P20 & FP – **Investigation of Novel biomarkers and Definition of role of microbiome In Graves' Orbitopathy (GO) (INDIGO): Microbiota analysis of patients at recruitment**

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Background: In the past years, the microbiome of patients and animal models for inflammatory diseases and autoimmune conditions was extensively investigated. In the context of autoimmune thyroid diseases (AITD), the gut microbiota is thought to be implicated in the development of conditions, such as Hashimoto's thyroiditis[1]. However, little is known about its role in Graves' disease (GD), characterized by thyroid-stimulating auto-antibodies (TSAb) causing hyperthyroidism and in the concomitant eye disease Graves' orbitopathy (GO). Since the balance between pro-inflammatory Th17 cells and anti-inflammatory T-reg cells seems to be compromised in the presence of specific pro-inflammatory bacterial strains[2], we hypothesized that the presence or the absence not only of pathogens, but also of symbiotic and commensal bacteria can favour an immune response more prone to inflammation and conducive to GD, and possibly sustain its progression to GO[3]. To address such hypothesis, we characterized the gut microbiota of GD (n=65) and GO (n=56) patients, enrolled within the EU INDIGO project from different centres across Europe, and compared to that of matched healthy controls (n=42).

Methodology: Metataxonomics (16S rRNA gene sequencing) was performed on total bacterial DNA extracted from both patients and controls' faecal samples, targeting the V1-V2 plus bifidobacteria regions. QIIME pipeline was used to process the sequences, while statistical analysis was performed in R. A subset of faecal samples was also evaluated using traditional microbiology.

Results: The within-sample alpha and between-sample beta diversity indices were similar in patients and controls. However when considering the taxonomic composition, counts from the phylum *Bacteroidetes* were significantly more abundant in controls (38.5%) than in GD (24.2%) and GO (27.3%) ($p=0.012$), whilst *Firmicutes* were more abundant in GD (59%) and GO (60.5%) than controls (53.2%). Consequently, the *Firmicutes:Bacteroidetes* ratio was significantly higher in GD/GO than controls, but similar between GD and GO. At a genus level, *Bacteroides* was reduced in GD and GO patients with mild disease ($p=0.012$) compared to controls, but not between moderate-severe GO compared to controls. Reduction in the genus *Bacteroides* was observed also in two patients who developed GO during the study (BH adjusted $p<0.0001$), also confirmed using traditional microbiology techniques. Furthermore, *Enteroccoccus gallinarum* counts, a pathobiont reportedly involved in triggering autoimmunity[4], though low overall, were significantly higher in GD and GO than controls.

Conclusions: Our data illustrate substantial perturbation of the gut microbiota in GD/GO, which may be driven by hyperthyroidism. Reduction in the genus *Bacteroides* was also recently reported in the GO mouse model compared

to control mice[5].Future analyses will explore correlations between taxonomic profiles and TSAB auto-antibodies levels, thyroid function and GO disease severity and whether they are affected by treatment.

References

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