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The Analysis of Urine Proteomic and Metabonomic Patterns for the Diagnosis of Cystitis

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Introduction

The advent of systems biology approaches that have stemmed from the sequencing of the human genome has led to the search for new methods to diagnose diseases. A recent development has been the use of proteomic and metabonomics patterns to discriminate healthy from disease states. We have developed and contrasted the use of both proteomic and metabonomics patterns for the development of a high-resolution urine-profiling test for the detection of interstitial (IC) and bacterial cystitis (BC). The bioinformatic-based methodology uses the information from mass spectrometry (MS) and high-resolution proton nuclear magnetic resonance spectral patterns to distinguish IC- and BC-affected patients from non-affected individuals. Using bioinformatic-clustering algorithms we were able to discriminate the spectral patterns associated with the three different physiological states with a success rate of almost 70%.

Methods

Urine samples acquired from IC- and BC-affected, as well as healthy patients were normalized with respect to pH and osmolality. After applying and processing the sample onto a weak cation exchange proteinchip the proteome patterns of each sample was acquired using surface enhance laser desorption ionization time-of-flight mass spectrometry (SELDI TOF-MS). The metabonomics profiles of the same urine samples were acquired using a 500 MHz ^{1}H-NMR spectrometer. The resulting proteomic and metabonomics profiles were analyzed using a K-means clustering algorithm to discriminate spectra generated from the urine samples collected from either the healthy or cystitis-affected individuals.

Results

Variability was observed between the proteomic and metabonomics profiles of the urine samples acquired from healthy, IC-affected, and BC-affected individuals. Spectra from the three conditions, however, also showed a variability within those acquired for each condition making the identification of a unique disease marker that is diagnostic for each of the states impossible with the sample cohort used in this study. The proteomic and metabonomic data was analyzed using bioinformatic tools designed to recognize key differences within the spectral patterns from each set of samples. By applying a genetic algorithm and self-organizing cluster algorithm, we were able to correctly diagnose urine samples as acquired from healthy or IC-affected individuals with 100% sensitivity and specificity. Applying a K-means clustering algorithm to the ^{1}H-NMR data allowed the correct diagnose of IC-affected, BC-affected, or healthy individuals with a success rate of almost 70%. In addition, many of the key frequency values that allow the different states to be discriminated were also identified in the ^{1}H-NMR spectra either by their frequency value or through two-dimensional NMR experiments. While the proteomic analysis required much less sample, the sample preparation for the metabonomic analysis was much simpler and

the results led to the identification of some key metabolites that were differentially abundant in the different sample sets.

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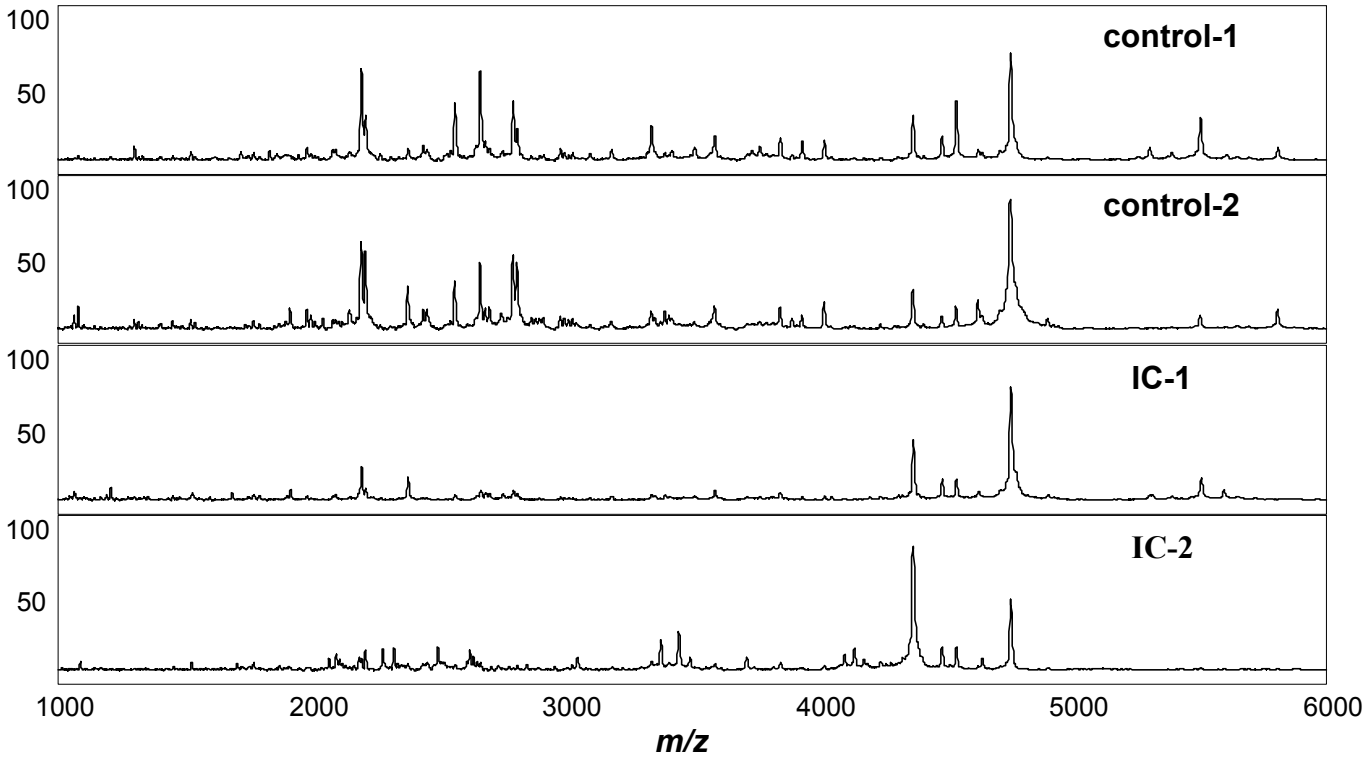

Figure 1. Comparison of SELDI-TOF MS spectra of urine samples obtained from healthy (i.e. control) and IC-affected individuals.