Low back pain patients with Modic type 1 changes exhibit distinct bacterial and autoimmune subtypes that can be distinguished by cytokines in the blood

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INTRODUCTION: Modic type 1 changes (MC1) are painful vertebral endplate bone marrow (BM) lesions frequently observed on magnetic resonance images in sub-populations of chronic low back pain (CLBP) patients. They occur adjacent to degenerated intervertebral discs (IVDs) and co-locate with endplate damage. The etiopathogenesis remains unknown and disease-modifying treatments do not exist. Clarifying the etiopathogenesis of MC1 may help identify risk factors for MC1 and aid in the development of disease-modifying treatments. There are indications for bacterial (*Cutibacterium* acnes (*C. acnes*)-mediated) and autoimmune MC1 etiologies. In the bacterial etiology it is suggested that anaerobic aerotolerant *C. acnes* invade structurally damaged IVDs leading to IVD infection, accumulation of bacterial virulence factors, pro-inflammatory cytokine production by IVD cells, and endplate resorption. Comingling of inflammatory mediators with the adjacent BM leukocytes potentially induces an immune response in the BM. Conversely, since the nucleus pulposus of the IVD is an immune privileged site, it is suggested that autoimmunity might also be an etiopathological factor. Loss of compartment separation, e.g., through endplate damage, and loss of immune tolerance potentially induces an autoimmune response against IVD tissue in the BM. Despite the plausibility for bacterial and autoimmune MC1 etiologies, evidence of their existence in clinical populations does not exist. This is because the immune profile of MC1 BM in relation to adjacent intradiscal *C. acnes* load is unknown. We hypothesized that i) MC1 patients have either "low" or "high" intradiscal *C. acnes* load, ii) that immune cell signatures in the adjacent BM differ between patients with "low" vs. "high" *C. acnes* load, and iii) that patients with "high" and "low" *C. acnes* load can be distinguished with blood cytokine profiles.

METHODS: CLBP patients (n=38) with and without MC1 undergoing spinal fusion at the Balgrist University Hospital, Switzerland, were included in this study. From all patients, IVDs adjacent to MC1 (n=34) and control (n=11) were obtained and intradiscal *C. acnes* genome copy numbers were quantified with 16S qPCR. From a subset of these patients, a BM aspirate from a MC1 and an intra-patient control vertebrae was collected with Jamshidi needles using the pedicle screw trajectories prior to screw insertion and cells were separated from plasma by centrifugation. To compare the BM cell transcriptome of MC1 patients with "low" (n=3+3) and "high" (n=3+3) *C. acnes* load, red blood cells were lysed, RNA sequencing was performed, and differential expression analysis was conducted. Genes were considered to be differentially expressed (DEGs) for p<0.01. First, a *C. acnes* "low" and "high"-separated overrepresentation analysis (ORA) between MC1 and intra-patient control BM was performed to identify overrepresented cell types. Next, gene expression of MC1 was normalized to intra-patient control and compared with gene set enrichment analysis (GSEA) between groups to identify biological processes (BPs) and pathways that differed most between groups. Terms were considered significantly enriched for FDR <0.05. Concentrations of 20 innate and adaptive immunity cytokines were measured in blood (n=19) and BM plasma (n=13+13) with MesoScale U-Plex (Mesoscale Diagnostics). Blood plasma and normalized BM plasma (ΔMC1-control) concentrations were compared between *C. acnes* "low" and "high" groups with Mann-Whitney U test and corrected for multiple comparison using Bonferroni correction. Correlations between intradiscal *C. acnes* copies and blood cytokine concentrations were calculated with Spearman correlation. Area Under the Curve (AUC) of the Receiver Operating Characteristic was computed for each cytokine that correlated with intradiscal *C. acnes* load using simple logistic regression. A logistic regression model was calculated for the combination of cytokines that correlated significantly with intradiscal *C. acnes* load.

RESULTS: <u>IVD analysis:</u> None of the control IVDs exceeded 870 *C. acnes* copies/gram IVD (median: 262, [117,682]; 99% CI (0, 870)). Overall, MC1 IVDs had similar *C. acnes* copy numbers (423, [132,1866]; p=0.28), but with 14 IVDs (38.9%) having >870 copies/gram IVD. Based on the upper 99% CI limit of the control group (870 copies/gram IVD), the MC1 group was dichotomized, resulting in a *C. acnes* "low" group (211, [37,364] copies/gram IVD) with similar copy numbers as control IVDs, and a *C. acnes* "high" group (2278, [1352,3324]), whose copy numbers were 10.8-fold higher, on average (**Figure 1**). MC1 patients of both groups did not differ in age, BMI, disability, back- and leg pain, IVD- and endplate degeneration, nor in the proportions of females, smokers, patients with prior back surgery / epidural- or facet joint infiltrations.

