

Sarcopenia is Observed with Imaging Biomarkers in Preclinical Model of Mixed Osteoblastic-Osteolytic Bone Metastases

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Disclosures: The authors have no disclosures

INTRODUCTION: Metastasized cancer cells disturb the bone remodeling process, leading to increased formation of low-quality bone (osteoblastic bone lesions). In addition to changes to the bone, 61% of patients with prostate cancer experience sarcopenia (generalized muscle weakening). Systemic treatment with docetaxel (DXT) and bisphosphonates [Zoledronic Acid {ZA}] for prostate cancer are further known to have an impact on muscle and bone health. Although the muscle and bone have many shared signaling pathways and undergo biomechanical crosstalk, research on the relationship between the atrophy of both tissues, known as osteosarcopenia, has been limited. Prostate cancer patients experiencing osteosarcopenia have decreased bone and muscle volume and increased fat deposition in the tissues. These are associated with higher patient mortality and morbidity, increased frequency of falls and fractures, and an overall lowered quality of life. This study aims to quantify changes and identify imaging biomarkers from the psoas muscle in the presence of metastatic prostate cancer.

METHODS: Twenty-four athymic, 6-week-old male rats were randomly assigned to the following groups: healthy control (H-C; n=3); healthy treated (H-DXT; n=4), (H-ZA; n=2) and ACE-1 cell injected control (ACE-1-C; n=3); ACE-1 cell injected treated (ACE-1-DXT; n=8) and ACE-1-ZA; n=4). Institutional approval was obtained, and the ARRIVE guidelines were followed. Under inhalation anaesthesia 15 rats received an intra-cardiac injection of luciferase-transfected ACE-1 canine prostate cancer cells ($1.5 \times 10^6/200 \mu\text{l}$). Grip tests (Grip Strength Test, Bioseb) to evaluate muscle strength were conducted on rats on days -1, 13, and 20 post-tumor cell injection to test the functional capacity of the muscles prior to *in vivo* μMRI (NanoScan, Mediso, with Gadolinium contrast) and μCT (NanoScan, Mediso) imaging to monitor changes to the psoas muscle and vertebrae. Bioluminescence imaging (IVIS Spectrum) monitored tumour formation and progression upon luciferin injection on days 14 and 21 post-tumour cell injection (Figure 1). Rats were injected with docetaxel (5 mg/kg, i.v.), or zoledronic acid (Zometa; 60 $\mu\text{g/kg}$, s.c.) on day 9 post-tumour cell injection. All animals tolerated the procedures well. The rats were sacrificed on day 21, and muscle and bone tissue were harvested. Psoas muscles were manually segmented from fused *in-vivo* MR/CT images to investigate imaging biomarkers like muscle volume and attenuation and normalized to the L2 vertebrae volume (Figure 2). The vertebrae were imaged *ex vivo* using high-resolution μCT ($\mu\text{CT}100$, Scanco, 34.4 μm isotropic voxels) to assess trabecular bone architectural properties. Formalin-fixed bone tissue was decalcified using 10 % EDTA solution. Haematoxylin eosin (H&E) staining and immunohistochemistry staining identified the tumor cells within the bone. In addition to the H&E staining, the psoas muscle was stained with Oil Red O to visualize lipid content (Figure 3).

RESULTS: Bioluminescence and *ex vivo* μCT images confirmed mixed osteoblastic-osteolytic bone metastases in 7 rats (Figure 1,2). The presence of sarcopenia in rats with metastatic disease was confirmed by significantly lower normalized psoas volume in cell-injected animals compared to healthy animals (two-factor ANOVA, $p < 0.0005$). However, no significant differences in muscle function were found. ZA treatment significantly increased BMD (two-factor ANOVA, $p < 0.005$) and decreased muscle attenuation (two-factor ANOVA, $p < 0.05$). Docetaxel treatments showed a smaller negative change in muscle attenuation over time compared to the ZA-treated rats, or controls ($p < 0.002$). Animals treated with docetaxel had a significantly more negative change in grip strength from before, to 3 days after treatment compared to non-treated, or ZA-treated animals ($p < 0.0001$).

DISCUSSION: The reduced growth in normalized psoas volume in animals with metastases compared to healthy control animals confirms the presence of sarcopenia. μCT psoas muscle attenuation analysis suggests that animals with bone metastases had higher fatty infiltration during disease progression, which was confirmed using histological analysis of the muscle. Previous data from the lab has shown that rats experience a stagnation in weight gain for a week after docetaxel injection, which may impact the decrease in grip strength during this time. Differences in psoas attenuation in the treatment groups indicate an effect on the muscle. Body composition parameters like muscle attenuation are known to be predictive of treatment response and tumor progression in the case of metastatic disease. These results indicate that the above-described model mimics the disease progression described in prostate cancer patients and can be used in further studies.

SIGNIFICANCE/CLINICAL RELEVANCE: Sarcopenia was observed with imaging biomarkers of muscle volume and attenuation in this preclinical model of spinal metastases secondary to prostate cancer. This study will provide a more thorough understanding of the relationship between treatments, osteopenia, sarcopenia, and prostate cancer tumour burden. The imaging biomarkers evaluated in this study may facilitate the earlier diagnosis of osteosarcopenia in prostate cancer patients, leading to earlier intervention and increasing the quality of life.

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ACKNOWLEDGEMENTS: This study was funded by the Canadian Institute of Health Research (CIHR) #180337

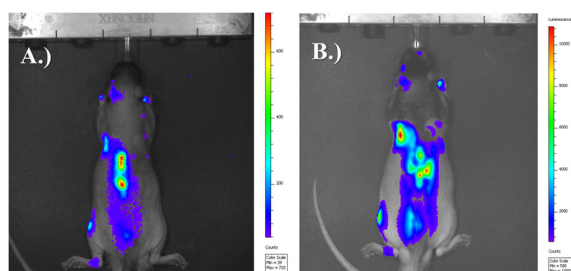


Figure 1. Bioluminescent image of ACE1 cell injected rats on day 14 (a) and 21 (b) respectively displaying tumor development.

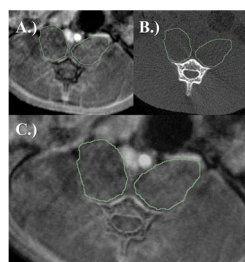


Figure 2. CT image (a), MR image (b), and co-registered CT & MR (c) image of rat vertebra with segmented psoas muscle in green.

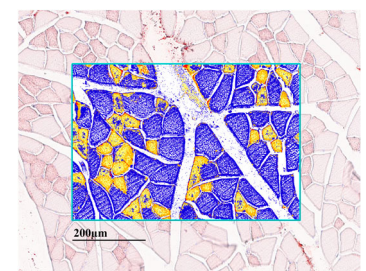


Figure 3. Oil Red O stain of psoas muscle showing fatty infiltration (yellow/orange) with positive pixel analysis in blue square.