Intervertebral Disc Microbiome in Modic Changes: lack of result replication underscores the need for a consensus in low-biomass microbiome analysis

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INTRODUCTION: The technological advancements have facilitated an untargeted approach to explore the microbiome in low-biomass samples with the use of next-generation sequencing. Specifically in tissue that was long thought to be sterile, such as the intervertebral disc, this has led to a paradigm shift and has sparked intense debates within the research community. Within the context of Modic changes (MC), Cutibacterium acnes (C. acnes) has been the pathogen in the spotlight. However, a study on the MC microbiome recently published by Rajasekaran et al. challenged this with the hypothesis that the resident IVD microbiome is in a state of dysbiosis in MC[1]. Therefore, it is crucial to ensure that such a paradigm shift changing finding is reproducible and is built upon a methodology that is robust, replicable, and accurate. Without a consensus pipeline, the results of different studies cannot be compared, and the groundbreaking potential is lost. The purpose of this study was two-fold: (i) to analyze the microbiome of the Modic type 1 (MC1), MC2, and non-Modic (nonMC) discs and (ii) to emphasize the importance of reaching a methodological consensus including decontamination strategies for low-biomass samples.
METHODS: A total of 70 discs (24 nonMC, 25 MC1 and 21 MC2) were collected from patients undergoing spinal fusion surgery. To control for contamination, 10 samples with buffer only were included throughout all steps. DNA was isolated from all 70 discs, the V3-V4 region was amplified and sequenced using Illumina NextSeq 2000. Reads mapping to the human reference genome GRCh38p13, resulting from off-target amplification, were filtered out (Walker et al., 2020). The remaining reads were trimmed for adapter content. The data was processed with the QIIME-2 pipeline and the obtained amplicon sequence variants (ASVs) were annotated with SILVA database. Bacterial contaminants were identified and removed with Decontam R package, using the prevalence method comparing the composition of the positive samples to the negative controls. For further analysis, ASVs were normalized for counts of ASVs in each sample divided by the total sum of reads in that sample with an added pseudocount. Phyla and gram-stain differences were compared with use of the Friedman test, followed by the Benjamini, Krieger, and Yuketeli multiple hypothesis testing on normalized ASV values.
RESULTS SECTION: Disc degeneration measured by Pfirrmann grade was similar in all groups ($p = 0.448$). Alpha-diversity presented no difference between the groups. Beta-diversity indicated that the nonMC group has a greater diversity compared to MC1 and MC2 (Figure 1). The total number of ASVs detected was 180 of which 80% were found in all samples. On a phyla level, proteobacteria were most prevalent, followed by firmicutes and actinobacteria. Firmicutes significantly decreased their abundance in MC1 and MC2 compared to nonMC discs (Figure 3). MC1 discs had less gram-positive bacteria compared to both nonMC ($q = 0.013$) and MC2 ($q = 0.001$), in contrast MC2 discs had significantly more gram-negative bacteria ($q < 0.001$) (data not shown). The widely debated C. acnes was identified in all three groups, without significant group differences (data not shown).
DISCUSSION: This is the first study which has replicated the investigation of the MC disc microbiome through use of next generation sequencing with a geographically different patient cohort and different bioinformatic methods. The comparison of the detected bacteria showed vast differences to the detected bacterial species of the patient cohort used by Rajasekaran et al. (2023). However, a reduced bacterial diversity in MC discs and an increase in gram-negative bacteria is also reported suggesting a common characteristic. This suggests a more favorable environment for gram-positive bacteria in general. The large differences between the two studies emerge either from (i) geographical and ethnical differences, from (ii) sample processing, or from (iii) bioinformatic analysis differences. In order to use the unprecedented potential of metagenomics and to address the clinically relevant questions, disc sample processing and bioinformatic analysis pipeline need to be harmonized.
Overall, the study not only adds further evidence that the disc has its own unique microbiome but also finds differences in the MC adjacent IVDs which may specifically be important in the context of clarifying the MC etiology. To delve deeper into this still largely unexplored field and provide further clarity on the etiology of MCs, it is essential to establish a consensus for a low-biomass microbiome analysis pipeline.
SIGNIFICANCE/CLINICAL RELEVANCE: The direct comparison of our findings to other studies emphasizes the differences in results generated either through methodological or ethnicity-based differences and highlights the importance of standardization that needs to be implemented in this field.

REFERENCES:

Rajasekaran, S., Vasudevan, G., Easwaran, M., Devi Ps, N., Anand K S, S. V., Muthurajan, R., Tangavel, C., Murugan, C., B T, P., Shetty, A. P., & Kanna, R. M. (2023). "Are we barking up the wrong tree? Too much emphasis on Cutibacterium acnes and ignoring other pathogens"— a study based on next-generation sequencing of normal and diseased discs. *Spine Journal*. https://doi.org/10.1016/j.spinee.2023.06.396

Walker, S. P., Barrett, M., Hogan, G., Flores Bueso, Y., Claesson, M. J., & Tangney, M. (2020). Non-specific amplification of human DNA is a major challenge for 16S rRNA gene sequence analysis. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-73403-7

IMAGES AND TABLES:

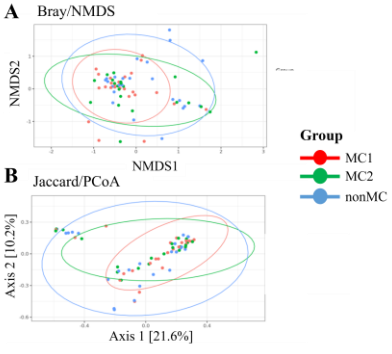

Figure 1. Beta diversity shows lower diversity in MC1. (A) Bray dissimilarity based on abundance and (B) Jaccard distance based on presence and absence of samples.

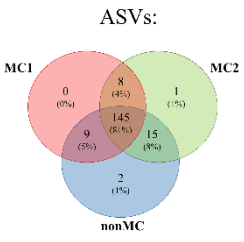

Figure 2. ASVs detected per group.

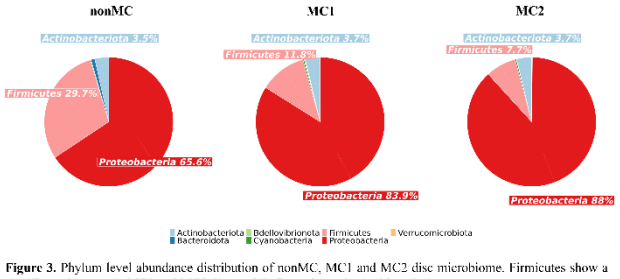

Figure 3. Phylum level abundance distribution of nonMC, MC1 and MC2 disc microbiome. Firmicutes show a significant decrease in MC1 and MC2 discs, while Proteobacteria significantly increase.