

## Title: Biochemical monitoring of bone sarcomas Patients Infected with COVID-19 Pandemic

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Statement of the Problem: In both preclinical and clinical settings, histological images are now digitalized into high resolution images. Big data sets of images seek digital tools for fast and precise analysis and diagnosis. Machine learning (ML)-based software are commonly used for various images analysis: detection, segmentation and classification. Here, we describe advantages and disadvantages of ML-supervised based digital histopathology image tools based on the literature review.

Review-based observations: ML-based software can significantly reduced image analysis time and inter-operator variability. However, we and other have experienced some limitations. Supervised ML is strongly encouraged for homogeneous staining quantifications, in which the pathologist can control the learning phase and choose appropriate input and output data (quality control). Subsequent, ML algorithms need to be well trained on a large amount of high-quality labeled images, to accurately segment and classify each image. The chosen images should include enough diversity to be representative of the entire dataset.

In addition, the choice of ML-algorithm is fundamental, and it reflects the complexity of the desired histological analysis. If a complex analysis is needed, more complex ML-based tools should be applied. For example, for simple staining quantification ML-FIBER is considered as easy-to use, fast and reproducible but lack of complex analysis and it requires specific image formats as input. Other software must be considered to quantify the image features. For instance, Ilastik software uses a random

forest classifier in the learning step, which helps to characterize by a set of generic (nonlinear) features (color and texture) and it supports up to three spatial plus one spectral dimension, calculating all dimensions in the feature analysis. Additionally, higher image processing can require deep neural networks in order to extract higher-level features from the raw input (used for cell characterization).