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CASE SERIES

# Markers of Tissue Deterioration and Pain on Earth and in Space

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**Purpose:** Pain is an understudied physiological effect of spaceflight. Changes in inflammatory and tissue degradation markers are often associated with painful conditions. Our aim was to evaluate the changes in markers associated with tissue deterioration after a short-term spaceflight.

**Patients and Methods:** Plasma levels of markers for systemic inflammation and tissue degeneration markers were assessed in two astronauts before and within 24 h after the 17-day Axiom Space AX-1 mission.

**Results:** After the spaceflight, C-reactive protein (CRP) was reduced in both astronauts, while INFγ, GM-CSF, TNFα, BDNF, and all measured interleukins were consistently increased. Chemokines demonstrated variable changes, with consistent positive changes in CCL3, 4, 8, 22 and CXCL8, 9, 10, and consistent negative change in CCL8. Markers associated with tissue degradation and bone turnover demonstrated consistent increases in MMP1, MMP13, NTX and OPG, and consistent decreases in MMP3 and MMP9.

**Conclusion:** Spaceflight induced changes in the markers of systemic inflammation, tissue deterioration, and bone resorption in two astronauts after a short, 17-day, which were often consistent with those observed in painful conditions on Earth. However, some differences, such as a consistent decrease in CRP, were noted. All records for the effect of space travel on human health are critical for improving our understanding of the effect of this unique environment on humans.

**Keywords:** spaceflight, astronaut, cytokine, interleukin, chemokine, bone turnover

## Introduction

Pain is an understudied physiological response to spaceflight, even though it is commonly reported by astronauts during and after missions to space.[1] It is well known that musculoskeletal tissues need loading to maintain a good physiology,[2] and degradation of bone and cartilage are associated with painful conditions, such as osteoarthritis, low back pain, and osteolytic bone metastasis.[3] Microgravity decreases physical demand on the body, resulting in a decrease in muscle mass[4] and alterations in the intervertebral discs, leading to back and neck pain,[5,6] bone loss,[7] and other symptoms like immune and bone turnover dysfunction in the elderly.[8,9] In general, changes in the musculoskeletal system occur relatively slowly, making it difficult to study the effects of short-duration flights. Nevertheless, inflammatory markers associated with tissue deterioration often increase early in the process, and bone resorption markers specifically were shown to significantly increase within 10 to 14 days of spaceflight,[7] suggesting their potential usefulness for assessing the initiation of the degradation processes in musculoskeletal system during short-duration spaceflight. Importantly, interleukins (IL), in

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particularly IL-6,[10] chemokines, cytokines that regulate osteoclast function, tumor necrosis factor (TNF) α, receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG),[11] as well as other inflammatory markers,[12] have been shown to be consistently associated with pain in clinical research.

The aim of this study was to evaluate the changes of systemic inflammation markers associated with tissue deterioration in general and bone resorption specifically after exposure to microgravity for a 17-day-long spaceflight in astronauts on the first all-private Axiom Space AX-1 mission to the International Space Station. This study is complementary to the recent report that, for the first time, investigated the somatosensory changes in the Axiom AX-1 astronauts.[1]

# Materials and Methods

## Population and Consent

This study was approved by the Institutional Review Board (IRB) of the McGill University Health Center (MUHC: 2022–7768), the IRB of the National Aeronautics and Space Administration (NASA STUDY 00000403) by the University of Texas - M.D. Anderson (Reliance Agreement M.D. Anderson - NASA 2021–1179). All study procedures were conducted according to the guidelines of the 2013 Declaration of Helsinki. Two crew members of Axiom Space's AX-1, launched on April 8, 2022, at 11:17 AM ET and splashed down on April 25, 2022, at 1:06 PM ET agreed to participate. The informed consent briefing was in October 2021, informed consent forms were signed on December 2021, the revised informed consent forms (an increase in the total blood collection from 30 mL to 37 mL at each time point) were signed in March 2022. The crew members remained in orbit for seventeen days and completed 240 orbits. Validated pain questionnaires, qualitative interviews and quantitative sensory testing were performed before, during and after the space mission and reported in Sauer et al.[1]

## Specimen Collection and Processing

Written consent was obtained from two male astronauts to collect peripheral venous whole blood samples using BD P100 blood collection tubes within two weeks of departure, and 24 h after landing, centrifuged at 1300 G, room temperature for 10 min. Plasma was aliquoted into dedicated cryotubes and stored at −80°C until further processing.