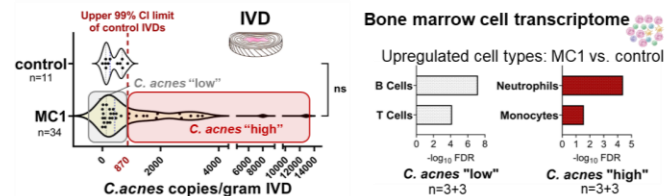

Figure 1: Distribution of *C. acnes* copies/ gram IVD in control and MC1 IVDs. Red line: Upper 99% CI limit of control (870 copies).

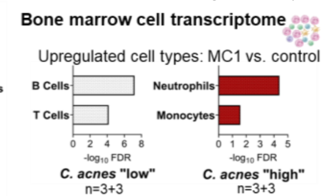

Figure 2: Transcriptomic BM cell analysis. ORA comparing MC1 to controls stratified for *C. acnes* "low" (left) and "high" (right) MC1 patients.

Bone marrow plasma cytokines

Table 1: BM cytokine levels. *C. acnes* "low" (n=5+5) and "high" (n=8+8) $\Delta_{MC1\text{-}control}$ BM cytokine levels (pg/ml) of proteins with significant / trend towards significant (ENA-78) inter-group differences.

	IL-8	ENA-78	IL-18	IP-10	M-CSF	MIP-1α	MIP-1β
C. acnes "low" (pg/ml)	**-11**, [-22,-6]	**-76**, [-450,-32]	**-255**, [-1211,-160]	**-189**, [-199,-157]	**-6**, [-18,-5]	**-6**, [-6,-4]	**-36**, [-46,-26]
C. acnes "high" (pg/ml)	**8**, [2,26]	**117**, [-2,463]	**42**, [27,233]	**33**, [-12,175]	**4**, [3,9]	**-0.6**, [-2,8,3]	**18**, [1,32]
"low" vs. "high" FDR	0.01	0.07	0.01	0.01	0.01	0.01	0.01

<u>BM analysis:</u> ORA revealed upregulated adaptive immune cell signatures (T / B cells) in *C. acnes* "low" and innate immune cell signatures (neutrophils, monocytes) in *C. acnes* "high" BM comparing MC1 to intra-patient controls (**Figure 2**). GSEA showed that the top enriched BPs in the *C. acnes* "low" group was "adaptive immune response" (normalized enrichment score (NES)=2.1, FDR=0.00) whereas it was "neutrophil degranulation" (NES=-1.5, FDR=0.00) in the *C. acnes* "high" group. GSEA of pathways further showed significantly enriched T helper subset differentiation pathways in *C. acnes* "low" MC1 patients like "Th1 and Th2 cell differentiation" (NES=2.1, FDR=0.00) and "Th17 cell differentiation" (NES=2.1, FDR=0.00) (not shown). Compared to the *C. acnes* "low" MC1 patients, BM plasma of the *C. acnes* "high" group had increased BM protein levels of epithelial-neutrophil activating peptide (ENA-78), interleukin (IL)-8, IL-18, interferon gamma-induced protein 10 (IP-10), macrophage colony stimulating factor (M-CSF), macrophage inflammatory proteins (MIP)-1α, and MIP-1β. All pro-inflammatory cytokines/chemokines related to neutrophil and macrophages/monocyte function or production (**Table 1**).

<u>Blood analysis:</u> IL-13, a cytokine that associates with autoimmune diseases, was increased in MC1 patients with "low" (n=10) vs. "high" (n=9) intradiscal *C. acnes* load ("low": 1.8 pg/ml, [0.9,3.5], "high": 0.0 pg/ml, [0.0,0.0], FDR=0.02). All other measured cytokines did not differ between the groups. IFN-y (ρ=-0.64, p-value=0.004), IL-12p70 (ρ=-0.54, p-value=0.018), and IL-13 (ρ=-0.71, p-value=0.0006) correlated negatively with intradiscal *C. acnes* load in the MC1 IVD. IL-13 distinguished best between the two groups (AUC=0.89, sensitivity=100%, specificity=70%, p=0.00). In combination with IFN-y and IL-12p70, the discriminatory ability was further increased (AUC=0.92, sensitivity=89%, specificity=80%, p=0.00).

DISCUSSION: Here, we found that two biological MC1 subtypes exist, (i) a bacterial subtype, with high *C. acnes* concentration in the MC1-adjacent IVD and with an activation of the innate immune system in the MC1 BM, and (ii) an autoimmune subtype, with low intradiscal *C. acnes* concentration in the MC1-adjacent IVD and an activation of the adaptive immune system in the MC1 BM. Finally, we found blood plasma cytokines that were able to distinguish these subtypes with high accuracy. A limitation is the inability to investigate whether biological MC1 subtypes represent different stages of the same subtype, since collection of IVD and adjacent BM biospecimens of MC1 patients over time is not feasible. For this reason, we cannot draw conclusions about the etiology in MC1 patients and we here refer to biological MC1 subtypes.

SIGNIFICANCE/CLINICAL RELEVANCE: In conclusion, we show that a bacterial and an autoimmune subtype exists in MC1 patients. This has large clinical implications for the development of subtype-specific biomarkers and treatment strategies.