Serum biomarkers for Modic changes in patients with chronic low back pain

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Purpose: Lumbar Modic change (MC) can serve as a diagnostic marker as well as an independent source of chronic low back pain (CLBP). This study aimed to test for the existence of serum biomarkers in CLBP patients with MC.

Methods: Age- and sex-matched CLBP patients with confirmed MC on lumbar MRI (n=40) and pain-free controls (n=40) were assessed. MC were classified into M1, predominating M1, predominating M2, and M2. MC volumes were calculated. Fasting blood samples were assessed for inflammatory mediators, signalling molecules, growth factors and bone turnover markers. Serum concentrations of 46 biomarkers were measured.

Results: Median concentrations of interleukin (IL)-15 ($p<0.001$), IL-8 ($p<0.001$), tumor necrosis factor (TNF)-alpha ($p<0.001$), Eotaxin-1 ($p<0.05$), Eotaxin-3 ($p<0.001$), monocyte chemotactic protein (MCP)-1 ($p<0.05$), macrophage inflammatory protein (MIP)-1alpha ($p<0.01$), TEK receptor tyrosine kinase (Tie)-2 ($p<0.001$), vascular cell adhesion molecule (VCAM)-1 ($p<0.001$), RANTES ($p<0.001$), C telopeptide of type I collagen (CTX)-1 ($p <0.001$), vascular endothelial growth factor (VEGF)-C ($p<0.001$), VEGF-D ($p<0.05$), fms-related tyrosine kinase (Flt)-1 ($p<0.01$) and intercellular adhesion molecule (ICAM)-1 ($p<0.01$) were significantly higher among controls. IL-1sRII (23.2 vs. 15.5 ng/ml, $p<0.001$) and hepatocyte growth factor (HGF)-1 (169 vs. 105 pg/ml, $p<0.01$) concentrations were significantly higher among patients. Type or volume of MC were not associated with biomarker concentrations.

Conclusions: This is the first study to assess the blood serum biomarker profile in individuals with CLBP with MC. Several biomarkers were suppressed while two markers (IL-1sRII and HGF) were elevated among MC patients, irrespective of MC type or size, with CLBP compared to asymptomatic controls.

Keywords: serum; biomarkers; magnetic resonance imaging; Modic change; Zoledronic acid; chronic low back pain

BACKGROUND

Low back pain (LBP) is the leading cause of disability globally, and it's societal impact is expected to increase as the average lifespan lengthens.[1,2] LBP may be classified according to the duration of pain. As such, pain lasting for more than three months is noted as chronic low back pain (CLBP). For nearly all patients presenting with LBP, the specific nociceptive source of pain cannot be identified, and consequently, the affected patients are regarded as having 'non-specific' LBP.[1]

Modic change (MC) is a vertebral subchondral bone marrow lesion visualized on magnetic resonance imaging (MRI), which can be classified based by signal intensities on both T1- and T2-weighted imaging as follows: type 1 MC (M1) represent an active oedemic inflammatory state; type 2 MC (M2) demonstrate a red-to-yellow fat conversion of the bone marrow; and type 3 MC (M3) are related to the development of sclerosis.[3-6] Several population-based studies have reported a significant positive association between MC and LBP, while many others report only a weak relationship or none at all.[7-14] In spite of the inconsistencies with regards to the association of MC and LBP, MC has been proposed to represent a specific degenerative imaging phenotype associated pain in the neighbouring discs, with inflammatory M1 being a potentially better diagnostic marker than M2 or M3.[15-19] As such, MC have been noted to be one of the most clinically-relevant LBP phenotypes.

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes as well as pharmacologic responses to a therapeutic intervention.[20] Biological signal molecules found in serum have already been studied increasingly for diagnostic purposes of other musculoskeletal disorders, such as osteoarthritis and rheumatoid arthritis.[21-24] Serum biomarkers of interest consist mainly of inflammatory and immunomodulatory cell-signalling molecules, such as, cytokines and metabolites of bone and cartilage turnover. These molecules are released to the circulation by the affected tissues and certain cells of the immune system.[25-27] We hypothesized that inconsistencies in the association of lumbar

MC with CLBP could be resolved with establishment of MC-specific serum biomarker profiles, which could provide an appropriate and cost-efficient method to augment MRI-diagnostics for differential diagnosis and follow-up of patients suffering from CLBP. To the authors' knowledge, only one study on serum biomarkers of MC has been published thus far. Based on a study by Rannou *et al*[28] consisting of CLBP patients, serum high-sensitive C-reactive protein (hs-CRP) concentration was higher among patients with M1 compared to M2 or patients without MC. The aim of the current study was to build on that work, and test for the existence of other serum biomarkers that are significantly elevated or reduced in CLBP patients with MC in comparison to matched healthy controls.

