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Exploratory urinary metabolomics of type 1 leprosy reactions

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SUMMARY

Background: Leprosy is an infectious disease caused by *Mycobacterium leprae* that affects the skin and nerves. Although curable with multidrug therapy, leprosy is complicated by acute inflammatory episodes called reactions, which are the major causes of irreversible neuropathy in leprosy that occur before, during, and even after treatment. Early diagnosis and prompt treatment of reactions reduces the risk of permanent disability.

Methods: This exploratory study investigated whether urinary metabolic profiles could be identified that correlate with early signs of reversal reactions (RR). A prospective cohort of leprosy patients with and without reactions and endemic controls was recruited in Nepal. Urine-derived metabolic profiles were measured longitudinally. Thus, a conventional area of biomarker identification for leprosy was extended to non-invasive urine testing.

Results: It was found that the urinary metabolome could be used to discriminate endemic controls from untreated patients with mycobacterial disease. Moreover, metabolic signatures in the urine of patients developing RR were clearly different before RR onset compared to those at RR diagnosis.

Conclusions: This study indicates that urinary metabolic profiles are promising host biomarkers for the detection of intra-individual changes during acute inflammation in leprosy and could contribute to early treatment and prevention of tissue damage.

1. Introduction

Leprosy is a complex infectious disease often resulting in severe, life-long disabilities.[1] It presents in different clinicopathological forms[2,3] and is considered a major threat in developing countries by the World Health Organization (WHO), remaining endemic in Africa, South America, and Asia. Every year approximately 220 000 new patients are still diagnosed, and this incidence rate has been essentially stable over the last decade. Furthermore, with increasing migration, new cases are also being detected in developed countries, where initial misdiagnosis is likely to occur.[4–6]

Although leprosy can be treated effectively with multidrug therapy (MDT), it is complicated by acute inflammatory episodes called leprosy reactions. These immunological complications, occurring before, during, and after treatment in 30–50% of the patients, represent the major cause of leprosy-related neurological damage.[7,8] Two types of reaction are recognized: reversal reactions (RR) or type 1 reactions and erythema nodosum leprosum (ENL) or type 2 reactions. RRs, which affect 30% of leprosy patients at least once,[9] coincide with characteristic CD4+ T-cell infiltrations of skin and nerve lesions.[10,11] Prompt diagnosis and treatment aids recovery significantly, thereby reducing the risk of permanent disability.[12,13] Unfortunately, reactions are frequently misdiagnosed due to decreased expertise within integrated health services.[9] Therefore, sensitive tests based on dependable biomarkers for early diagnosis of reactions are urgently needed.

Since leprosy endemic areas are often lacking in sophisticated laboratories, it is imperative to develop diagnostic tests that are suitable for the field setting. Similarly, home-monitoring of chronic diseases with inflammatory episodes requires ease in test performance. Besides the selection of suitable biomarkers, an

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important prerequisite for such tests is the ease of obtaining samples. Using urine samples rather than venous blood will avoid the need for a trained phlebotomist, reduce costs, and improve ease of use. However, host-derived biomarkers for mycobacterial diseases based on urine have not yet been reported.

The rapidly evolving field of metabolomics provides a technological basis for the comprehensive analysis of urinary metabolites and the discovery of disease-associated biomarkers in urine. The feasibility of the approach has already been established in clinical studies.[14,15] Indeed, it has been shown that the metabolic composition of urine reflects the physiological status of an organism and as such can be a useful readout of multiple (patho)physiological processes.[14–16]

To investigate whether disease-specific metabolites could be detected in urine, as a non-invasive body fluid, and to identify associations between such urinary metabolites and the occurrence of leprosy reactions, an exploratory metabolomics analysis was performed in leprosy patients in Nepal.

2. Materials and methods

2.1. Study participants

Recruitment took place in Nepal (Mycobacterial Research Laboratories, Kathmandu).[17] Leprosy prevalence was 1.1–0.79/10 000, new case detection rate (NCDR) 1.67– 1.15/10 000 (Annual Report 2012–2013, Leprosy Control Division, Department of Health Services, Kathmandu). Patients and healthy individuals from the same area (endemic controls) were recruited on a voluntary basis between February 2008 and March 2013 (Tables 1 and 2). Leprosy was diagnosed based on clinical, bacteriological, and histological observations and classified by skin biopsy according to Ridley and Jopling.[1] Clinical monitoring for reactions was performed during monthly clinic visits. Clinical and demographic data were collected in databases with a special emphasis on standardizing data collection and the definition of reactions between the cohorts. For patients who presented with a reaction, the type, severity, skin and/or nerve involvement, number of lesions, and relapse were noted in accordance with state-of-the-art clinical expertise and international consensus scoring.[18] Endemic controls were assessed for the absence of clinical signs and symptoms of leprosy and tuberculosis (TB). Staff of leprosy and TB clinics were excluded.