## Specimen Analysis

Thirty-five analytes were selected for multiplex protein analysis. The analysis was performed according to the manufacturer's instructions (ThermoFisher) using three custom kits: PPX-24-MX9HKUR containing brain derived neurotrophic factor (BDNF), epithelial-derived neutrophil-activating peptide 7 (CXCL5), myeloid progenitor inhibitory factor 2 (CCL24), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN) gamma, hematopoietin 1 (IL-1 alpha), lymphocyte activating factor (IL-1 beta), IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, IL-21, IFN gamma-induced protein 10 (IP-10/CXCL10), monocyte chemoattractant protein (MCP) −3, macrophage derived chemokine (MDC/CCL22), monokine induced by gamma IFN (CXCL9), macrophage inflammatory protein 1-alpha (CCL3), macrophage inflammatory protein 1-beta (CCL4), matrix metalloproteinase (MMP) 1, MMP-13, and TNFα; granulocyte chemotactic protein-2 (CXCL6), MCP-1, TNF superfamily member 14 (LIGHT), beta nerve growth factor (NGF-beta) and MCP-2; and CRP, MMP-3, transforming growth factor (TGF) beta, MMP-9, Osteopontin and regulated on activation, normal T cell expressed and secreted (RANTES/CCL5). Protein concentrations in pg/mL were acquired from a MAGPIX (Luminex) using 30 ul of plasma. Four analytes (LIGHT, NGF-beta, TGF-beta and GRO-alpha) were below the detection threshold and were therefore omitted from the analysis. Median fluorescence intensity (MFI) was measured, and the concentration from each analyte was calculated using a 7-point standard curve by the ThermoFisher ProcartaPlex analysis platform. ELISA kits were used for the following analytes: human cross linked N-telopeptide of type I collagen (NTX, Cedarlane MBS705111); human procollagen I C-terminal (PICP, Cedarlane MBS2702057), human osteoprotegerin (OPG, ABclonal Science Inc RK00317) human TRANCE/TNFSF11/RANKL (ABclonal Science Inc, RK00341), and human osteocalcin (ABclonal Science Inc, RK09258).

## Rapid Literature Review Methodology

Rapid reviews were performed on October 10, 2023, in Medline (Ovid) using the search strategies that combined a) the list of biomarkers measured in the study, and b) pain as a MESH term and keyword. Resulting studies were screened by a single reviewer and were included in the analysis if they were performed with human subjects, measured biomarkers of interest in plasma or serum, and reported association with pain outcomes. From each manuscript, we extracted data for publication first author and date, disease studied and total number of study subjects, pain measurement method, reported biomarkers and the direction of association between pain and each biomarker (positive, negative, or undetermined (variable or no association)). The diseases were combined as follows: abdominal pain (AP): appendicitis, abdominal pain and kidney pain; back pain (BP): chronic low back pain, spinal tuberculosis, and lumbar disc herniations; chronic malignant pain (CMP): cancer: chronic non-malignant pain (CNMP): complex regional pain syndrome, chronic widespread pain, depression, fibromyalgia, and neuropathic pain; chest pain (CP): myocardial infarction and coronary syndromes; head and neck pain (HNP): temporomandibular disorder and migraines; musculoskeletal pain (MSP): foot pain, growing pain, shoulder pain, and non-arthritis joint pain; osteoarthritis (OA); and pelvic pain (PP): chronic pelvic pain syndrome, endometriosis, labor pain, and prostatitis.

## Data Analysis and Presentation

Raw data are presented in tables; for figures, percentage difference from pre-flight values was calculated and $d = \left(x_{postflight} - x_{preflight}\right) * 100/x_{preflight}$. Figures for disease-marker association were developed using Matlab using the following rules: the word's size is proportional to the number of included studies that report it; each connecting line represents an article, and the width of the line is proportional to the study size. To assign the association of specific biomarkers with pain, each study was given a value of 1 for a positive association, 0 for no association, and −1 for a negative association. For each biomarker, the sum of 2 or greater was interpreted as a general positive association with pain (red color), the sum of −2 or less as a general negative association (blue color), and a sum of −1, 0 or 1 as no association (grey color). No statistical analysis was performed for the experimental values obtained from the two astronauts.