METHODS

The Oulu University Hospital ethics committee had approved the study protocol 18.8.2008 (121/2008).

Study population

The study population consisted of patients with CLBP and a confirmed MC on lumbar spine (L1-S1) MRI (n = 40) and voluntary subjects with no current symptoms of the lower back and no MC on MRI (n = 40). Patients' inclusion criteria were CLBP with duration at least three months, LBP intensity of at least six on a 10-cm Visual Analog Scale (VAS) or an Oswestry Disability Index of at least 30%, and an MC (M1, M2 or mixed M1/2) on MRI performed within no more than six months prior to enrolment. The exclusion criteria included renal impairment, hypocalcaemia, hypersensitivity to bisphosphonates or the ingredients of the infusion product, the presence of red flags, nerve root entrapment, willingness for early retirement and childbearing potential.[29] Controls were recruited to the study from the staff of Oulu University Hospital and University of Oulu, and were matched to the patients for age and gender. The patients with CLBP and control group participants were recruited between November 2008 and March 2011. Confounding factors such as

smoking, physical workload and level of physical activity were inquired. All participants gave their written consent to the study.

Magnetic resonance imaging

Patients' MRIs were performed in the district of the Oulu University Hospital with five 1.5 T units (GE Signa Twinspeed, General Electric Medical Systems, Milwaukee, WI, USA; Philips Achieva and Philips Intera, Philips Medical Systems, Eindhoven, The Netherlands; Siemens Avanto and Siemens Espree, Siemens Medical, Erlangen, Germany), a 0.34 T unit (Siemens Magnetom C, Siemens Medical, Erlangen, Germany) and a 0.23 T unit (Philips Panorama, Philips Medical Systems, Eindhoven, The Netherlands). Twenty-nine patients were scanned with 1.5 T equipment, eleven patients with ~~at~~ low~~-~~-field MRI~~strength.~~ Imaging protocols varied somewhat due to the multiple units used but the protocols were established to spine imaging. The imaging parameters of the sagittal T1-weighted (T1 W) turbo spin-echo (TSE) or fast spin-echo (FSE) sequences with fluid attenuation inversion recovery (FLAIR) were repetition time (TR) 1800–2270 ms/inversion time (TI) 860 ms/echo time (TE) 9–29 ms (N = 16), and without FLAIR: TR 326–793 ms/TE 8–18 ms (N = 23). The imaging parameters of the sagittal T2-weighted (T2 W) TSE/FSE sequences were TR 3000–4500 ms/TE 105–130 ms (N = 39). The imaging parameters of the short tau inversion recovery sequences (STIR) were, for example, TR 3400/ TI 150/ TE 70. The spacing, including slice thickness and slice gap, was 4.4–6.2 mm in all sequences.[29,30]

Controls subject MRIs were performed in the Oulu University Hospital with 1.5 T equipment (GE Signa HDx, General Electric Medical Systems, Milwaukee, WI, USA). The imaging parameters of the sagittal T1-weighted (T1W) FSE sequences with FLAIR were TR 2274.4–2276.6 ms/TI 860 ms/TE 20.1 ms. The imaging parameters of the sagittal T2-weighted (T2W) FSE sequences were TR 3500 ms/TE 114.7 ms. The imaging parameters of the STIR were TR 4400/ TI 150/ TE 48.2 ms. The spacing, including slice thickness and slice gap, was 5.0 mm in all sequences.