2.2. Ethics

This study was performed in accordance with the Declaration of Helsinki (2008 revision). Participants were informed about the

Table 1
Study cohort in Nepal[a]

Category[b]	Number	Leprosy type	
EC	34	-	
No Rxn t = 0	28	16 TT/BT	12 BL/LL
No Rxn t = end	11	4 TT/BT	7 BL/LL
RR t = 0	7	3 BT	4 BL/LL
RR t = x	24	12 BT	12 BL/LL
RR t = end	19	8 BT	11 BL/LL
Total	123		

TT, tuberculoid; BT, borderline tuberculoid; BL, borderline lepromatous; LL, lepromatous.

[a] Newly diagnosed leprosy patients without reactions (no Rxn) were sampled before (t = 0) and after treatment (t = end); leprosy patients developing reactions during the study were sampled in the absence of any clinical signs of reactions and at least 3 months before RR (t = 0), at RR diagnosis before steroids (t = x), or after multidrug therapy and RR at least 1 month after the end of steroids (t = end).

[b] EC, endemic control; Rxn, reaction; RR, reversal reaction.

Table 2
Endemic control (EC) and patient information

	PGL-I (OD)
EC	0.025
EC	0.073
EC	0.008
EC	0.020
EC	0.059
EC	0.041
EC	**0.153**
EC	0.004
EC	0.017
EC	0.006
EC	0.003
EC	0.093
EC	0.035
EC	0.019
EC	0.036
EC	0.032
EC	0.014
EC	**0.258**
EC	**0.179**
EC	**0.233**
EC	0.000
EC	0.008
EC	0.006
EC	0.000
EC	**0.568**
EC	**0.257**
EC	0.024
EC	0.046
EC	0.000
EC	0.012
EC	0.066
EC	0.019
EC	**0.203**
EC	0.147

		PGL-I (OD)	classification
no Rxn	t = 0	0.050	TT/BT
no Rxn	t = 0	0.045	TT/BT
no Rxn	t = 0	**2.919**	BL/LL
no Rxn	t = end	**1.325**	BL/LL
no Rxn	t = 0	0.078	TT/BT
no Rxn	t = 0	**0.636**	TT/BT
no Rxn	t = 0	**3.146**	BL/LL
no Rxn	t = 0	0.009	TT/BT
no Rxn	t = 0	**0.203**	TT/BT
no Rxn	t = end	0.057	TT/BT
no Rxn	t = 0	0.020	TT/BT
no Rxn	t = 0	**3.147**	BL/LL
no Rxn	t = 0	**1.349**	BL/LL
no Rxn	t = 0	**1.700**	BL/LL
no Rxn	t = 0	0.055	TT/BT
no Rxn	t = 0	0.032	TT/BT
no Rxn	t = 0	**0.225**	TT/BT
no Rxn	t = 0	**2.550**	BL/LL
no Rxn	t = end	**1.309**	BL/LL
no Rxn	t = 0	**0.305**	TT/BT
no Rxn	t = end	**0.190**	TT/BT
no Rxn	t = 0	**0.199**	TT/BT
no Rxn	t = end	0.118	TT/BT
no Rxn	t = 0	0.010	TT/BT
no Rxn	t = 0	**2.565**	BL/LL
no Rxn	t = end	**0.304**	BL/LL
no Rxn	t = 0	**0.193**	BL/LL
no Rxn	t = 0	**3.012**	BL/LL
no Rxn	t = end	**2.025**	BL/LL
no Rxn	t = 0	**1.229**	BL/LL
no Rxn	t = end	0.020	BL/LL
no Rxn	t = 0	0.117	TT/BT
no Rxn	t = end	0.034	TT/BT
no Rxn	t = 0	**0.803**	BL/LL
no Rxn	t = end	**0.315**	BL/LL
no Rxn	t = 0	**1.161**	BL/LL
no Rxn	t = end	**nt**	BL/LL
no Rxn	t = 0	**0.159**	TT/BT
no Rxn	t = 0	**0.171**	TT/BT

