

Urinary Biomarkers of Asthma Inflammation in Children: The Use of NMR Spectroscopy

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ABSTRACT

Asthma is a chronic inflammatory disease of the airways that leads to airway obstruction. Accurate knowledge of the daily level of inflammation could directly impact treatment of the child. Existing methods for determining inflammation levels (*i.e.* lung function tests and bronchoscopy) are not practical for all patients especially the very young. We hypothesized that the unique cellular activity present in the asthmatic lung would be reflected in unique metabolites generated and excreted in the urine. Clinical information and urine samples were collected from the Stollery Children's Hospital pediatric outpatient asthma clinic (66 children, ages 4-16). By chart review age, sex, history/physical exam from each visit, medication dosage, atopic status, and spirometry were collected in order to correlate clinical presentation with urine NMR data. We measured 39 possible metabolites in urine samples from children over two visits. As expected most metabolites were similar between visits. In contrast, one metabolite unique to mast cell metabolism, 1-methylhistamine, demonstrated a possible correlation with patient's clinical improvement or worsening between visits. While this work is still preliminary, we believe urine NMR analysis offers an opportunity for non-invasive monitoring of airway inflammation in patients of any age.

INTRODUCTION

- Asthma is a chronic inflammatory disease of the airways leading to airway obstruction
- Current therapies like inhaled corticosteroids are used to control the level of inflammation of the airways, but are associated with several adverse side-effects such as weight gain, growth retardation, glucose intolerance, and hypertension
- Determining when to start inhaled corticosteroids or to adjust the dosage requires an accurate knowledge of the patient's current level of airway inflammation
- Current methods for determining the level of airway inflammation can be invasive and are not suited for the very young
- Due to the limitations of the current methods a patients status must be determined from physical exams and pulmonary function tests.
- ¹H-NMR spectroscopy identifies compounds in solution based on their unique resonant frequencies when placed in a static magnetic field.
- We hypothesized that the unique airway inflammation of asthma creates distinct metabolic products that could be measured in a non-invasive urine sample using NMR analysis

METHODS

Pediatric Sample Collection: Informed consent was obtained from children attending the Stollery Childrens Hospital (66 patients) in accordance with guidelines established by the University of Alberta Health Research Ethics Board.

Sample Preparation: Urine samples were stored at -80°C until time of analysis. Urine samples were thawed in a biosafety fume hood and a 630 µl aliquot was removed and placed in a 1.5 ml Eppendorf tube followed by the addition of 70 µl of a reference buffer solution ((4.9 mM DSS (disodium-2, 2-dimethyl 2-silapentane-5-sulphonate) and 100 mM imidazole in D₂O) Sigma-Aldrich, Mississauga, ON). Each sample was then brought to a pH of 7.0 +/- 0.1 using HCl and NaOH. An aliquot of 600 µl was taken and transferred to a standard 5 mm glass NMR tube (Wilmad, NJ, USA).

NMR Analysis: All ¹H-NMR spectra were acquired on a 600 MHz Inova (Varian Inc, Palo Alto, Ca.) spectrometer equipped with a 5 mm triple-resonance (HCN) probe with Z-axis gradient coil. One-dimensional ¹H-NMR spectra were collected at 25°C with a tnoesy pulse sequence (one-dimensional, three pulse NOESY, with a transmitter pre-saturation delay of 900 ms for water suppression during the pre-acquisition delay and 100 ms mixing time), and a spectral width of 8012 Hz.

Clinical Data: Clinical data for each patient was collected by chart review, including age, sex, history/physical exam from each visit, medication dosage, atopic status, and lung function (FEV1, FVC, FEF 25-75%, FEV1/FVC).

Metabolomic Analysis: Thirty-nine known metabolites were identified according to chemical shift and spin couplind patterns using Chenomx NMR Suite Professional. Preliminary analysis has shown an inverse correlation between 1-methylhistamine and a patients clinical improvement.

CLINICAL/PATIENT INFORMATION

Clinical Presentation

MRDC

Study No. BRS 1302
Clinic Patient No. 14911223
Storage Profile U of A Hospital
Hospital No. U of A Hospital

PI Individual Patient Analytical Sample

Study No. BRS 1302
Date 11/23/2007
Litter Sample #

Comments: [Text describing patient history and symptoms]

Height 105%
Weight 105%

Patient's Current Medication

MRDC

Study No. BRS 1302
Clinic Patient No. 14911223
Storage Profile U of A Hospital
Hospital No. U of A Hospital

PI Individual Patient Analytical Sample

Medications: [List of medications]

Pulmonary Function

MRDC

Study No. BRS 1302
Clinic Patient No. 14911223
Storage Profile U of A Hospital
Hospital No. U of A Hospital

PI Individual Patient Analytical Sample

Medications: [List of medications]

Cell Work-Up: [List of tests]

Pulmonary Function: [List of tests]