# Results and Discussion

The participants were healthy male astronauts 53 and 64 years of age with no history of chronic pain, spinal surgery, or peripheral nervous system disorder. After the 17-day long spaceflight, CRP was reduced in both astronauts, while INFγ, GM-CSF, TNFα, BDNF, and all measured interleukins were consistently increased (Tables 1 and 2, Figure 1A). To examine the reported association of these markers with painful conditions, we performed a rapid review, which retrieved 362 studies, of which 42 were included in the final analysis.[13–54] TNFα, IL-6, and CRP were reported to be positively associated with pain, specifically with CNMP, PP, CP, and OA (Figure 1B). Of interest, even though TNFα and IL6 were consistently increased in astronauts after the spaceflight, CRP was consistently decreased. Thus, with the notable exception of CRP, spaceflight-associated changes in cytokines were like those observed in painful conditions.

**Table 1** Spaceflight-Induced Changes in Cytokines and Growth Factors. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

| | CRP | INFγ | GM-CSF | TNFα | BDNF |
|---|---|---|---|---|---|
| A1 pre | 1369.58 | 29.68 | 107.51 | 8.86 | 60.96 |
| A1 post | 813.95 | 150.44 | 276.22 | 45.80 | 80.32 |
| A2 pre | 1695.19 | 33.25 | 98.08 | 10.17 | 20.15 |
| A2 post | 920.07 | 131.26 | 216.73 | 37.33 | 36.76 |

**Table 2** Spaceflight-Induced Changes in Interleukins. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

| | IL1a | IL1b | IL2 | IL4 | IL6 | IL8 | IL10 | IL17a | IL21 |
|---|---|---|---|---|---|---|---|---|---|
| A1 pre | 4.38 | 9.87 | 36.69 | 16.57 | 53.48 | 5.58 | 7.37 | 35.56 | 245.09 |
| A1 post | 7.51 | 49.92 | 89.56 | 66.33 | 121.88 | 10.25 | 27.14 | 141.05 | 349.82 |
| A2 pre | 1.10 | 16.40 | 27.83 | 16.82 | 51.48 | 1.50 | 8.73 | 39.69 | ND |
| A2 post | 3.69 | 50.85 | 68.75 | 54.81 | 119.12 | 5.74 | 22.45 | 116.57 | 58.84 |

Chemokines demonstrated variable changes, with consistent positive changes in CCL3, 4, 8, 22 and CXCL8, 9, 10, consistent negative change in CCL8, and variable or no changes in CCL5, 24, and CXCL5, 6 (Tables 3 and 4, Figure 2A). To examine reported association of these markers with painful conditions, we performed a rapid review which retrieved 211 studies, of which 17 were included in the final analysis.[18,39,55–68] Several chemokines, including CCL3, 4, 5, and 24 and CXCL5 and 10, were reported to be negatively associated with pain, specifically with CNMP, PP, BP, and OA (Figure 2B). CXCL8, most reported for its association with painful conditions, was also reported to be both positively and negatively associated with pain outcomes. Thus, spaceflight-associated changes in chemokines are different from those observed in painful conditions.

Markers associated with tissue degradation and bone turnover were similarly variable, with consistent increases in MMP1, MMP13, NTX, and OPG, consistent decreases in MMP3 and MMP9, and variable changes in PICP, OPN and OCN (Tables 5 and 6, Figure 3A). To examine reported association of these markers with painful conditions, we performed a rapid review which retrieved 373 studies, of which 24 were included in the final analysis.[56,69–91] Bone