Image analysis

MRIs were analyzed for type and volume of each MC from sagittal images at a clinical workstation (Neaview Radiology, version 2.23, Neagen Corporation, Finland). MC type was assessed using T1W and T2W images, and the MRI scans were classified as previously described: M1 showed low signal intensity (SI) on T1W and high SI on T2W and STIR images, M2 showed high SI on both T1W and T2W images and low SI on STIR images, and M3 showed low SI on both T1W and T2W images.[9] MC type was divided into four groups: M1 (100%), predominating M1 (M1/2 (65:35%)), predominating M2 (M1/2 (35:65%)) and M2 (100%). The first two were considered M1-dominant, and the latter two M2-dominant. M1 and M2 were defined as consisting totally of oedemic or fatty signal changes, respectively; whereas predominating M1 and predominating M2 were defined as mixed Type M1/2 MC with more oedemic or fatty signal changes, respectively. The proportion of M3 was so low that it was excluded from the analyses. The area (cm^2) of the MC was measured slice-by-slice from T2W images by a workstation area tool. The volume (cm^3) of the MC was calculated by multiplying the area with the slice spacing. For the interobserver reliability, a Fellow in musculoskeletal radiology (JJ) and an experienced musculoskeletal radiologist (JN) analyzed the images of 19 randomly selected patients. Interobserver reliability was substantial for both raw MC type classification (M1, predominating M1, predominating M2 and M2; linearly weighted kappa 0.65) and for dichotomized data (M1-dominant vs. M2-dominant; kappa 0.73). The reliability of the volume measurements was excellent (intraclass correlation coefficient 0.92) as described earlier.[30]

Since some individuals had multiple MC, a primary MC was defined to represent the most likely LBP generator. The severity of the lesion was assumed as follows: M1 > predominating M1 >

predominating M2 > M2. In cases when patients with multiple MC had the same types at different levels, the larger MC was selected as the primary MC. In this study, the characteristics of the primary MC were only considered.

Analysis of serum biomarkers

Whole blood was withdrawn at noon from fasting patients using serum collection tubes. All subjects were told not to exercise, eat or drink in the previous evening and the morning before blood withdrawal. Blood samples were allowed to clot at room temperature for 30-60 min and then centrifuged at 2500 x g for 10 min at 4°C. The resulting serum supernatant was stored in aliquots first at -20°C and transferred within 2-20 hours to -70°C. Serum samples (n = 80) were taken for the assessment of selected inflammatory and signalling molecules, growth factors and markers of bone turnover. Participants were told to restrict strenuous leisure-time activity during the previous day. Blood sampling of controls was performed within six months (SD = 3.8) of MRI scans. Serum concentrations of 38 biomarkers were measured using electro-chemiluminescent based multi-array immunoassays from MesoScale Discovery (MSD, Rockville, MD, USA) following the protocol of the manufacturer: Chemokines panel: eotaxin, eotaxin-3, interferon-gamma-inducible protein (IP)-10, macrophage inflammatory protein (MIP)-1-alpha, MIP-1-beta, monocyte chemotactic protein (MCP)-1, MCP-4, macrophage derived chemokine (MDC)-1, regulated on activation normal T-expressed and secreted (RANTES), thymus and activation regulated chemokine (TARC); cytokines panel: granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-5, IL-7, IL-12/23p40, IL-15, IL-16, IL-17A, tumour necrosis factor (TNF)-beta; inflammation panel: interferon (IFN)-gamma, IL-1-alpha, IL-1-beta, IL-2, IL-6, IL-8, IL-12p70, TNF-alpha, IL-4, IL-10, IL-13; angiogenesis panel: vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, TEK receptor tyrosine kinase (Tie-2), fms related tyrosine kinase 1 (Flt-1), basic fibroblast growth factor (bFGF); and vascular injury panel: intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, serum amyloid A (SAA). For analysis of interleukin 1 receptor antagonist

