Supplemental Material

EXPERIMENTAL

Biomarker development approach

The initial phases of biomarker development described by Hewitt et al.[1] were adapted for use in the current studies. Phase 1 consisted of sample collection from healthy cats and cats with feline interstitial cystitis (FIC) to determine if any differences in IRMS spectra could be identified. Effects of sex and stress on the spectra were investigated, and the results were compared with spectra obtained from the serum of cats with non-bladder diseases. After these pilot studies, serum was obtained from healthy humans and humans with interstitial cystitis (IC) for analysis and comparison. Effects of sex were investigated on biomarker patterns in patients and control subjects of both species, and the effects of disease activity in cats with FIC. Phase 2 consisted of developing procedures to improve performance of the assay by using ultrafiltration to remove serum constituents that might not be related to the disease diagnosis. The predictive accuracy of the calibration models was validated with an independent test set of samples representative of the classes modeled with the training set. Coded samples from (F)IC and healthy subjects were provided for diagnosis based on the IR spectral data and multivariate model. These results were compared to the real identity of the samples to determine accuracy and robustness of the infrared technique.

Cats

All cats were individually housed in stainless steel cages (69.9cm x 78.1cm x 74.9cm), fed a commercial cat food in amounts sufficient to maintain a moderate body condition, and water was provided ad libitum. Baseline conditions consisted of enriched cages, daily free playtime (weekdays) in a room with boxes, toys, climbing and scratching opportunities, treats, individual interaction with one person familiar to them (JS) and classical music for 2–3 hours per day. All cats were kept under constant temperature (72 F +/– 4 F) and lighting schedule (12 L: 12 D). The husbandry schedule remained constant (care from 0700-1000) with one caretaker Monday–Friday and a second one on the weekends. The enrichment was carried out by one individual (JS) familiar to the cats.

Cats were subjected to 3 days of psychological stress to test for effects of stress on the biomarker pattern. The cats had no enrichment or individual attention, and routine feeding and cleaning was conducted at varied and unpredictable times by caretakers that were unfamiliar to the cats. Temperature and lighting remained the same. On the third day food was removed at 1700 hours. At 0900 the following morning, all cats were brought to the lab in a cat carrier and anesthetized with isoflurane for sample collection.

To compare results with data obtained from cats with other diseases, we obtained serum from seven client-owned cats with chronic, non-bladder diseases from the clinical laboratory of the Ohio State University Veterinary Teaching Hospital. Serum from 10 FIC (5 female, 5 male)

and 10 healthy cats (5 female, 5 male) were used to evaluate the effect of serum filtration, and for LC–MS analysis.

Humans

A. Harvard Urological Diseases Center

Male Participants: Serum samples were obtained from 9 men with chronic prostatic pain syndrome type IIIA (CPPS-IIIA) that had at least "moderate" symptoms, defined as a total National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) score of 15 or greater. The eligibility criteria were identical to those of the NIH Chronic Prostatitis Collaborative Research Network.[2] Five healthy asymptomatic men from the general population were matched to the patients by age and served as voluntary controls. In addition to an NIH-CPSI score of 0, the controls were required to have a prostate-specific antigen level of less than 2.5 ng/mL and normal digital rectal examination findings.

Female participants: Serum samples were obtained from 9 women with IC, and from 6 age- and sex-matched healthy control subjects.

B. The Ohio State University (OSU)

Healthy women – Sera from 10 healthy women were collected from employees of the Ohio State University College of Veterinary Medicine (OSU-CVM). *Women with IC* – Sera were obtained from four women members of a local Interstitial Cystitis support group and one woman employee of the OSU-CVM with IC. *Women with other urologic disorders* – Serum samples for comparison with IC were collected from three patients from the Division of Urology at the Ohio State University Medical Center; one with chronic urinary tract infection, one with urge incontinence, and one with neurogenic bladder.

Sample preparation and analysis: Serum was separated by centrifugation and samples were immediately stored at –20°C to maintain their integrity until analysis. Serum (1:1 v/v serum:water) solutions (~5 µL), complete or fractioned by centrifugation, were placed (0.5 ul) on SpectCONC-IR (Tienta Sciences, IN) slides and dried under vacuum to produce dried serum films (DSF). Serum was fractionated by centrifugation through a cellulose membrane of 10,000 nominal molecular weight limit at 16,000 x g for 10 min using a microcentrifuge (Model 5415R, Eppendorf, Westbury, NY), followed by vacuum drying of 1 µL of the filtrate over the SpectCONC-IR microscope slides.

Procedures were slightly modified for human samples from The Ohio State University, which resulted in comparable results and enhanced signal strength. Blood samples were collected into plain glass hematocrit tubes after finger stick using an Accu-Chek Softclix lancet device (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's directions. Serum was then separated by centrifugation and stored at –20°C until analysis (within 2 days). Samples from employees at the OSU-CVM were collected, centrifuged, and analyzed in the same day. Sera were used to prepare DSF using a 1:5 serum/water solution. The serum solution was

loaded onto a cellulose membrane filter device with a 10,000 nominal molecular weight limit and fractionated as described above. These filtrates (0.5 µL) were applied to microscope slides (SpectRIM™) with a highly reflective surface, and films were obtained by vacuum drying.

References

1 S. M. Hewitt, J. Dear and R. A. Star, *J. Am. Soc. Nephrol.*, 2004, **15**, 1677–1689

2 M. S. Litwin, M. McNaughton-Collins, F. J. Fowler, Jr., J. C. Nickel, E. A. Calhoun, M. A. Pontari, R. B. Alexander, J. T. Farrar and M. P. O'Leary, *J. Urol.*, 1999, **162**, 369–375