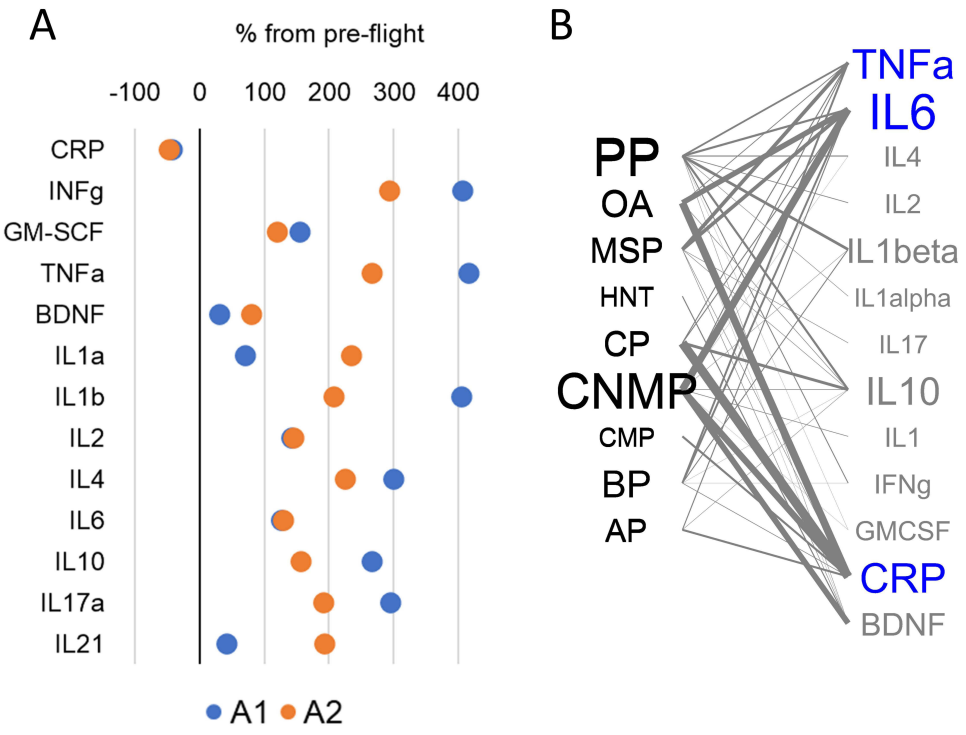


**Figure 1** Spaceflight-induced changes in cytokines and growth factors. (**A**) For each astronaut (A1 and A2), the percentage change from pre-flight was calculated and plotted for reported cytokines and growth factors. (**B**) Map of associations between painful conditions and the reported cytokines and growth factors. The size of the letters is proportional to the frequency of mention; the thickness of the connecting lines is proportional to the total number of patients in reported studies, bleu font indicates consistently positive association, grey variable association or no association.

**Abbreviations**: PP, pelvic pain; OA, osteoarthritis; MSP, musculoskeletal pain; HNT, head and neck pain; CP, chest pain; CNMP, chronic non-malignant pain; CMP, chronic malignant pain; BP, back pain; AP, abdominal pain.

**Table 3** Spaceflight-Induced Changes in CCL Chemokines. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

| | CCL3 | CCL4 | CCL5 | CCL7 | CCL8 | CCL22 | CCL24 |
|---|---|---|---|---|---|---|---|
| A1 pre | 13.04 | 60.96 | 357.15 | 37.65 | 4.24 | 130.39 | 60.60 |
| A1 post | 18.32 | 80.32 | 178.94 | 69.62 | 2.51 | 229.86 | 64.04 |
| A2 pre | 1.98 | 20.15 | 289.24 | 47.16 | 6.07 | 143.47 | 62.00 |
| A2 post | 6.77 | 36.76 | 308.97 | 72.72 | 4.90 | 168.19 | 63.35 |

**Table 4** Spaceflight-Induced Changes in CXCL Chemokines. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

| | CXCL5 | CXCL6 | CXCL9 | CXCL10 |
|---|---|---|---|---|
| A1 pre | 122.39 | 7.51 | 28.51 | 27.84 |
| A1 post | 194.40 | 6.88 | 34.56 | 36.75 |
| A2 pre | 279.20 | 14.30 | 1.98 | 30.96 |
| A2 post | 242.47 | 11.21 | 6.77 | 65.34 |

resorption markers (BRM) and bone formation markers (BFM) were positively associated with pain in OA, MSP, and BP, and MMP9 was positively associated with CP and PP (Figure 3B). Thus, the increase in bone resorption marker NTX in astronauts is similar to the commonly reported increase in BRM in painful musculoskeletal conditions, however MMP9 was consistently decreased in astronauts and bone formation marker, PINP was variably affected.

