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# Global proteomics profiling of serum and synovial fluid identifies biomarkers associated with improved PainDetect scores after intraarticular gold for management of painful knee osteoarthritis

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## Background and aims:

In a pilot study, we have shown that intra-articular gold micro-particles can reduce knee osteoarthritis pain for up to two years and found significant proteomic changes in serum and synovial fluid within eight weeks (1). This study aimed to identify serum and synovial proteomic biomarkers associated with improvements in PainDetect scores (nociceptive versus neuropathic characteristics) following intra-articular injection of gold microparticles in painful knee osteoarthritis.

Dissolucytotic metallic gold (DMG) ions have an immune-suppressive effect in laboratory testing (1). Gold may decrease inflammation because of various mechanisms such as regulation of the NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway (1). (Figure 1 - 4).

## Methods:

Thirty patients with moderate KOA were included and had intra-articular 20 mg gold microparticles (72.000 particles, 20-40 µm in diameter) injected using the patient's synovial fluid (SF) as the carrier. Proteomic profiling on SF and serum samples before and after eight weeks of treatment. PainDetect is scored from 0 to 38, with total scores of less than 13 considered to represent nociceptive pain, 13-18 possible neuropathic pain, and > 18 that represent neuropathic pain.

Synovial fluid and serum samples were trypsin-digested and analyzed using a discovery proteomics LC-MS workflow on a timsTOF Pro mass spectrometer operated in diaPASEF mode. Raw mass spectrometry data were then matched against the human UniProt database using the directDIA algorithm in Spectronaut for the identification and quantification of proteins. A sparse Partial Least Squares (sPLS) model was adapted to integrate proteomics data (expression of up to 600 proteins) with the PainDetect score and explore relationships between the two datasets. The linear combination of proteins with the highest correlation to PainDetect score was identified and used to predict treatment response. Finally, a functional enrichment analysis was made in Metascape to assess the biological relevance of the synovial fluid proteins that were covarying with the PainDetect score. Biological processes/terms were regarded as significantly enriched at a $p < 0.01$.

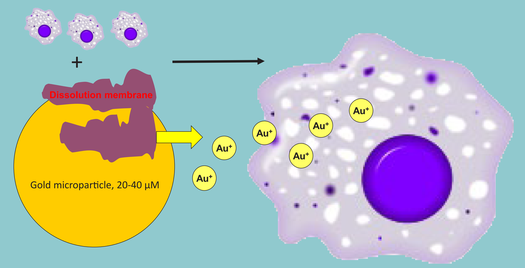


**Figure 1.** *Macrophages controls the dissolution membrane which liberate the gold ions by oxidation of the surface. Once the ions are liberated, most likely as Au(CN) , they are free to diffuse through the immediate microenvironment. The gold-loaded molecules are taken up into the cells, primarily macrophages, mastcells and histocytes.*

**Figure 2.** *Figure 2. Close to the gold implant gold-loaded molecular clusters are located outside cells. The two loaded cells are believed to be macrophages loaded with gold ions. The gold ions accumulate primarily in the lysosymes.*

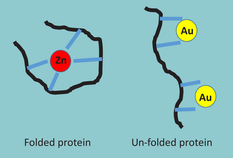


**Figure 3.** *Once in the intercellular fluid and the intracellular compartments, the gold ions act in the same ways that have been demonstrated for systemically administered gold ions. The effect is related to the ability of the gold ions to unfold the protein structures.*

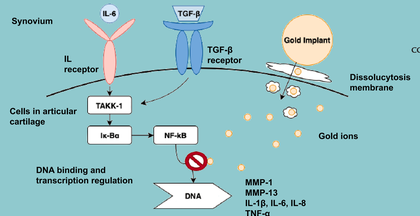


**Figure 4.** *Gold ions suppress inflammation locally by affecting certain signalling molecules and binding enzymes essential for the inflammatory process. The DNA binding activity and transcription regulation of NF-kB is abolished when AU- ions replace Zn2+ ions.*

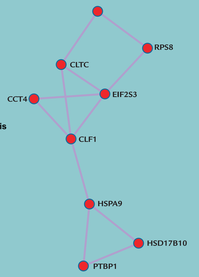


**Figure 5.** *Molecular complex suggestive of neural tissue regeneration and modulation.*

## Results:

The Pearson correlation coefficient between the PainDetect score and the linear combination of 17 selected synovial fluid proteins was $r(34) = 0.92$, $p < 0.001$. The linear combination of synovial fluid proteins and the PainDetect score partly explains the differences between baseline and follow-up samples. The functional enrichment analysis revealed that the 25 proteins were associated with 1) gene and protein expression by JAK-STAT signaling after interleukin-12 stimulation, 2) metabolism of RNA and axon guidance, 3) signaling by FGFR2, 4) platelet degranulation, 5) negative regulation of peptidase activity and proteolysis, 6) transport of small molecules and 7) negative regulation of cell differentiation. The Molecular Complex Detection algorithm incorporated in Metascape identified a subset of nine proteins known physically to interact with each other in a functional protein complex (CFL1, CLTC, EIF2S3, HSD17B10, HSPA9, PTBP1, RPS8, UBB, CCT4) (Figure 5). The most significantly enriched biological processes among these proteins were 1) axon guidance ($p < 0.001$), 2) nervous system development ($p < 0.001$) and 3) metabolism of RNA ($p < 0.001$).

The Pearson correlation coefficient between PainDetect score and the linear combination of nine selected serum proteins was $r(46) = 0.79$, $p < 0.001$. Both the linear combination of serum proteins and PainDetect score explained the difference between baseline and follow-up samples. The functional enrichment analysis revealed that the seven proteins were associated with multiple immune response-related biological processes including complement cascade and regulation hereof.

## Conclusions:

We identified linear combinations of serum or synovial biomarkers that changes significantly alongside PainDetect scores following gold micro-particle treatment for KOA. Both the serum and synovial fluid biomarkers were associated with immune-related processes. The synovial fluid biomarkers were also associated with other biological processes, but of particular interest was the identification of multiple members of a molecular complex that is suggestive of neural tissue regeneration and modulation following gold micro-particle treatment. The study further demonstrates the feasibility of utilizing protein biomarker signatures in future clinical decision-making.

COI statement: none

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