Patient's Cell Work Up

MRDC

Study No. BRS 1302
Clinic Patient No. 14911223
Storage Profile U of A Hospital
Hospital No. U of A Hospital

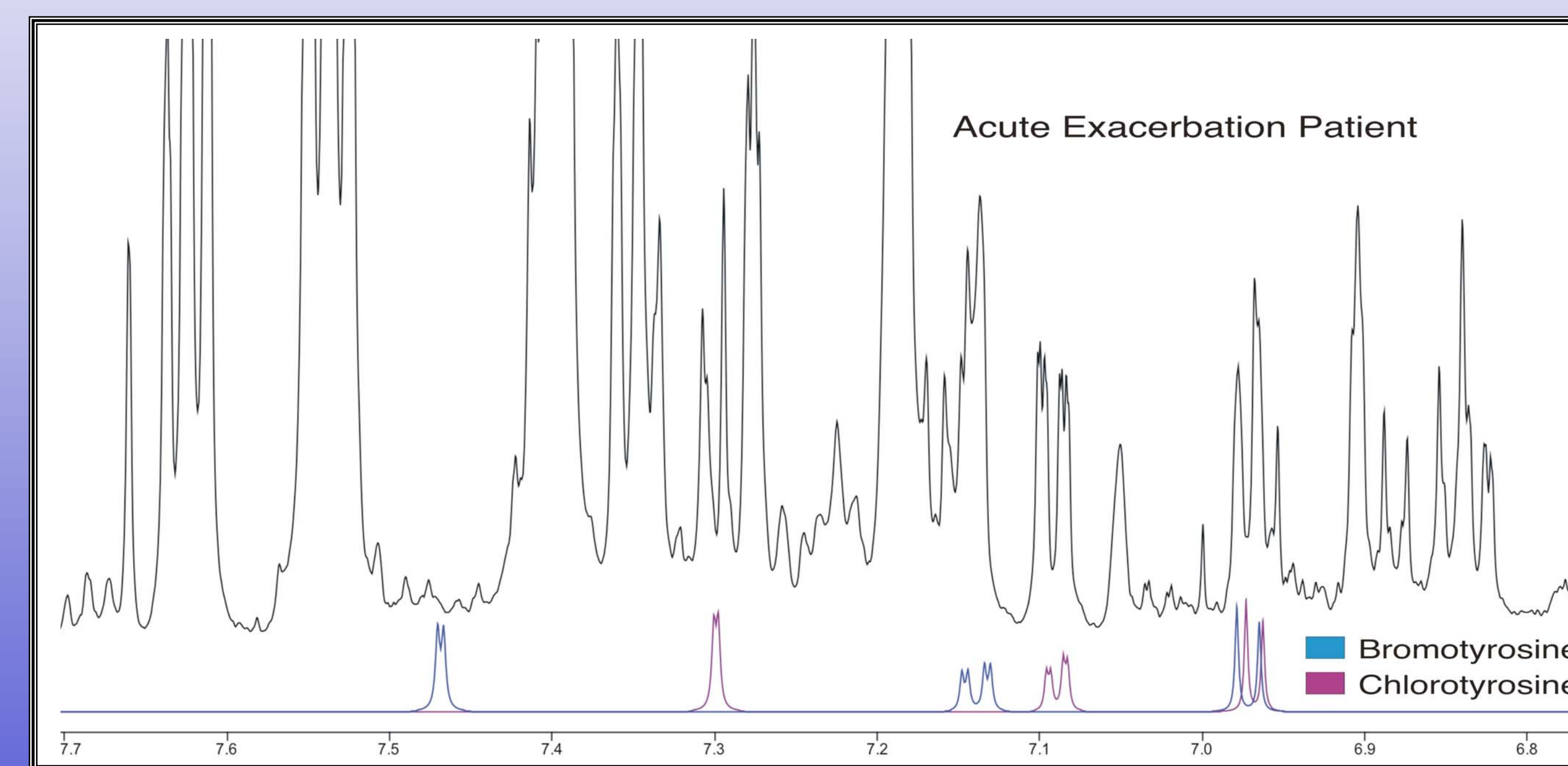
PI Individual Patient Analytical Sample

Medications: [List of medications]

Cell Work-Up: [List of tests]

