Summary of MIMSA

Project Title: Role of oral microbiome & mucosal immunity in COVID-19 disease: diagnostic and prognostic utility in South Asian populations (MIMSA)
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CI: Professor Stephen Challacombe. **PIs**: Dr David Moyes, Dr Saeed Shoaie, Professor Mark Ide, Professor Newell Johnson
Project Indian Lead PI: Dr. Priya Kannian
Project Acronym: MIMSA

Background

There is strong evidence of disparities in susceptibility to SARS-CoV-2 infection and subsequent morbidity and mortality to COVID-19 amongst groups of different ethnic origin. In the UK, South Asian populations have been identified as a particularly susceptible group, with a higher percentage mortality in contrast to similar populations in India, even after taking into account the effect of socio-economic level and of co-morbidities. The extent to which social, environmental and biological factors differ in the two populations and contribute to differences in outcomes from COVID-19 is not known. Our hypothesis was that differences in natural immunity and in the microbiome (all your bacteria, viruses and fungi) might be responsible. SARS-CoV-2 first infects mucosal surfaces (the linings of the body). Our research compares the oral microbiome, salivary innate (naturally present and not stimulated by infection) and specific (stimulated by infection) mucosal antibody responses among SARS-CoV2-positive patients and controls in the UK and India. Our aim was to determine whether specific factors were involved and if so whether their measurement could be useful for diagnosis and for prediction of susceptibility. We compared mucosal and systemic immunity in order to help reveal biomarkers for risk of disease progression so as to enable early initiation of treatment. We also hypothesised that pre-existing oral disease might be related to COVID severity.

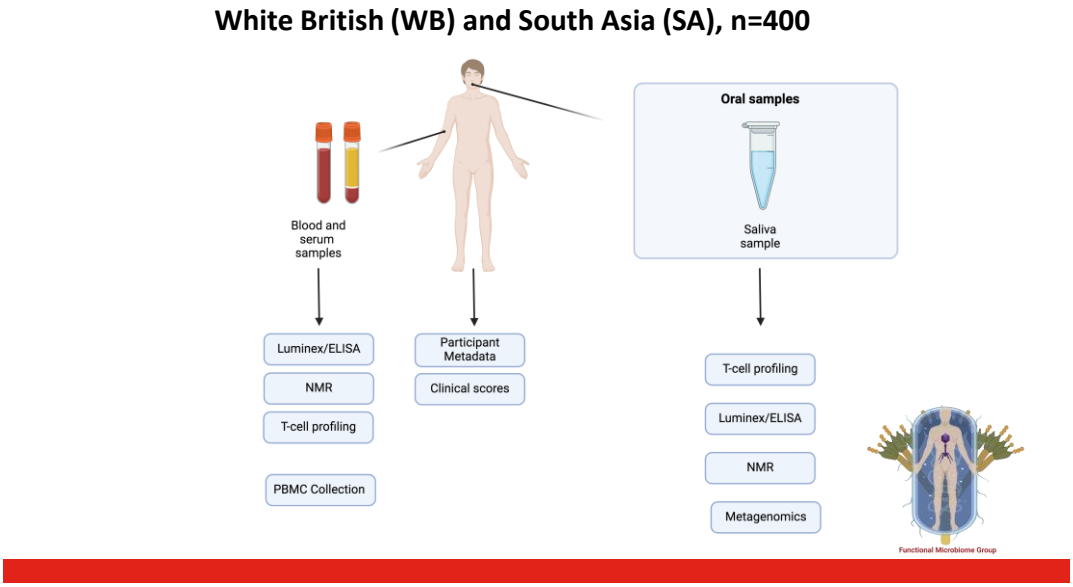
The study is part of a UK-India partnership and data from the UK is compared with that derived from a similar study with our collaborators in India.

Methods

We collected samples of stimulated saliva (we called stimulated whole mouth fluid) and blood from 303 participants in London and 203 in Chennai, India. In over one third of participants we obtained further samples over three months so that we had 118 longitudinal samples in the UK and 86 in Chennai in addition to the baseline samples. By utilising modern techniques we were able to assay antibodies (ELISA techniques), cytokines (the factors by which immune cells talk to each other)(flow cytometry with cytokine bead arrays) and

immune cells themselves (fluorescent activated cell sorting FACS) in only a few mls of the SWMF. Further by extracting DNA, we could quantify 800 species of bacteria (the microbiome), 400 of which had never been grown as well as their products (metabolome). We compared all the immune factors in SWMF with those in blood. The COVID severity ranged from healthy controls who denied ever having COVID, to those with mild, moderate or severe disease. The majority had recovered from COVID but groups with active disease were also included. All the immune factors were compared in South Asian (SA) heritage in the UK and India, and in White British heritage (WB) in the UK. Analyses were also performed in relation to age and gender as well as vaccination responses, longitudinal responses and reproducibility.

