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POTENTIAL USE OF BIOMARKERS IN CLINICAL PRACTICE: ALPHA DEFENSIN AND SYNOVIAL FLUID

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Alpha defensin in synovial regards potential biomarker for identification of periprosthetic joints infections (PJI). The only commercially available test is an in situ immunocromatographic device. Orthopedics use the device under their direct responsibility, either as part of pre surgery patient assessment or, intra operatively, to make treatment decisions according to the likelihood of infection. In this diagnostic path flow, the availability of a method not prone to interferences, with high sensitivity and specificity, can be of great help to clinicians. LC-MS methods are considered to meet needs in terms of analytical performance and are widely used to measure new biomarkers. The aim of our study was to implement a methods to accurately measure Alpha defensin in synovial fluids. Several issues had to be addressed: the synovial fluid matrix viscosity, the need of a standard Alpha defensin peptide and a valid Internal Standard (IS), the definition of analytical protocol and, finally, the method validation. As preliminary step the uniqueness of peptides derived from trypsin digestion of Alpha defensin was checked by liquid chromatography - time of flight mass spectrometry. Synovial fluids samples from primary knee arthroplasty were used as negative matrix; a synthetic matrix (simulant) was produced and measures of spiked samples were run in parallel. The quantitative analysis was performed with two different instruments (liquid chromatography with time of flight or triple quadrupole mass spectrometry, LC-QTOF or LC-MS/MS) and two different methods (negative matrix spike and simulant synovial fluid spike) by spike of different concentrations of synthetic marker peptide. The quantitative method was developed with dynamic range from 0.1 to 100 µg/ml.

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