(IL-1RA), epidermal growth factor (EGF), hepatocyte growth factor (HGF), interferon-alpha (IFN-A), interleukin-2 receptor (IL-2R) and monokine induced by gamma-interferon (MIG), a Cytokine Human Magnetic Multi-Plex Panel (Thermo Fisher Scientific, Waltham, MA, USA) was used according to the instructions given by the manufacturer. Receptor activator of NF-κB ligand (RANKL) was measured with a commercial Luminex single plex kit (Human RANKL single plex kit, MilliplexTM Map, Merck Millipore, Billerica, MA, USA), and interleukin-1 receptor, type II (IL-1RII) with a human IL-1RII Quantikine Elisa kit (R&D Systems, Minneapolis, MN, USA). All Luminex assays were performed using Luminex xMAP Technology (Bio-Plex 200 System; BioRad, Hercules, CA, USA). For IL-1RII Elisa assay, the absorption was read at 450 nm with an ELISA plate absorbance reader and the correction at 570 nm (Multiskan MS, Thermo Scientific, Vantaa, Finland). Plasma alkaline phosphatase activity (AFOS) was analyzed using IFCC recommended enzymatic method (Advia, Siemens, Germany). Serum highly sensitive CRP (hs-CRP) was analyzed using nephelometric instrument (BN ProSpec, Siemens, Germany). Intact procollagen I N-terminal propeptide (iPINP) and C telopeptide of type I collagen (CTX-I) analyses were performed using IDS-iSYS Multi-Discipline Automated System (IDS, Bolton, U.K.).

Statistical analysis

Mann-Whitney's U-test was used to compare the differences of biomarker concentrations between the patients and controls, and within patients between M1- and M2-dominant MC. Benjamini-Hochberg procedure was used to correct for multiple comparisons. Spearman's correlation coefficient was used to test for association between MC volume and biomarker concentration. A p-value, respectively q-value for corrected p-values, of <0.05 was considered significant. The area-under-the-curve (AUC) of the receiver operating characteristics (ROC) was calculated for all biomarkers in the direction of higher biomarkers predicting presence of MC.

RESULTS

Table 1 presents the characteristics of the 40 patients and 40 matched pain-free volunteers. The patients were more obese, but age, gender and smoking distributions were similar. Seven of the patients had clinically relevant osteoarthrosis (knee or hip) requiring hospital admission compared to none of the controls. Twenty-seven (67.5%) patients had a M1-dominant lesion and 24 (60%) had a single MC. Primary MC occurred most commonly (72%) at L4/5 and L5/S1.

Biomarker analyses

The results of 11 biomarkers (EGF, GM-CSF, IL-1A, IL-1B, IL-1RA, IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13) were below the limit of quantification for more than half of the samples in the whole study and thus they were excluded from the final results. **Table 2** presents the results of all measured 39 serum biomarkers. In the bone panel, median concentration of CTX-1 was significantly higher among controls ($p<0.001$; Supplementary Figure 1; ~~**Figure 1**~~) while AFOS tended to be higher among patients ($p=0.065$). The concentrations of RANKL and iPINP did not differ significantly between patients and controls. In the chemokine panel, median concentrations of Eotaxin-1 ($p<0.05$), Eotaxin-3 ($p<0.001$), MCP-1 ($p<0.05$), MIP-1A ($p<0.01$), MCP-1 ($p=0.012$), and RANTES ($p<0.001$; Supplementary Figure 2; ~~**Figure 2**~~) were significantly higher among controls, while concentrations of IP-10, MIP-1B, MCP-4, MDC-1, TARC and MIG-1 did not differ between patients and controls. In the cytokine panel, the median concentration of IL-15 ($p<0.001$; Supplementary Figure 3; ~~**Figure 3**~~) was significantly higher among controls, while concentrations of IL-7, IL-12/23p40, IL-16, IL-17A and TNF-B did not differ between patients and controls. In the anti-inflammatory panel, the concentration of IL-1sRII (23.2 vs. 15.5 ng/ml, $p<0.001$; Supplementary Figure 4; ~~**Figure 4**~~) was significantly higher among patients. In the pro-inflammatory panel, the median concentrations of IL-8 ($p<0.001$) and TNF-A ($p<0.001$; Supplementary Figure 4; ~~**Figure 4**~~) were significantly higher among controls, while concentrations

of IFN-A, IFN-G, IL-2R and IL-6 did not differ between patients and controls. In the angiogenesis panel, the concentrations of Tie-2 ($p<0.001$), VEGF-C ($p<0.001$), VEGF-D ($p<0.05$) and Flt-1 ($p<0.01$) were significantly higher among controls, while concentration of HGF-1 (169 vs. 105 pg/ml, $p<0.01$; Supplementary Figure 5; ~~**Figure 5**~~) was significantly higher among patients. Patients and controls did not differ with respect to VEGF-A and bFGF. In the vascular injury panel, concentrations of VCAM-1 ($p<0.001$) and ICAM-1 ($p<0.01$; Supplementary Figure 6; ~~**Figure 6**~~) were significantly higher among controls. Hs-CRP tended to be higher among patients (1.0 vs. 0.7 mg/l, $p=0.069$), while concentration of SAA was similar among patients and controls **(Table 2).**

