Evaluations of human prostate cancer metabolomic profiles with a testing cohort.

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Introduction: In a 2005 publication on the investigations of human prostate cancer (PCa) metabolomics we reported improved diagnostic and prognostic abilities presented by metabolomic profiles when compared with individual metabolites measured from intact tissue HRMAS MRS from a study of 199 prostate specimens from 82 biopsy proven cancer patients[1]. That study led to two major conclusions that metabolomic profiles established through the measurements of prostate tissue metabolites can differentiate 1) tissue specimens with and without cancer glands, and 2) patient pathological stages. However, the utility of this first proposition of using PCa metabolomic profiles in the clinic could only be categorized as a generation of the hypothesis of the potential existence of PCa metabolomics. In other words, it only proposed the metabolomic structure from the analyses of a training cohort, and did not evaluate another independent cohort to test the hypothesis.

Earlier this year (2010), and in conjunction with our proposal of metabolomic imaging for the detection of human PCa, we realized the limitation of concentration based PCa metabolomic profiles in imaging applications where the determination of metabolic concentrations is not as straight forward as in the analysis of tissue samples. Consequently, we reanalyzed the training cohort for the PCa detection ability of metabolomic profiles established through relative metabolite intensities, as shown in Figure 1[2]. Although this recent study in 2010 may be viewed to a certain degree as a test of the training cohort proposed in 2005, the comparison was not precisely controlled. Here, we report an analysis of a testing cohort that was measured precisely following the exact experimental procedures that guided the 2005 study.
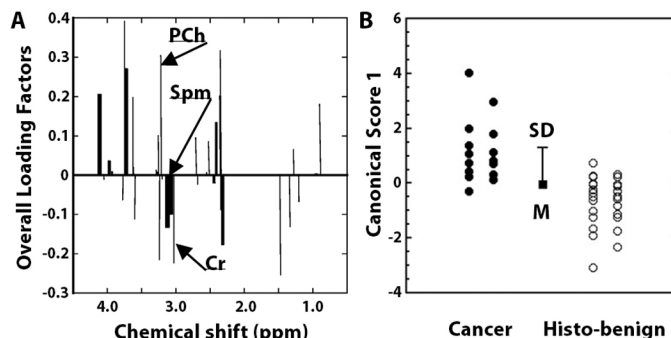

Figure 1. Prostate cancer metabolomic profiles of relative metabolic intensities at 14T. [2]

Methods: *MR Spectroscopy*. A Bruker (Billerica, MA) AVANCE spectrometer operating at 600MHz (14.1T) was used for all MR experiments. Tissue samples were placed into a 4mm rotor with 10 l plastic inserts. 1.0µl D_2O was added for field locking. Spectra were recorded at 3ºC with the spectrometer frequency set on the water resonance, and a rotor-synchronized DANTE experimental protocol was applied with spinning at 600 and 700Hz (±1.0Hz)[3]. 32 transients were averaged at a repetition time of 5s. Spectra will be analyzed by an in-house MatLab based program. All FIDs will be subjected to 1 Hz apodization; metabolite resonances will be integrated and calibrated according to the external and internal standards. Metabolomic profiles previously proposed (e.g. Figure 1) are tested for tissue pathologies and patient clinical statuses. *Histopathology*. After spectroscopy, tissue samples were fixed in formalin, embedded in paraffin, cut into sets of 5µm sections at 100µm intervals, and stained with hematoxylin and eosin. Volume percentages of histological features (cancer, stroma, benign ducts, and necrosis) were analyzed and quantified by a pathologist.

Results: Similar to the training cohort, the current testing cohort included 192 prostate samples from 83 biopsy proven PCa patients. Following the HRMAS spectral analysis, pathology analysis of these tissue samples revealed that cancer cells only exist in 11 of the 192 samples due the well known heterogeneity of PCa. Numerical and statistical analyses of the corrections between spectral results and tissue pathology, as well as patient pathological stages are current underway.

Conclusions: With the development and maturation of cancer metabolomics, it is extremely important to apply quality controls for the report of clinical data with randomized patient cohorts. This study represents one such approach. Biomolecular studies and parameters, aka biomarkers, may only discover their clinical utility if they can survive the test of an independent testing cohort.

References: 1. L.L. Cheng et al. *Cancer Res* 2005; **65**(8)**:** 3030-3034. 2. C.L. Wu et al. *Sci Transl Med* 2010; **2:** 16ra18. 3. J.L. Taylor et al. *Magn Reson Med* 2003; **50**(3)**:** 627-632.