Raman Micro-spectroscopy: Potential for Diagnosis and Prediction of Prostate Cancer Outcome

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Rationale. Raman spectroscopy (RS) has been reported to permit distinction of cancerous prostate lesions from healthy tissue and benign changes such as BPH. However, the question as to whether this technology has the potential for clinical utility with regard to a) accuracy of early diagnosis and prediction of outcome and, b) practicality for clinical diagnostic routine, has hitherto not been fully assessed. We have tested an advanced confocal Raman micro-spectroscopy system (BioRam™) to assess, in a retrospective study, whether the technology can distinguish aggressive from non-aggressive prostate cancer on prostatectomy tissue from Gleason 6 patients for whom long term outcome data were available. In addition, based on previous work on aggression-specific biomarker sets derived from tumor-surrounding tissue (stroma), we wanted to assess whether RS was capable of detecting aggression-indicating signals in the stroma. The overall rationale of our project is the evaluation of the performance of RS technology in the prostate cancer diagnostic arena with the goal of improving early diagnosis and prediction of outcome.

Purpose. To investigate whether Raman Micro-spectroscopy, applied to prostatectomy tissue, is useful in the prognosis of prostate cancer outcome, i.e. can this technology distinguish aggressive (fast biochemical relapse) from non-aggressive (no-biochemical relapse in 60 months) disease in Gleason 6 patients with special emphasis on analyzing prostate cancer stroma.

Materials and Methods. In a retrospective study on 30 prostatectomy patients with known outcome (Departments of Urology and Pathology, SLK Kliniken, Heilbronn), we analyzed prostatectomy tissue with Gleason 6 scores: 15 cases with non-aggressive disease (no biochemical relapse in 60 months, i.e. PSA always <0.2); 7 cases with very aggressive disease (average time to biochemical relapse 4.3 months); 8 cases with aggressive disease (average time to relapse 30.1 months) on a CellTool BioRam™ Raman confocal micro-spectrometer. Several tumor areas and stroma areas at increasing distances from tumor were measured for each patient and data analyzed by bio-statistical methods.

Results: RS analysis was performed on de-paraffinized prostatectomy tissue following designation of cancerous lesions by a certified pathologist. Clusters of approximately 50 cells in eptithelial tumor areas and several stroma areas were measured. Spectrometric data were averaged for each cluster and, following unblinding, analyzed by bio-statistical methods. The overall accuracy of distinction of tumor measurements between aggressive and non-aggressive disease was 84% ($p = 0.031$). In this study specificity was defined as to how well the test distinguished non-

aggressive disease from rapid relapse cases and yielded 91%. Analysis of the stroma measurements yielded similar results whereby the overall accuracy and specificity as defined above were dependent on distance from tumor with accuracy of 82% and highest specificity of 94% at a distance up to 100 micron from tumor. At a distance up to 500 micron specificity is in the 80% range. Initial comparison of the spectral data between the very aggressive, aggressive and non-aggressive patients (see Material and Methods) indicated that distinction of these cohorts with RS is possible as well.

Conclusions. The results indicate that Raman micro-spectroscopy is indeed capable of distinguishing aggressive from non-aggressive prostate cancer with high specificity by analyzing tumor and adjacent stroma tissue. The ability to diagnose in stroma will be further investigated as it holds promise for reducing false negative biopsy analysis, e.g. when the biopsy misses the tumor. We now plan to carry out a confirmatory study with larger patient cohorts, include analysis of biopsy tissue and map the extend of the measurable changes in stroma more extensively.