Correlation between MRI-derived Quantitative Biomarkers and Circulating Tumor Cells in Prostate Cancer

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Target Audience: Physicians and scientists interested in prostate cancer basic and imaging research.

Purpose: Circulating tumor cells (CTC) are rare malignant cells found in the peripheral blood of patients with a wide range of solid tumors such as breast, colorectal, lung, prostate, ovarian, pancreatic, liver, and bladder[1]. CTCs constitute *seeds* for subsequent growth of additional tumors (metastasis) in vital distant organs, triggering a mechanism that is responsible for the vast majority of cancer-related deaths[2]. One could hypothesize that ***(i)*** the number of CTCs could be prognostically important by potentially estimating the total burden of disease and/or tumor invasiveness. Other plausible hypotheses are that CTC counts are related to ***(ii)*** tumor angiogenesis, which is well documented to be essential for tumor growth, invasion and metastatic spread; and ***(iii)*** tumor water-diffusion properties, which are considered surrogate markers of tumor cellularity and cellular membrane integrity. In this study we tested these hypotheses by: ***(i)*** using multiparametric (MP-MRI) to delineate the tumor in the prostate and estimate the tumor volume; ***(ii)*** utilize Dynamic Contrast Enhanced MRI (DCE-MRI) for quantitative assessment of tissue perfusion and vascular permeability; and ***(iii)*** estimate tissue diffusion properties via Diffusion Weighted Imaging (DWI). The imaging biomarkers are related to CTC counts in patients enrolled in a contemporary randomized clinical trial for definitive radiotherapy of prostate cancer.

Methods: CTCs and MP-MRI were analyzed in twelve patients participating in a Phase II randomized clinical trial: Hypofractionated External beam Image Guided Highly Targeted Radiotherapy – the HEIGHT trial. The objective of the trial is to compare the efficacy of administering a hypofractionated MRI-targeted external beam boost to standard dose and fractionated definitive external beam radiation for intermediate to high risk prostate patients. Multiparametric (MP)-MRI was acquired on Siemens (Erlangen Germany) 3T Trio System (n=6) and GE (Waukesha, WI) 3T Discovery MR750 System (n=6). It consisted of: ***(i)*** Axial T2w-MRI of the pelvis: resolution 1.25×1.25×2.5 mm; Field of View (FOV): 320×320 mm; slice thickness=2.5mm (no gap); 72 slices; ***(ii)*** Dynamic Contrast Enhanced MRI (DCE-MRI)–12 series of T1w at 30-34s temporal resolution; and ***(iii)*** DWI - Single-shot echo-planar imaging, utilizing the diffusion-module and fat-suppression pulses. Water diffusion in three directions was measured by using *b* values of 50, 500, and 1000 s/mm^2. Apparent Diffusion Coefficient (ADC) maps were automatically calculated utilizing imaging console software. In-house software was used to define tumor volumes based on MP-MRI in the prostate using spatially explicit quantitative image analysis[3]. CTC enumeration protocol included the collection of 20 mL of blood in two 10 mL serum BD Vacutainer tubes (Becton Dickinson and Co, NJ). In order to preserve CTCs, the blood sample was transferred to 7.5 mL CellSave™ Preservative Tube (Immunicon, PA). Samples were processed for CTC enumeration as previously described [4]. Briefly, the sample was fixed in 1% end-concentration of formalin for 10 minutes and processed through a microfiltration platform for CTC capture. Filter was washed post-filtration with 1×DPBS (Life technologies Corp, CA) and subjected to pan-Cytokeratin CD45 (Dako North America Corp, CA) immunofluorescence staining and cover-slipped with ProLong Gold antifade mounting media with DAPI (Life Technologies, CA). CTCs were then identified as Cytokeratin positive/DAPI positive/CD45 negative cells with additional morphology criteria.