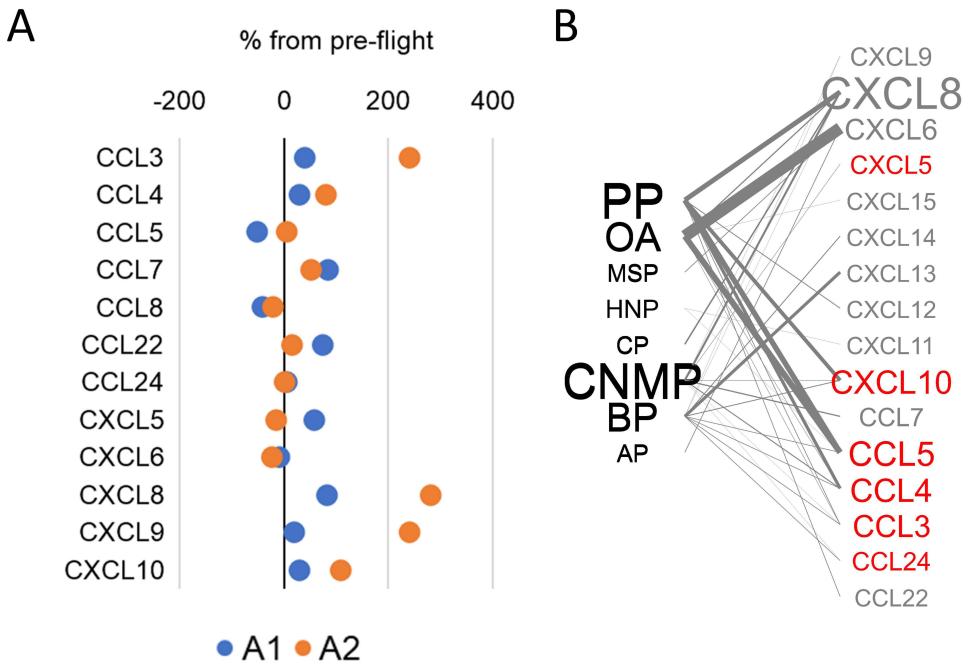


**Figure 2** Spaceflight-induced changes in chemokines. (**A**) For each astronaut (A1 and A2), the percentage change from pre-flight was calculated and plotted for reported chemokines. (**B**) Map of associations between painful conditions and the reported chemokines. The size of the letters is proportional to the frequency of mention; the thickness of the connecting lines is proportional to the total number of patients in reported studies, red font indicates consistently negative association, grey font: variable association or no association.

**Abbreviations**: PP, pelvic pain; OA, osteoarthritis; MSP, musculoskeletal pain; HNT, head and neck pain; CP, chest pain; CNMP, chronic non-malignant pain; BP, back pain; AP, abdominal pain.

**Table 5** Spaceflight-Induced Changes in MMPs. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

| | MMP1 | MMP3 | MMP9 | MMP13 |
|---|---|---|---|---|
| A1 pre | 9.16 | 642.40 | 677.05 | 116.27 |
| A1 post | 42.65 | 454.07 | 506.42 | 281.24 |
| A2 pre | 13.64 | 760.83 | 449.93 | 133.96 |
| A2 post | 36.61 | 320.70 | 307.30 | 246.02 |

**Table 6** Spaceflight-Induced Changes in Bone-Related Factors. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

| | NTX | PICP | OPN | OCN | OPG |
|---|---|---|---|---|---|
| A1 pre | ND | 185.20 | 6072.36 | 3.73 | 2.64 |
| A1 post | 26.00 | 284.67 | 6857.75 | 4.76 | 4.10 |
| A2 pre | ND | 195.40 | 10,810.12 | 4.69 | 3.11 |
| A2 post | 14.59 | 130.53 | 4551.06 | 4.95 | 4.42 |

## Limitations

Astronauts are exposed to multiple stressors, including microgravity, noise and vibrations, sleep disruptions, and confinement.[92] Within the scope of the study, it is impossible to attribute the observed changes to any of the specific stressors. This case study provides data for two astronauts only, making it impossible to reach concrete conclusions.