Table 2 (*Continued*)

		PGL-I (OD)	classification
RR during study	t = 0	0.133	TT/BT
RR during study	t = x	**nt**	TT/BT
RR during study	t=0	**1.764**	BL/LL
RR during study	t=x	**2.072**	BL/LL
RR during study	t=end	0.095	BL/LL
RR during study	t = 0	0.143	TT/BT
RR during study	t = x	**0.259**	TT/BT
RR during study	t = end	**1.391**	TT/BT
RR during study	t = 0	**0.866**	BL/LL
RR during study	t = x	**2.814**	BL/LL
RR during study	t = end	0.098	BL/LL
RR during study	t = 0	0.139	TT/BT
RR during study	t = x	**3.398**	TT/BT
RR during study	t = 0	**0.307**	BL/LL
RR during study	t = 0	**0.214**	BL/LL
RR at recruitment	t = x	**1.619**	BL/LL
RR at recruitment	t = end	**0.747**	BL/LL
RR at recruitment	t = x	**2.205**	BL/LL
RR at recruitment	t = end	**0.208**	BL/LL
RR at recruitment	t = x	0.017	TT/BT
RR at recruitment	t = end	0.021	TT/BT
RR at recruitment	t = x	**0.786**	BL/LL
RR at recruitment	t = end	**0.156**	BL/LL
RR at recruitment	t = x	0.034	TT/BT
RR at recruitment	t = x	0.037	TT/BT
RR at recruitment	t = x	**0.764**	BL/LL
RR at recruitment	t = end	0.003	BL/LL
RR at recruitment	t = x	0.067	TT/BT
RR at recruitment	t = end	**0.184**	TT/BT
RR at recruitment	t = x	0.124	TT/BT
RR at recruitment	t = end	0.111	TT/BT
RR at recruitment	t = x	0.054	TT/BT
RR at recruitment	t = end	0.030	TT/BT
RR at recruitment	t = x	**1.669**	BL/LL
RR at recruitment	t = end	**1.550**	BL/LL
RR at recruitment	t = x	0.035	BL/LL
RR at recruitment	t = end	**0.505**	BL/LL
RR at recruitment	t = x	**no**	TT/BT
RR at recruitment	t = end	0.088	TT/BT
RR at recruitment	t = x	0.148	TT/BT
RR at recruitment	t = end	0.061	TT/BT
RR at recruitment	t = end	**3.099**	BL/LL
RR at recruitment	t = x	**1.848**	BL/LL
RR at recruitment	t = x	**0.228**	BL/LL
RR at recruitment	t = end	**nt**	BL/LL
RR at recruitment	t = x	**0.363**	BL/LL
RR at recruitment	t = end	0.113	BL/LL
RR at recruitment	t = x	**0.286**	BL/LL
RR at recruitment	t = x	**0.217**	TT/BT
RR at recruitment	t = end	0.062	TT/BT

$OD_{450} > 0.2$ are considered positive; positive values are indicated in bold; nt = not tested.

study objectives, the samples, and their right to refuse to take part/withdraw from the study without consequences for their treatment. Written informed consent was obtained before enrolment. All patients received treatment according to national guidelines. Ethical approval for the study protocol was obtained through the Nepal Health Research Council (NHR #751).

2.3. Recruitment

Newly diagnosed, untreated leprosy patients without clinical reactions were enrolled. Blood and urine were collected before MDT (t = 0). Patients who presented reactions within 3 months of the start of therapy were excluded to avoid profile analyses of patients with latent reactions. If patients presented with reactions after more than 3 months of MDT, samples were taken before the initiation of anti-reactional therapy (t = x). Newly diagnosed leprosy patients who visited clinics with RR were recruited (t = x), but consequently lacked t = 0 samples. Blood (serum) and urine were collected after MDT and/or steroid therapy from all patients (t = end). For patients with RR this was done at least 1 month after the completion of steroid therapy to avoid assessment of the effect of steroids. All patients were assessed for the absence of reactions at 3 months after t = end. For patients showing clinical signs of reactions within 3 months after t = end, this time point was excluded. In the case of a patient death, or if the patient moved or withdrew from the study thereby preventing follow-up, their samples were excluded. Urine and sera were stored at −80 °C.