Table 1: Correlation between CTC counts and imaging biomarkers, based on MP-MRI (n=12)

Imaging Markers	CTC r (p-value)
Anatomical Imaging (T2-weighted MRI)	
Prostate Volume (cc)	0.24 (0.226)
Peripheral Zone Volume (cc)	-0.39 (0.105)
Central Region Volume (cc)	***0.43 (0.081)***
Tumor Volume (cc)	-0.048 (0.441)
Peripheral Zone (% of Prostate)	**-0.75 (0.002)**
Central Region (% of Prostate)	**0.75 (0.002)**
Tumor Volume (% of Prostate)	-0.30 (0.171)
Perfusion (DCE-MRI)	
K^{trans} (Tumor) (min^{-1})	**0.79 (0.001)**
k_{ep} (Tumor) (min^{-1})	***0.42 (0.087)***
v_e (Tumor) (%)	**0.53 (0.038)**
K^{trans} (NAT PZ) (min^{-1})	***0.47 (0.061)***
k_{ep} (NAT PZ) (min^{-1})	-0.24 (0.226)
v_e (NAT PZ) (%)	***0.41 (0.092)***
K^{trans} (NAT CR) (min^{-1})	0.17 (0.298)
k_{ep} (NAT CR) (min^{-1})	-0.31 (0.163)
v_e (NAT CR) (%)	**0.60 (0.019)**
Diffusion (DWI)	
ADC Prostate – Median (μm^2/s)	**0.60 (0.019)**
ADC Tumor – Median (μm^2/s)	***0.44 (0.076)***

Abbreviations: NAT = Normal-Appearing Tissue; PZ = Peripheral Zone; CR = Central Region

Results: Median age of the patients was 73 years (range: 64-86); ten patients had Gleason Score of 7 and two 8. Four patients were T-category T1 and eight – T2. The median pre-treatment PSA was 5.9 ng/mL (range: 1.4-15.5). CTCs mean±std was 33±43 (4(33%) pts had undetectable CTCs). The following volumes were determined on T2-weighted MRI via manual contouring (mean±std): prostate: 45.01±20.47; peripheral zone (PZ) volume: 13.13±6.8; central region (CR=transition+central zones): 31.87±17.47. The fractions (%) of PZ and CR from the prostate volumes were also calculated: PZ: 0.30±0.14 and CR: 0.69±0.14. The tumor volume (mean±std: 1.38±0.87) was on average 3.37% from the prostate volume. From DCE-MRI, the following pharmacokinetic parameters characterizing perfusion were estimated: Ktrans – volume transfer constant between blood plasma and Extracellular Extravascular Space (EES); k_{ep} – rate constant between EES and blood plasma; v_e – volume fraction of EES[5]. Besides in the tumor, the perfusion parameters were also calculated for manually contoured normal-appearing tissue (NAT) volumes in PZ and CR. The median ADC values for prostate and tumor volumes were on average 1311 and 1045 μm^2/s, resp. Correlations and p-values between CTC counts and imaging biomarkers are presented in **Table 1**. The CTC counts were *not* correlated with tumor volume, while the majority of tumor perfusion and diffusion parameters were significantly ($p<0.05$) or marginally ($p<0.1$) correlated with the CTCs. Interestingly, the CR volume was positively correlated with CTCs and several perfusion/diffusion biomarkers of the tumor environment (e.g. prostate, PZ and/or CR) had tendencies for significant correlations.

Conclusions: This is, to our knowledge, the first study comparing CTC counts with *in vivo* measurements of tumor volume, perfusion and diffusion. Albeit with relatively small sample size, an intriguing relationship is emerging: the perfusion and diffusion characteristics of the tumor, rather than the tumor volume itself are correlated with the CTC counts. Our analysis also demonstrates a relationship between the overall morphology and physiology of the prostate and the CTC numbers and thus, their potential role in tumor progression and metastatic spread.

References: [1]Jemal et al, CA Cancer J Clin 58:71-96,2008; [2]Alix-Panabieres et al, Clinical chemistry. 2013;59:110-8; [3]Lin et al, Clin Cancer Res. 2010;16:5011-8; [4]Stoyanova et al, Pract Radiat Oncol, 2013; [5]Tofts PS, Stoyanova R: In: *ESMRMB Congress, Leipzig*; 2011.

Funding: Florida Bankhead Coley Cancer Research Program, Team Science Project 1BT03