The MC type was not associated with serum biomarker concentrations after correction for multiple comparisons (Supplementary Table 1). The concentrations of ICAM-1 and SAA were correlated negatively with total volume of MC and M1 volume (Supplementary Table 2).

One-at-time (OAT) sensitivity analysis for multiple MCs and o~~O~~steoarthritis (OA) resulted in the same set of significant biomarkers, except that Eotaxin-1 ($p=0.095$ and~~,~~ $p=0.057$ for OAT of multiple MC and OA, respectively) and VEGF-D ($p=0.1554$ and~~,~~ $p=0.202$, respectively) were not significant anymore after Benjamini-Hochberg correction. When excluding both, multiple MCs and OA, additionally HGF-1 was not significant anymore ($p=0.083$).

DISCUSSION

We questioned whether serum biomarkers varied with the presence and type of vertebral MC in CLBP patients. Our data demonstrate that several biomarkers were suppressed while two markers (IL-1sRII and HGF) were elevated among MC patients with CLBP compared to asymptomatic controls. None of the biomarkers were related to MC type. Only two markers of the vascular injury panel (ICAM-1 and SAA) correlated negatively with MC size, with their concentration being lower in larger MC or M1.

In the bone panel, CTX-1 was suppressed among patients with MC. M1 lesions are associated with vascular granulation tissue within the bone marrow and high bone turnover, while M2 lesion reflects fatty replacement of the bone marrow and reduced bone formation.[6,31] Our study sample of CLBP patients consisted mainly of M1-dominant mixed lesions. In related work, we have previously observed endplate sclerosis in mixed MC lesions.[32] Sclerosis with reduced bone formation would explain suppressed CTX-1 level observed in the current study.

Of cytokines, chemokines and proinflammatory mediators, IL-15, Eotaxin-1, Eotaxin-3, MCP-1, MIP-1A, MCP-1, RANTES, IL-8 and TNF-A were suppressed among MC patients. For MC, either infectious or autoimmune etiologies have been suggested both etiologies presupposing structural damage of the endplate.[31,33] A recent award-winning paper found a fibrogenic and pro-inflammatory cross-talk between MC bone marrow and adjacent discs.[34] Several studies indicate that M1 discs are characterized by the production of increased amounts of pro-inflammatory cytokines such as M-CSF, GM-CSF, IL-1A, IL-1B, IL-6, and IL-8.[34-36] These studies were, however, conducted using disc or bone marrow tissue samples, and it remains to be seen whether elevated levels of pro-inflammatory biomarkers may be observed in serum samples. In this current study, anti-inflammatory marker IL-1sRII, a soluble decoy receptor of IL-1, was significantly elevated among CLBP patients with MC, and thus could potentially be used as a biomarker of MC. The AUC for IL-1sRII was very high (AUC = 0.835). However, IL-1sRII was also elevated among patients with short-term upper extremity disorders and thus may not be MC-specific.[37] Thus, further studies are needed to further clarify the role of IL-1sRII in the diagnosis of MC. Interestingly, in an Australian acute LBP cohort the combination of elevate TNFA concentration and depression was associated with poorer prognosis, while elevated CRP/IL-6 was related to good recovery.[38] It may well be, however, that the 'window of opportunity' to detect inflammatory serum markers exists only in the early phase of MC development.