2.4. Serology

Antibodies against disaccharide-octyl bovine serum albumine (ND-O-BSA), a synthetic analogue of phenolic glycolipid I (PGL-I), were determined in the sera, as described previously.[19]

2.5. Urinary creatinine levels

Assuming that urinary creatinine excretion is constant across and within individuals, creatinuria was determined as a normalizer.[20] For all urine samples, the levels of creatinine were determined using the CREA Plus Test according to the manufacturer's protocol (Roche Diagnostics, Indianapolis, IN, USA).

2.6. Metabolomic analysis of urine samples by ultra-performance liquid chromatography electrospray ionization time-of-flight mass spectrometry (UPLC-ESI-TOF MS)

Urine samples (10 ml) from the cohorts in Nepal were stored in 15-ml sterile polypropylene tubes (Greiner Bio-one; catalogue number 188271) and kept frozen until analysis. Urine samples were prepared for non-targeted analysis of small molecules and natively occurring urinary peptides. The urinary metabolomics analysis was performed as described previously.[21] Chromatographic and mass spectrometry procedures are described in the **Supplementary Material**.

2.7. Liquid chromatography mass spectrometry (LC-MS) data analysis

LC-MS data files were exported as mzXML files and aligned using the alignment algorithm msalign2 tool[22] developed in-house (http://www.ms-utils.org/msalign2/); peak picking was performed using XCMS package (The Scripps Research Institute, La Jolla, CA, USA).[14]

The data matrix generated was imported into SIMCA-P 13.0 software package (Umetrics, Umeå, Sweden). The data were mean-centred and unit variance-scaled prior to statistical analysis. The validity and the degree of over-fitting of the partial least square-discriminant analysis (PLS-DA) models were checked using a 200 permutations test.

The statistical analysis was performed in R version 3.1.3. To identify metabolites of interest, rational chemical formulae were generated based on internally calibrated monoisotopic masses within 10 mDa mass error, using the SmartFormula tool within the Data Analysis software package (Bruker Daltonics).

3. Results

3.1. An overview of the dataset

An exploratory metabolomics study is a method to obtain an unbiased view of a research question thereby addressing it beyond the existing paradigm. The data evaluation typically consists of several steps, namely evaluation of analytical consistency, an overview of the main sources of variance, and supervised modelling following the main lines of the study design. The score plot of the initial principal component analysis (PCA) model built on the entire dataset showed that the first two principal

components of the model covered 53% of the variance (**Supplementary Material**, Figure S1). A tight clustering of the quality control (QC) samples (a pool of all the samples within a study) provides an estimate of the fraction of analytical variance within a dataset. Next, a PCA model was built without QC samples (11 components explained approximately 60% of variance; **Supplementary Material**, Figure S2). However, neither the differences between the endemic controls and leprosy patients, nor those between patients with and without leprosy reactions, influenced the first two components.

3.2. Comparison of urine samples derived from endemic controls and leprosy patients

The initial PCA model (**Supplementary Material**, Figure S2) failed to describe any tendencies in the data associated with the study design, which is likely due to the fact that the cohort consisted of a cross-sectional and a longitudinal component (**Supplementary Material**, Figure S3). Therefore, the analysis was restricted to samples obtained at first recruitment (t = 0) to determine whether the urinary metabolic profiles reflected any differences between endemic controls and leprosy patients. Figure 1A shows a PCA model built on this selection of the samples. A visual inspection of the score plot for the first two components covering 42% of the variance showed no obvious grouping according to the disease status, although a clear tendency was present. Thus, a PLS-DA model with disease status as a class variable was built for the baseline samples. The statistical parameters of the model (R2X = 0.58, R2Y = 0.99, Q2 = 0.748) and an additional model diagnostic based on analysis of variance of the cross-validated residuals (CV-ANOVA) (F = 8.23 and p = 1.2e−08) indicated a valid model (Figure 1B). In contrast, creatinine levels of all individuals in this study did not provide discriminating potential (**Supplementary Material**, Figure S5), thereby indicating that the urinary biomarkers identified in this study are disease (state)-specific. This shows that the urinary metabolic profiles can indeed be useful for discriminating between leprosy patients and endemic controls. However, it should be taken into account that other mycobacterial infections may lead to similar metabolites as identified in leprosy patients. Therefore future studies should include the analysis of TB patients from the same region.