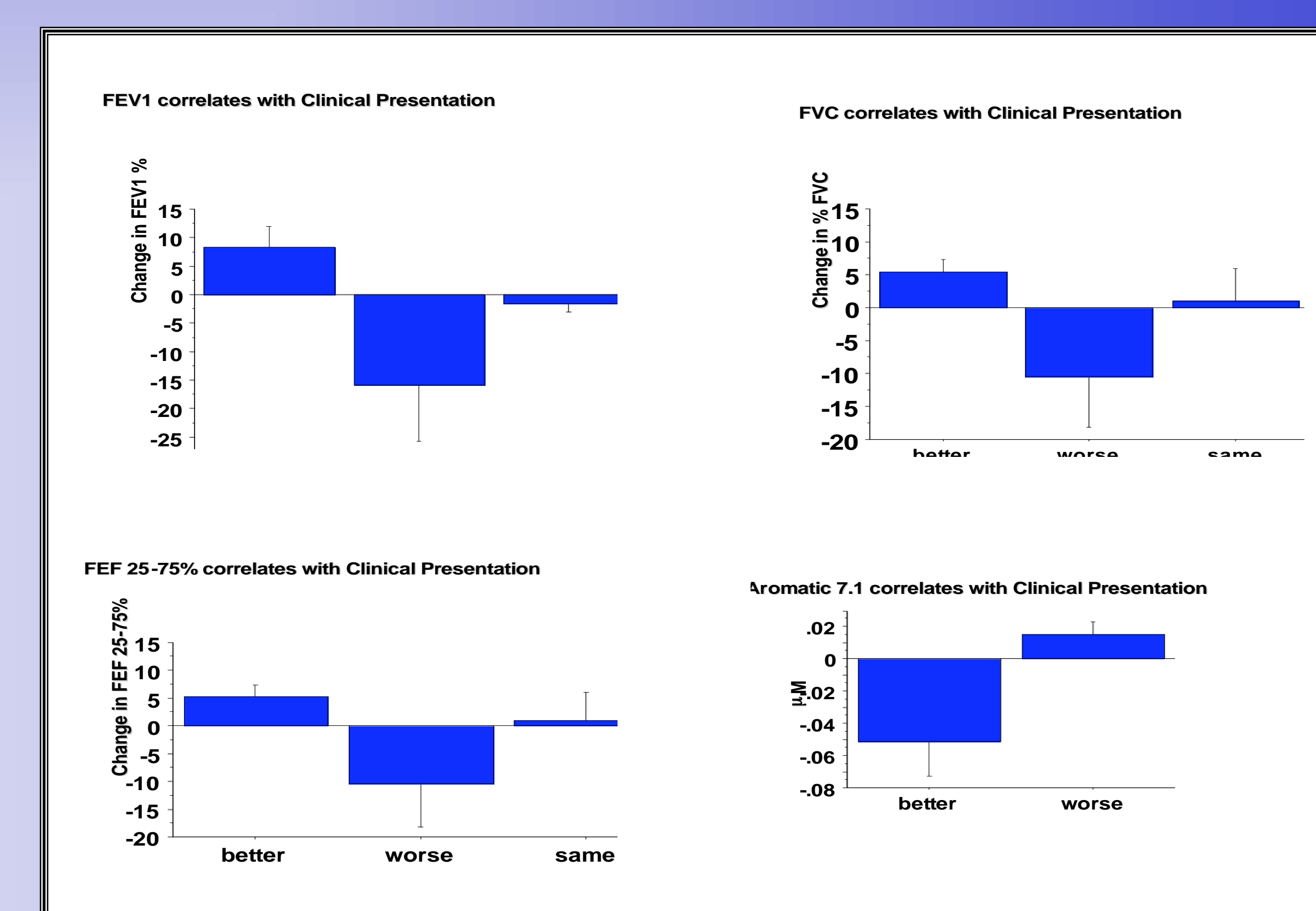
Pulmonary Function: [List of tests]

Clinical information was compiled on every patient for each visit where a urine sample was obtained. Information such as pulmonary function, medication/dose, sex, age, allergic status, clinical presentation, etc was collected to provide a patient profile for each visit. This profile was used to provide information on each urine sample obtained in relation to the status of the patients asthma.



¹H-NMR spectra of urine (600MHz, 25°C). Selected spectrum of an asthma patient overlaid with halo-tyrosine standard spectra. Urine spectrum demonstrates possible identification of modified tyrosine residues. Compounds identified in patient urine includes pathophysiologically relevant metabolites as well as novel biomarkers currently undergoing biochemical description

RESULTS



Pulmonary function tests performed in the pediatric clinic correlate with asthma severity, clinical improvement, and metabolite concentrations identified in 1D ¹H-NMR spectra of patient urine. Metabolites under investigation include known inflammatory markers 1-methylhistamine, adenosine, and novel compounds like 'yellow-7.1'.

CONCLUSIONS

- Clinical metabolomic investigations require careful, secure, and detailed information from patient recruitment to final disease correlation
- 1D ¹H-NMR analysis of asthma urine reveals an extremely rich metabolite make-up including some as yet unknown compounds
- Metabolites identified and quantified by NMR correlate with clinical presentation, symptom improvement, and clinical pulmonary function tests.
- For example, a decrease in the concentration of the biomarker for inflammation, 1-methylhistamine, correlates with a better clinical presentation in children with asthma. Thus, higher concentrations of 1-methyl histamine in the urine may predict an impending deterioration in an asthma patient's health
- Continuing work includes multivariate statistical analysis to determine the ability to diagnose and provide a prognosis through NMR analysis of asthma urine.. As well, additional metabolites are being identified by mass spectrometry