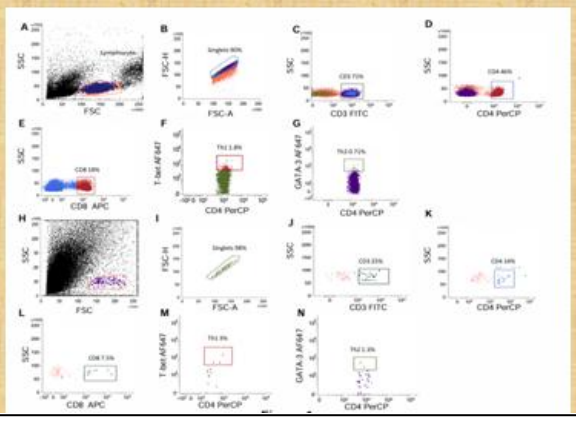
The saliva microbiome was collected in two different countries (UK and India) and shotgun metagenomics was performed to retrieve high-quality compositional and functional analysis of the microbiome. They were mapped against the non-redundant gene catalogue of the oral microbiome, consisting of more than 7 million genes. The produced gene counts were then used for retrieving the species profile for each sample and determining the abundances.

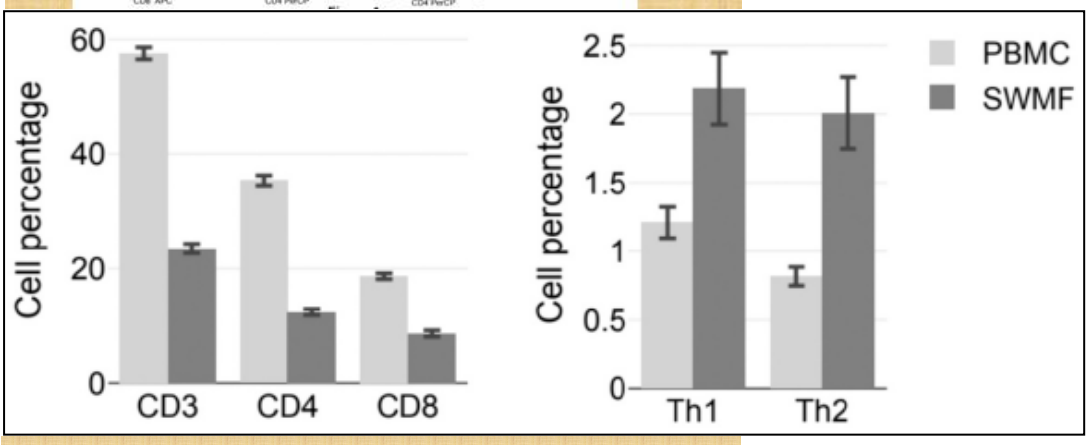


Results and key findings and significance:

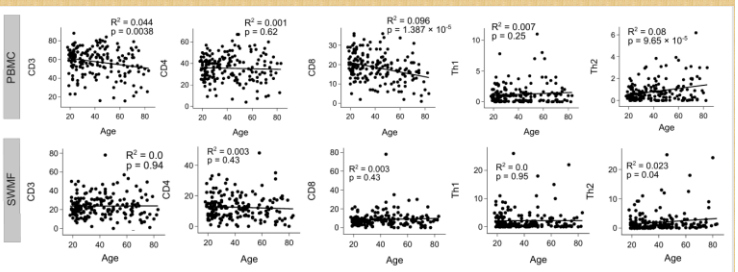
1. T cell phenotypes in mucosal secretions are different from those in blood and not related to age or gender. (Abs 1). *Very few studies have been able to reproducibly isolate, identify and quantify the immune cells in mucosal secretions. Almost all virus infections start at mucosal surfaces*

The gating strategy for flow cytometry using peripheral blood mononuclear cells (PBMCs) and stimulated whole mouth fluid (SWMF) samples. The cellular marker and the fluorochrome used are shown. A -G: PBMC sample. H -N: SWMF sample. The percentages of the CD3, CD4, CD8, Th 1 and Th 2 lymphocytes are shown. Linear scale was used for forward scatter (FSC) and side scatter (SSC) axes .