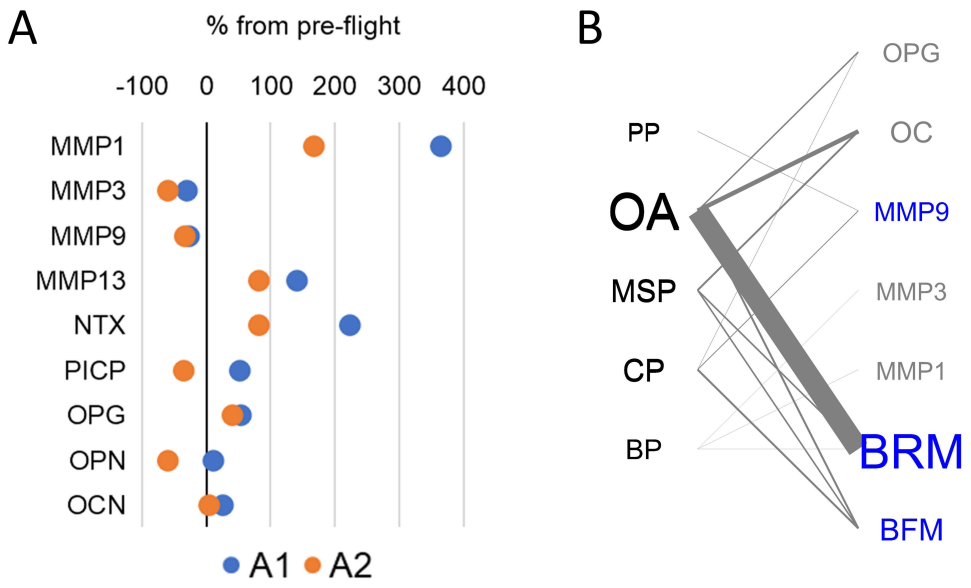


**Figure 3** Spaceflight-induced changes in tissue degradation and bone turnover factors. (**A**) For each astronaut (A1 and A2), the percentage change from pre-flight was calculated and plotted for reported factors. (**B**) Map of associations between painful conditions and the reported factors. The size of the letters is proportional to the frequency of mention; the thickness of the connecting lines is proportional to the total number of patients in reported studies, blue font indicates consistently positive association, red font indicates consistently negative association, grey font: variable association or no association.

**Abbreviations**: PP, pelvic pain; OA, osteoarthritis; MSP, musculoskeletal pain; HNT, head and neck pain; CP, chest pain; CNMP, chronic non-malignant pain; CMP, chronic malignant pain; BP, back pain; AP, abdominal pain.

However, our studies demonstrate for the first time the changes in the somatosensory system[1] and biomarkers associated with pain (current study), paving the way for future studies that can be combined to improve our understanding of the effects of spaceflight on pain. Another limitation is related to the lack of blood samples taken in flight due to difficulties in obtaining samples as well as in finding time in the busy schedule of astronauts in space missions.

## Conclusion

This study reports the changes in the markers of systemic inflammation, tissue deterioration, and bone resorption in two astronauts after a short, 17-day space travel. While some changes are consistent with those observed in painful conditions on Earth, we have noted some differences, such as a consistent decrease in CRP. Although no conclusions can be reached with two participants in the study, since space travel remains very costly and uncommon exposure to this unique environment, every record of the effect of space travel on human health is critical in improving our understanding of the effect of spaceflight on humans.

## Abbreviations

AP, abdominal pain; BDNF, brain derived neurotrophic factor; BFM, bone formation markers; BP, back pain; BRM, bone resorption markers; CCL24, myeloid progenitor inhibitory factor 2; CCL3, macrophage inflammatory protein 1-alpha; CCL4, macrophage inflammatory protein 1-beta; CMP, chronic malignant pain; CNMP, chronic non-malignant pain; CP, chest pain; CRP, c-reactive protein; CRP, C-reactive protein; CXCL5, epithelial-derived neutrophil-activating peptide 7; CXCL6, granulocyte chemotactic protein-2; CXCL9, monokine induced by gamma IFN; GM-CSF, granulocyte-macrophage colony-stimulating factor; HNP, head and neck pain; IFN, interferon; IL-1 alpha, hematopoietin 1; IL-1 beta, lymphocyte activating factor; IL, interleukins; IP-10/CXCL10, IFN gamma-induced protein 10; IRB, Institutional Review Board; LIGHT, TNF superfamily member 14; MCP, monocyte chemoattractant protein; MDC/CCL22, macrophage-derived chemokine; MFI, Median fluorescence intensity; MMP, matrix metalloproteinase; MSP, musculoskeletal pain; NGF-beta, beta nerve growth factor; NTX, human cross linked N-telopeptide of type I collagen; OA, osteoarthritis; OPG, osteoprotegerin; PICP, human procollagen I C-terminal; PP, pelvic pain; RANKL, receptor activator of nuclear factor κB ligand; RANTES/CCL5, normal T cell expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor.

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## Disclosure

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