COMPARATIVE ANALYSIS OF H&E AND FLUORESCENCE IMAGES OF BONES IN EXPERIMENTAL HEMATOGENOUS OSTEOMYELITIS MOUSE MODELS

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Hematogenous osteomyelitis (HOM) is defined as a bone infection that commonly affects the rapidly growing long bones, resulting in bone deformation followed by bone destruction. The most frequent pathogen responsible for HOM is *Staphylococcus aureus* (*S. aureus*), which enters the bone through bloodstreams [1]. Since the location of the causing pathogens is within the bone structure and frequently also within cells, HOM chemotherapeutic treatment is difficult to achieve and usually requires a longer treatment time. Therefore, exact knowledge about the localization of bacteria during the infection process is urgently needed to develop tailored treatment strategies bringing the appropriate antibiotic to the site of bacterial residence.

In this on-going study, we use an established mouse model of *S. aureus*-induced HOM [2]. Four conditions are investigated: (1) infected not treated, (2) infected treated successfully with antibiotics, (3) infected treated unsuccessfully, (4) uninfected controls. Longitudinal MRI over 6 weeks resulted in the visualization of bone deformation upon infection and its restoration upon antibiotic treatment. After 6 weeks, the mice were sacrificed and cryosections of the bones (femur) were produced after fixation and decalcification. One-half of the cryosections were stained with hematoxylin and eosin staining (H&E), revealing differences in bone structure, bone and immune cell characteristics among the different groups. The other half of cryosections were subjected to immunofluorescence labeling with two different combinations of primary antibodies prior to applying a specific fluorochrome-coupled secondary antibody, (a) anti-RUNX-2 (marker of osteoblast differentiation) + anti-*S. aureus* as a primary antibody, (b) anti-Tartrate-resistant acid phosphatase (TRAP, which has high expression levels in osteoclast) + anti-*S. aureus* alpha-hemolysin. Cell nuclei were counterstained using SYTOX-green and the actin cytoskeleton was labeled using fluorochrome-coupled phalloidin. The sections were analyzed using confocal microscopy and as an additional structural element, collagen was visualized using second harmonics generation microscopy upon excitation at 880 nm. Quantitative analysis of fluorescence images was compared to insights from H&E-stained sections and in-vivo MRI data. Characteristic differences between the treatment groups could be revealed.

References:

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