3.3. Treatment effect on urinary profiles

Taking advantage of the longitudinal sampling, regression models were built using the urinary samples of patients developing RR. The first model explored the differences between patients at t = 0 (at least 3 months before the reactions diagnosis) and at the time of the diagnosis of the reaction, before starting anti-reactional therapy (t = x) (Figure 2A). The model parameters (R2X = 0.496, R2Y = 0.995, Q2 = 0.691) indicated a valid model. However, the CV-ANOVA values were lower than those for the baseline model (F = 3.55, p = 3.8e−03). The next model (Figure 2B) was built on the urine samples from Nepalese leprosy patients at diagnosis of reaction before starting anti-reactional therapy (t = x) and at t = end, at least 1 month after the end of anti-reactional (steroids) therapy. Model parameters (R2X = 0.363, R2Y = 0.662, Q2 = 0.114) indicated low predictive power, which was supported by CV-ANOVA values (F = 1.25, p = 0.3).

3.4. Variable subset selection

One of the advantages of the multivariate regression model is the possibility of ranking the variables according to their contribution to the model. This ranking is often presented as a variable influence on projection (VIP) value calculated for every

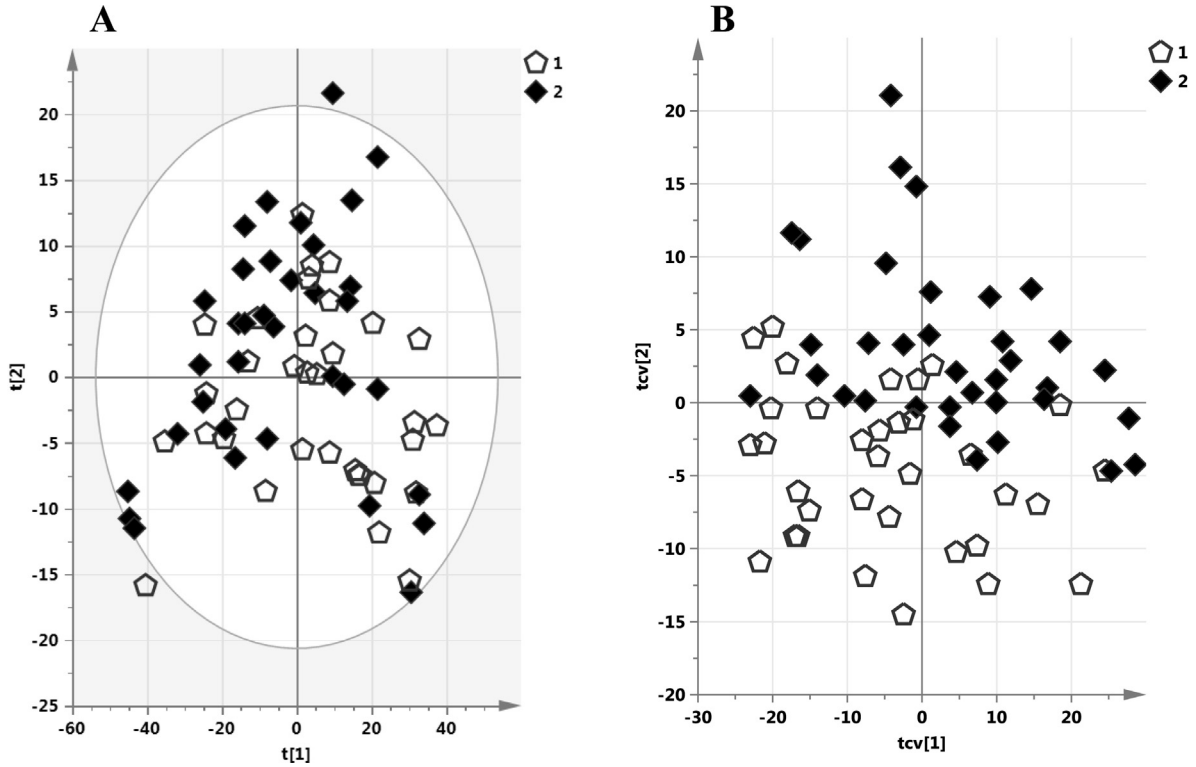

Figure 1. Urinary metabolic profiles can be used to discriminate between endemic controls and leprosy patients. (A) Score plot of a PCA model of urinary metabolomics data at baseline (seven components cover 0.58 of variance). (B) Cross-validated score plot of a regression (PLS-DA) model for the same dataset (R2Y = 0.528, R2Y = 0.99, Q2 = 0.748; F = 8.23, p = 1.2e−08).

