses to therapies.

A biomarker discovery study brings together different disciplines and therefore the success of such an approach relies on a proper cooperation and combination of expertise from different fields: epidemiology and medicine for an appropriate selection of the study cohort, analytical chemistry for the development and application of the appropriate technology, bioinformatics for data management and analysis and the extraction of the relevant information necessary to perform the biological interpretation. In addition to an appropriate selection of the study cohort, standardisation and automation of sample collection and handling are important aspects in biomarker discovery studies. Several recent initiatives within the proteomics community have been started to call attention to this for urinary peptidomics and proteomics (1). We believe that the method we have developed for urinary peptidomics, comprising desalting and pH-normalisation, use of cartridges with high peptide binding capacity and state-of-the-art mass spectrometry, is not only relevant to other parasitic infections but also to other diseases and is well suited to be applied in large clinical studies. Obviously, one of the major hurdles of biomarker discovery studies using material from field studies is the often high variability in factors related to the population (age, gender, etc), the infection (intensity, time course) and possible co-morbidities. Still, using our workflow, we were able to correlate the presence of haemoglobin peptides in urine samples with *S. haematobium* infection, even with a higher sensitivity than a standard micro-haematuria test. More importantly, using this method, we could specifically and sensitively classify *S. mansoni*-infected children within a large study cohort and identify

hitherto unknown putative biomarkers. For the analysis of the generated data set, the application of novel data processing tools (Discrete Wavelet Transformation (DWT) with Support Vector Machine (SVM)) resulted in higher classification power than other currently available methods. Several peaks, responsible for the discrimination of *S. mansoni*-infected from non-infected children, were identified as collagen peptides but due to the confounding factors mentioned above, it is at present unclear whether they are related to morbidity. However, based on our data, a controlled experiment using a rodent model could specifically focus on the relationship between the decrease of collagen peptides in urine of infected animals and the morbidity status.

In addition to the studies on peptidomic changes in urine of infected individuals, we presented the first global examination of urinary metabolites in the context of *S. mansoni* infection in humans, aiming at a better understanding of this disease by interpreting the metabolic perturbation induced by the parasite, and the identification of new metabolic signatures for *Schistosoma* infection in human. The study confirms that NMR-based metabonomics is a powerful approach in monitoring infectious diseases in human and we were able to identify metabolic signatures of the human-*S. mansoni* interaction and propose several biologically plausible biomarkers.

However, for a more comprehensive picture, it would be advantageous that an increased number of metabolites, resulting from different pathways, are analysed. This is a challenging task since metabolites are represented by many different compound classes and the concentration of metabolites covers a large dynamic range. Thus, only a combination of different analytical methods will be appropriate for this purpose. In addition to NMR-based metabonomics, which has the advantage of being non-destructive, non-selective and fast, gas chromatography or liquid chromatography coupled to mass spectrometry may be considered. Mass spectrometry is more sensitive than NMR and the elaborated fragmentation facilitates the assignment of the metabolites that are already included in the "Metabolome Database" and ultimately the identification of so far unidentified metabolites, since still a large percentage of metabolites measured in metabonomics analysis remain unidentified. As a consequence, it is expected that the most interesting and important technological developments for future research in metabonomics analysis will be primarily related to metabolite characterisation.

The application of our peptidomics and metabonomics studies on schistosomiasis have provided some novel, valuable information but they are obviously only the first step and the putative biomarkers need verification, validation and ultimately, commercialization. One of the major impedes to clinical translation of peptide/protein biomarkers is the need to generate specific antibodies for a sensitive clinical test such as enzyme-linked immunoabsorbent assays (ELISAs). Although ELISAs are still considered the 'gold standard" for many clinical applications, the production of antibodies for a significant number of peptide candidates and the development of

specific assays using these antibodies are time consuming and expensive. However, the recent developments in mass spectrometry such as multiple-reaction-monitoring (MRM) (2;3) may represent an alternative to ELISAs. Using MRM-MS, a number of candidate biomarkers can be targeted simultaneously with a high sensitivity and specificity, but before general applicability in the clinical laboratory, the approach has still to be further developed and validated. As mentioned above, in our biomarker discovery study we identified several potential biomarkers such as collagen peptides but in a second step these peptides will require validation using the above mentioned methodologies.

In addition to the potential biomarkers identified with the global biomarker discovery approaches described above, we showed in this thesis that a more targeted approach, looking at glycosylation, also resulted in novel information on *S. mansoni* infection. Using elaborate mass spectrometry in combination with prefractionation methods, we have identified and characterized a set of human Apolipoprotein C-III peptides aberrantly glycosylated at the O-glycosylation site (Thr^{74}), in urine of *S. mansoni*- infected individuals. This intriguing observation might be related to changes in the glycosylation activity in the liver due to schistosomiasis-induced reactions associated with the accumulation of *Schistosoma* eggs. This would imply that these peptides might be considered as very suitable morbidity markers, although additional studies have to demonstrate whether they are specific for *Schistosoma* infection. On the other hand, it is tempting to speculate that the modified Apo C-III glycosylation is the result of the action of schistosomal glycosidases and glycosyltransferases and future studies could test this hypothesis. If this proved to be true, it will not only justify the use of these peptides as infection markers, but it would also give new insights in the molecular interplay between host and pathogen glycosylation. Interestingly, comparable phenomena are known for other parasites: *Trypanosoma brucei*, for example, has a transsialidase which can transfer sialic acid, a sugar they cannot synthesize themselves, from the host's glycoconjugates to glycosylphosphatidylinositols on the surface of the parasite (4).

So far, we have been unable to find the aberrant glycosylation of full-length Apo-C III and this is probably related to the fact that only a small fraction of the total pool of Apo C-III contains these unusual glycans. Possibly, the catabolism of these forms is less efficient than that of the most common forms, resulting in a relative enrichment of their degradation products in urine. Because the glycosylation machinery of the parasite is also very different from that of its host, we believe that similar processes may result in the appearance of egg derived glycoconjugates in urine of infected individuals. Obviously, characterization of the glycosylation of the major egg secreted proteins is thus of major importance. The three major egg secreted proteins are Kappa-5, Omega-1 and IPSE/ alpha-1 (5;6). In this thesis we showed that IPSE/ alpha-1 is glycosylated on

two N-glycosylation sites. Lewis X is the major terminal glycan motif in the antennae and the chitobiose core α1-3- -fucosylation is a typical helminth / plant antigenic core motif. This knowledge could be helpful in a targeted analysis of urine samples with the aim to identify hitherto unknown glycopeptides that could serve as very specific markers of *S. mansoni* infection.

In conclusion, the presented study is the first in which mass spectrometry and NMR are used for the analysis of a cohort of human *S. mansoni*-infected individuals with the aim to identify markers associated with morbidity and/or infection. This has resulted in the identification of a number of novel markers, which (probably) are related to the host rather than to the parasite, and thus should be considered as morbidity markers. Nevertheless, further studies are needed to evaluate the overall applicability of these putative biomarkers.

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