The consistency of downregulated inflammatory proteins and upregulated inflammation-suppressing proteins (IL-1sRII, HGF-1) may indicate active inflammation-controlling mechanisms taking place in MC.[39,40] Interestingly, a recent study also reported decreased levels of pro-inflammatory cytokines and chemokines in plasma of patients with CLBP compared to healthy controls; it was hypothesized that either the chronic nature of the disease or the potential long-term effect of anti-inflammatory medication could lead to the suppression of pro-inflammatory molecules.[41] For example, in osteoarthritis chronic inflammation causes cellular senescence and epigenetic modifications of cell in affected joints, altering responsiveness to inflammatory stimuli.[42] Given the chronic character of MC, epigenetic modifications likely play an important role in MC, too. Epigenetics in MC have not been studied yet but reports of consistently down-regulated NFκB in MC discs and bone marrow despite increased levels of pro-inflammatory cytokines support the notion of epigenetic modifications.[34]

In the angiogenesis and vascular injury panels, Tie-2, VEGF-C, VEGF-D, Flt-1, VCAM-1 and ICAM-1 were suppressed among patients with MC, while HGF-1 concentration was elevated. Furthermore, ICAM-1 and SAA were correlated negatively with total volume of MC and M1 volume, i.e. with a larger MC or M1 volume the concentrations of these two biomarkers were lower, which supports suppression of vascular injury markers among patients with MC. VCAM-1 and ICAM-1 are expressed by endothelial cells in response to TNF and IL-1 and help trans-endothelial migration of immune cells.[43] Therefore, reduced TNF, VCAM-1, and ICAM-1 in MC may relate to reduced vascularization and number of macrophages in herniated disc materials at levels with MC.[44] The reason for reduced neovascularization in the presence of MC is unknown. It may be that the broad down-regulation of proteins in the angiogenesis and vascular injury panel indicate that inhibition of vascularization may also occur in MC without disc herniation, and potentially even drives MC pathogenesis.

A study by Rannou *et al*[28] focusing on French patients found that serum hs-CRP was higher among individuals with M1 compared to M2 or patients without MC. In our study, hs-CRP tended to be higher – but non-significantly - among CLBP patients with MC, but type or size of MC did not influence hs-CRP concentration. CRP is an acute phase reactant produced by hepatocytes under transcriptional control of IL-6.[45] The concentration of CRP reflects the intensity of the pathological process stimulating its synthesis, the concentration falling rapidly after the off-set of the pathological stimulus. Subjects in the general population tend to have stable CRP concentrations characteristic for each individual, apart from occasional spikes presumably related to minor or subclinical infections, inflammation, or trauma.[46] An elevated CRP is included in the axial spondylarthritis classification criteria. However, an elevated CRP or erythrocyte sedimentation rate is present only about 40-50% of patients with axial spondylarthritis.[47] Of other spinal disorders, an elevated serum CRP/hs-CRP has been observed occasionally in sciatica and among patients with ossification of the posterior longitudinal ligament.[48,49] The discrepancy between our study and the French study[28] on hs-CRP may be due to more acute MC lesions in the French patient sample as CRP is an acute phase protein with a half-life of ca. 19 hours.[46] M1 lesions in our sample represent a more acute lesion type but nevertheless, we observed no difference in hs-CRP levels according to MC type or size.

Generally, M1 and M2 represent different stages of a pathogenetic mechanism. The investigated biomarkers were not sensitive to distinguish these stages but rather represent potential biomarkers for detecting M1 or M2 or mixed M1/M2. MC are dynamic and most MC were mixed type in this study.~~,~~ Thus,~~hence~~ specific biomarkers for M1 or M2 may not be sensitive for mixed type M1/M2 and hence fail to detect a large clinically important patient population. In contrast, a robust biomarker for M1, M2, and mixed M1/M2 may be a measure for the general pathomechanism of MC.