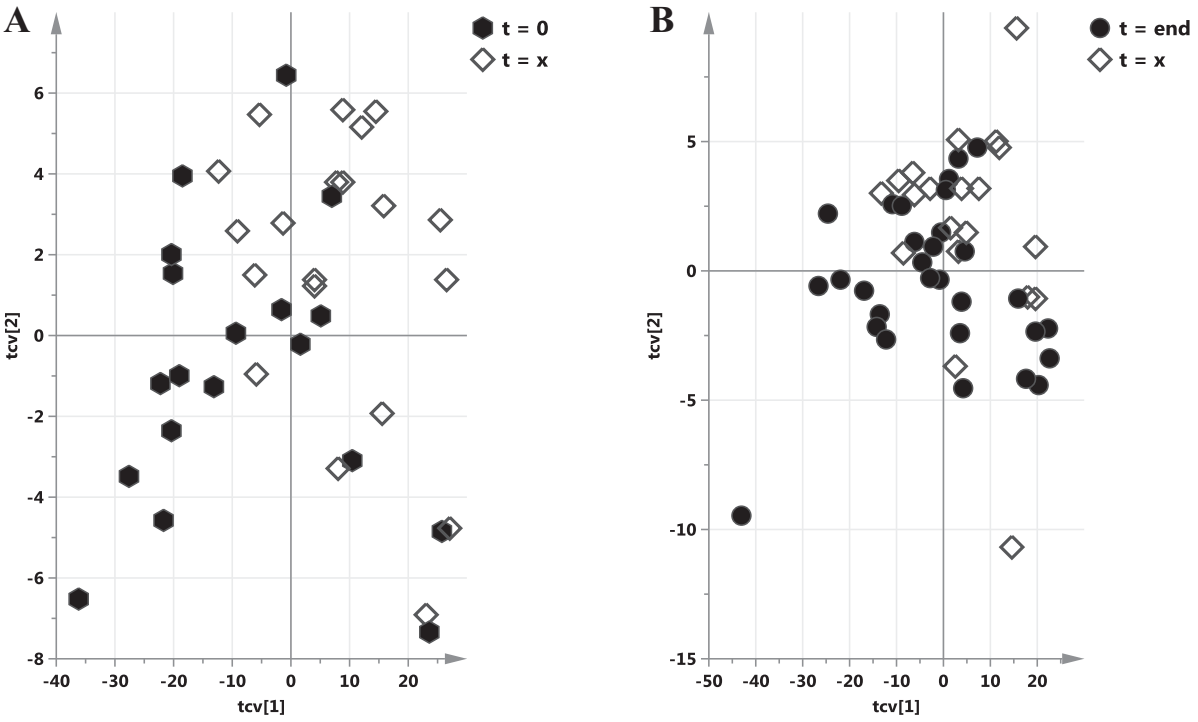

Figure 2. Urinary metabolic profiles reflect the status of the leprosy patients.
(A) Cross-validated score plot of a PLS-DA model built on the samples from all Nepalese leprosy patients in the absence of any clinical signs of reactions and at least 3 months before the reaction diagnosis (t = 0) and at the time point of the reaction diagnosis and before starting anti-reactional therapy (t = x). (B) Cross-validated score plot of a PLS-DA model built on the urine samples from Nepalese leprosy patients at the time point of the reaction diagnosis and before starting anti-reactional therapy (t = x) and at t = end at least 1 month after the end of anti-reactional (steroids) therapy.

variable. Following the logic of the analysis outlined above, it can be concluded that two models were statistically significant: the baseline model describing the differences between the endemic controls and the patients, and the model describing the changes in the urinary profiles of leprosy patients upon the development of a reaction. Since both models were built on the same matrix of variables, the degree of overlap between the VIP values was investigated. A selection of the variables for a comparison was made using a 'cut-off' VIP value of 1.7. The cut-off value was chosen empirically keeping in mind an imbalance between the number of observations and the number of variables. A Venn diagram of the preselected VIP values for both models (**Supplementary Material**, Figure S4) showed little overlap between the VIP lists. Therefore, it can be concluded that the difference in urinary compounds between the endemic controls and the leprosy patients, as well as the changes during development of reactions, are explained by a specific, barely overlapping set of metabolites.

