A proteomic approach to identify novel serum biomarkers of peri-implant osteolysis in a rat model

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Objective/Aim/Hypothesis:
Circulating biomarkers may offer a method to identify peri-implant osteolysis before bone loss causes aseptic implant loosening, the primary cause of total joint replacement failure in the mid- to long-term. However, there are currently no validated biomarkers for peri-implant osteolysis and studying biomarkers in patients remains challenging. Thus, the objective was to identify novel serum biomarkers of peri-implant osteolysis in a rat model of aseptic implant loosening.

Design/Approach/Methods:
In this IACUC-approved study, 33 male Sprague-Dawley rats (400 ± 12 g) were randomly allocated to four experimental groups (n=8-9/group). Titanium implants were placed in bilateral femora of three groups, which were then challenged with weekly intra-articular knee injections of either lipopolysaccharide-doped-polyethylene (LPS-PE) or cobalt-chromium-alloy (CoCr) particles, with particle-free vehicle (vehicle) serving as control. The remaining 8 rats were included as an intact age-matched control group that did not undergo surgery or injections. All rats were euthanized 6 weeks after surgery. The primary endpoint was serum protein expression assessed by mass spectrometry. Normalized and log-transformed total intensity raw protein data were extracted using the ScaffoldDIA desktop application. The false detection rate was set to 1% and a minimum of 2 peptides were required for protein identifications. Differential abundance analysis was performed using the *limma* package in the R programming language. An adjusted p value <0.05 was used to determine statistical significance. Pathway analysis software was used to explore cellular pathways enriched by proteins from the significantly altered protein sets.

Results:
Expression of 502 unique serum proteins was compared between groups. There were 24 proteins significantly altered in the CoCr-challenged group compared to the vehicle-control group. There were 52 proteins significantly altered in the CoCr- versus LPS-PE-challenged groups. Pathway analysis revealed significant interactions among proteins that were altered (all $p<0.001$). Further, while both PE and CoCr particles activated an innate immune response, CoCr particles also seemed to activate an adaptive immune response.

Conclusion:
There may be multiple novel candidate biomarkers for peri-implant osteolysis meriting further investigation. Differential activation of innate and adaptive immune responses by different particle types suggests there may be unique biomarkers associated with osteolysis caused by different materials.