Our study implements case-control design with a reasonably small sample size, which is a limitation. The absence of a CeLBP group without MC limits the interpretation of the results in respect of their specificity for MC as we were only able to assess the combined effect of CeLBP and MC. Nevertheless, we were able to detect clinically meaningful differences in serum biomarker concentrations and most of these biomarkers were robust for the presence of osteoarthritis or multiple MCs. Validity of the investigated serum biomarkers need to be investigated in larger longitudinal cohorts that stratify effects of MC and LBP on the investigated biomarkers. Another limitation is that outlier values of some biomarkers complicate the evaluation of the results. Patients and controls were matched for age and gender, but obesity was more prevalent among patients than controls. Adipose tissue is characterized with increased production of pro-inflammatory cytokines.[50] However, in our study the levels of inflammatory markers were mostly suppressed and, thus obesity most likely has not confounded the biomarker levels. The same reasoning fits for possible confounding effect of smoking and arthrosis, which were more prevalent among patients with mostly suppressed biomarker levels. Fi~~l~~eld strength of MRI scanners can affect sensitivity of MC detection [53]. At low-field MRI, MC1 were reported to be found three to four times more often, while at high-field MRI, MC2 was reported to be found twice as often. In our study, we found that the percentage of MC patients with primary MC being MC1 was only marginally higher at low-field MRI (low-field: 72.7%, high-field: 65.5%). Similarly, the percentage of MC patients with primary MC being MC2 was only marginally higher at high-field MRI (low-field: 34.5%, high-field: 27.3%). This difference was not significant (p=0.725).

A strength of our study is that all the analyses were performed with established, validated measurement assays in high-quality laboratories. Systemic biomarkers are prone to diurnal and activity-related variation in levels. In our study, all samples were taken at noon and both patients and controls were instructed not to eat or drink in the previous evening or in the morning before blood withdrawal. All subjects were instructed as well to avoid strenuous leisure-time activity

during the previous day. ~~Another strength is that we were very careful to exclude any control subject with even a small MC lesion.~~ Regarding the analysis of the multiplex assay data, a limit of quantification was defined based on the lowest calibrator point to avoid including data that were not suitable for reliable quantification. While this led to the exclusion of certain markers with overall low serum concentration, the values of the presented markers can be considered of minimal bias and imprecision.

As a conclusion, we observed that most biomarkers were suppressed among CLBP patients with MC, and that none of the measured biomarkers proved to be useful in distinguishing MC type. Comparing our data to recent work by others that report promising inflammatory biomarkers in subjects with intervertebral disc degeneration and LBP, we note inconsistent results across studies.[51,52] Consequently, further work is needed to establish whether any serum biomarkers have diagnostic and prognostic value for CLBP patients with MC.

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FIGURE LEGENDS

Fig. 1: The levels of C telopeptide of type I collagen (CTX)-1 in the patients with MC (n=40) or without MC (n=40). The data are shown by box plot showing median, interquartile range and minimum and maximum values. The median concentration of CTX-1 was significantly higher among controls.

Fig. 2: The levels of Eotaxin-1, Eotaxin-3, macrophage inflammatory protein-1-alpha (MIP-1A), monocyte chemotactic protein (MCP)-1 and regulated on activation normal T-expressed and secreted (RANTES) in the patients with MC (n=40) or without MC (n=40). The data are shown by box plot showing median, interquartile range and minimum and maximum values. The median concentrations of Eotaxin-1, Eotaxin-3, MIP-1A, MCP-1 and RANTES were significantly higher among controls.

Fig. 3: The levels of interleukin (IL)-15 in the patients with MC (n=40) or without MC (n=40). The data are shown by box plot showing median, interquartile range and minimum and maximum values. The median concentration of IL-15 was significantly higher among controls.

Fig. 4: The levels of interleukin (IL)-1sRII, IL-8 and tumour necrosis factor-alpha (TNF-A) in the patients with MC (n=40) or without MC (n=40). The data are shown by box plot showing median, interquartile range and minimum and maximum values. The median concentration of IL-1sRII was significantly higher among patients and the median concentrations of IL-8 and TNF-A were significantly higher among controls.

Fig. 5: The levels of TEK receptor tyrosine kinase (Tie-2), vascular endothelial growth factor (VEGF)-C, VEGF-D, fms related tyrosine kinase 1 (Flt-1) and hepatocyte growth factor (HGF-1) in the patients with MC (n=40) or without MC (n=40). The data are shown by box plot showing median, interquartile range and minimum and maximum values. The median concentrations of Tie-2, VEGF-C, VEGF-D and Flt-1 were significantly higher among controls, while the median concentration of HGF-1 was significantly higher among patients.

Fig. 6: The levels of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 in the patients with MC (n=40) or without MC (n=40). The data are shown by box plot showing median, interquartile range and minimum and maximum values. The median concentrations of VCAM-1 and ICAM-1 were significantly higher among controls.