Nevertheless, a number of practical limitations currently prevent us from providing the high confidence ID of the metabolites. First, the limited sample volumes rules out the possibility of in-depth de novo identification of the structures. Secondly, the available database contains very limited numbers of documented entries from the endemic areas, since this is based on reference databases created with samples of European or North American origin.[23] The MS/MS performed on the pooled samples (endemic controls, leprosy, and the samples at the time of reaction) helped to resolve structures of some features: a combination of the accurate mass and retention time provided suggestions for the others, but there is a large fraction of the features where the number of potential candidates is just too high to report. Under these circumstances a table consisting of multiple variables with only tentative IDs may appear misleading. Thus, the number of reported variables of the discriminating metabolites was reduced to an essential minimum, reporting them as unknown or tentatively identified features (*m/z* – retention time pair; Table 3). To this end, *p*-values were calculated (Mann–Whitney *U*-test with Benjamini–Hochberg correction for multiple testing) for all selected VIP values, for both the baseline (t = 0) and reaction development models. The variables that passed a commonly accepted significance level of 0.05 were used to build a sequence of the logistic regression models. The models were built in a stepwise manner, starting with a single variable and adding/deleting one variable at a time until the addition of the next variable failed to improve the model. The models were compared based on their Akaike information criteria (AIC) and Chi-square probability. The receiver operating characteristic (ROC) curves for the optimal models are presented in Figure 3.

4. Discussion

Biomarkers as reliable correlates of disease and the response to therapy are essential tools for early diagnosis of disease complications in chronic infections. Generally, the performance of one biomarker can be significantly enhanced by instead using a

Table 3
Overview of the discriminating compounds

Compound label	*m/z*	RT (min)	Comments
X836	564.266 ± 0.003	5.1	Most probably a peptide
X937	207.142 ± 0.001	5.2	A few possible ID matches like ammonium adduct of homocitrulline were rejected
X484	330.539 ± 0.002	4.1	
X405	636.086 ± 0.007	5.2	Triple charged feature, most probably a peptide

RT, retention time.

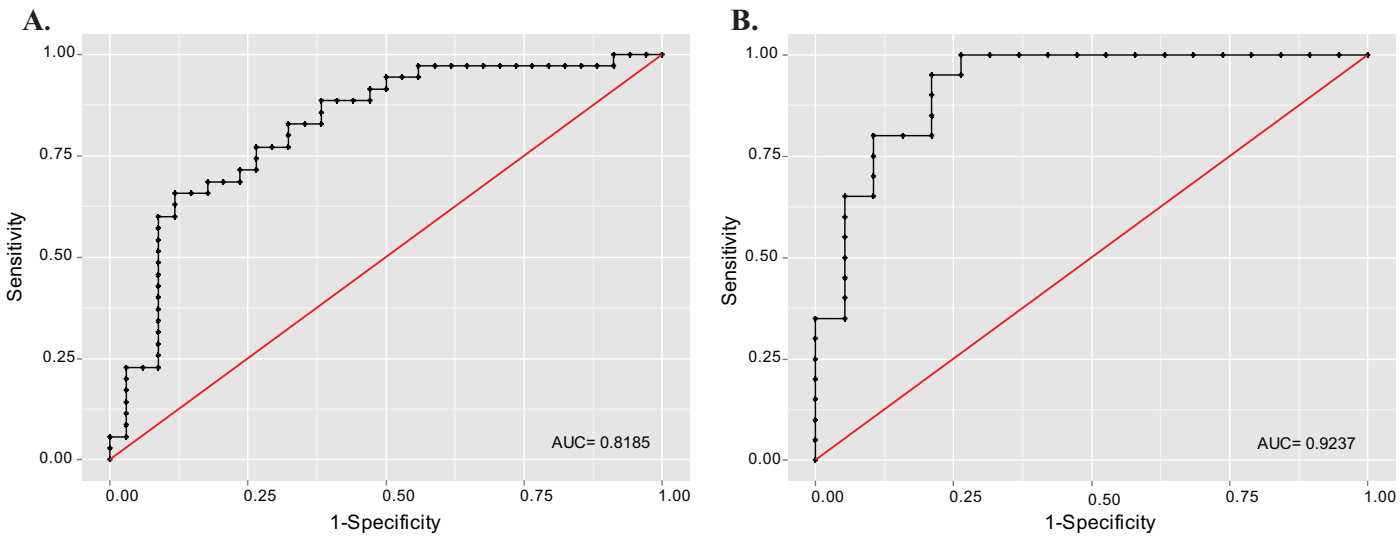

Figure 3. Receiver operating characteristic (ROC) curve plots for logistic regression models describing discrimination between the endemic controls and leprosy patients (A; Chi-square (2) = 27.737, p = 1.905225e−05) and the differences between t = 0 and t = x (B; Chi-square (2) = 24.367, p = 5.114389e−06).

custom-made grouping of independent biomarkers, designated a profile or signature.

The availability of sensitive and specific biomarkers that aid the early diagnosis of leprosy reactions as well as monitoring therapy, would be a strategic advantage enabling health care workers to identify, treat, and possibly prevent these episodes at an early stage, thereby reducing nerve damage. With this study, an attempt was made to extend a conventional area of the search for biomarkers of leprosy towards urine, aiming at non-invasive testing. For the first time in mycobacterial diseases, it was shown that the urinary metabolome can be used to discriminate endemic controls from patients. Moreover, the urinary metabolic signatures of patients developing RR were clearly different in the absence of RR before onset compared to those at diagnosis of this reaction. A simple comparison of the top VIP values from the baseline and treatment models shows that the differences between leprosy patients and the endemic controls, as well as changes during development of the reaction, are associated with different parts of the urinary metabolome. Although this report does not describe the first metabolomics analysis of leprosy patients, two previous reports used patient serum and were primarily targeted at testing an existing paradigm of leprosy pathogenesis (e.g., involvement of the signalling lipids).[24,25] Urine has been used so far either for monitoring dapsone metabolism,[26] or for targeted screening of *Mycobacterium leprae*-derived PGL-I.[27] In contrast, the present study had a clear exploratory character and aimed at new biomarkers for disease (state).

Thus, this first demonstration of the discriminative power of metabolomics analysis for leprosy is an important finding in the field, particularly since urine represents a more easily accessible sample than blood. The application of the metabolites identified using the approach described in this study in non-invasive field-friendly tests for leprosy would be very useful for monitoring treatment and for the early diagnosis of reactions. Since several lateral flow assays for the detection of cytokines/chemokines in blood samples have already been developed and field-tested,[28–32] it is envisaged that the translation of this metabolic biomarker study into field-friendly, non-invasive diagnostic tests will also be feasible.

These data prompt further investigations on urinary metabolites for leprosy and reactions, but also offer possibilities for TB, particularly in children in whom diagnosis using sputum is difficult.

Moreover, in view of the shared susceptibility genes between leprosy and other chronic diseases with acute inflammatory states, such as psoriasis[33] and Crohn's disease,[34] this leprosy-based metabolomics approach could be used as a model for other diseases. An unequivocal identification of the metabolites contributing to specific signatures will require confirmation in future studies. Although practical problems, such as the limited availability of material for confirmatory studies and a bias of the current metabolomics databases towards material collected in developed countries, hampered the identification of the exact metabolites based on the current data, the information provided in this study indicates the presence of different metabolic biomarker profiles for leprosy disease and reactions and offers promise for future studies.

In summary, RRs are a major cause of leprosy-related nerve impairment and bear similarities to acute inflammation-induced episodes in other infectious diseases. Since there is no laboratory test for the early diagnosis of these episodes, this longitudinal study on the occurrence of RR in leprosy patients showed, for the first time, that urinary metabolic profiles correlating with an early onset of RR can be identified. These metabolic biomarkers are promising tools for application in rapid, field-friendly tests,[29] allowing the detection of intra-individual changes during acute inflammatory responses in chronic diseases. Future studies should be performed to assess their predictive value for the early diagnosis of these episodes and to contribute to timely treatment and the reduction/prevention of tissue damage.

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Conflict of interest: The authors declare that they have no financial/commercial conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2016.02.012.

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