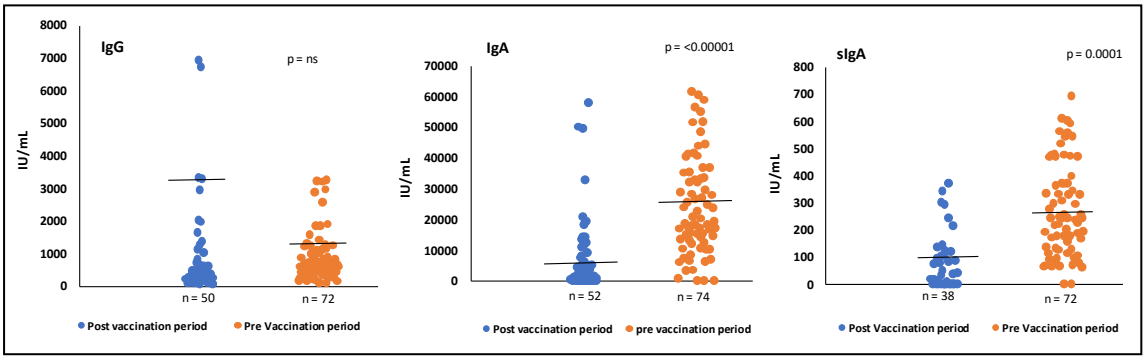


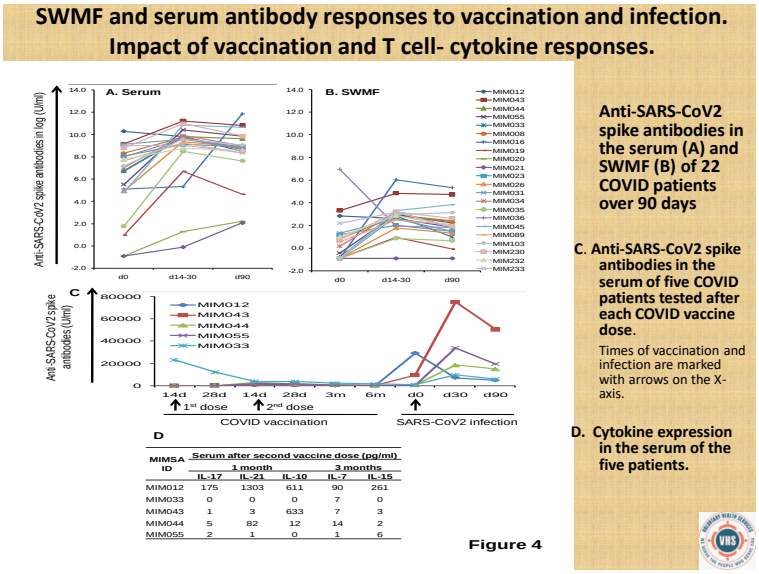


Relationship between percentage values of T lymphocyte phenotypes in stimulated whole mouth fluid (SWMF) and peripheral blood (PBMC) in relation to age in 194 subjects. Significant relationships shown between age and CD3, CD8 and Th2 in PBMC, and with Th2 in SWMF



2. Immunisation against SARS-CoV2 prior to infection resulted in higher IgG, IgA and SIgA antibodies in secretions, and a longer lasting SIgA response (Abs 5). *Antibodies at mucosal surfaces can be locally produced as well as come from serum and may be important in resistance to infections. This finding shows that immunisation followed by COVID resulted in significant mucosal antibody production which may protect against further SARS-CoV2 infection*

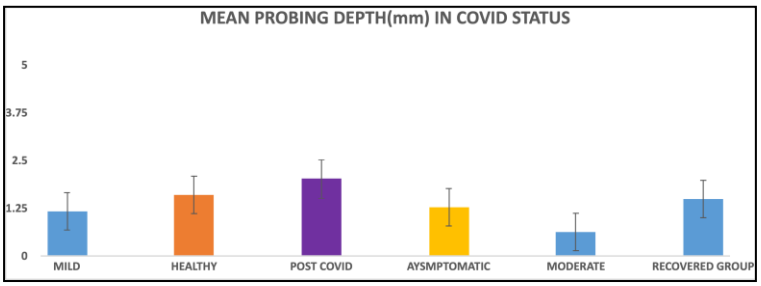




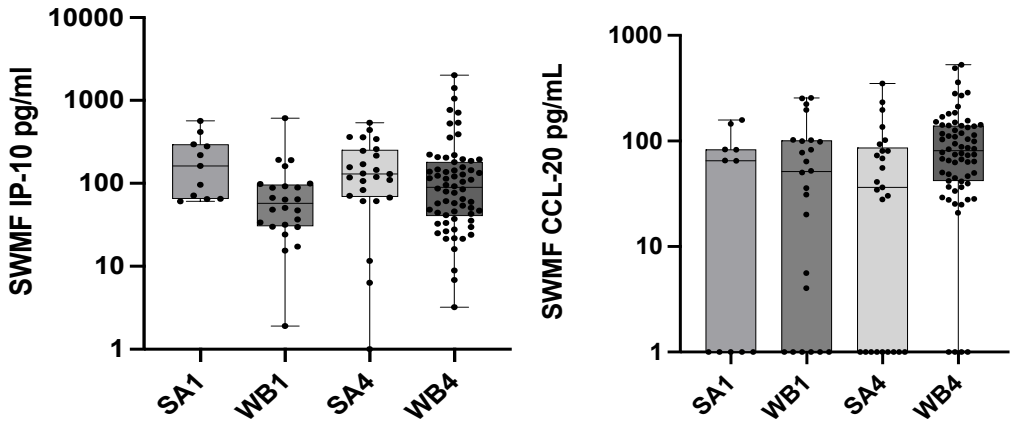
MIMSA ID	Serum after second vaccine dose (pg/ml)				
	1 month			3 months	
	IL-17	IL-21	IL-10	IL-7	IL-15
MIM012	175	1303	611	90	261
MIM033	0	0	0	7	0
MIM043	1	3	633	7	3
MIM044	5	82	12	14	2
MIM055	2	1	0	1	6

Figure 4

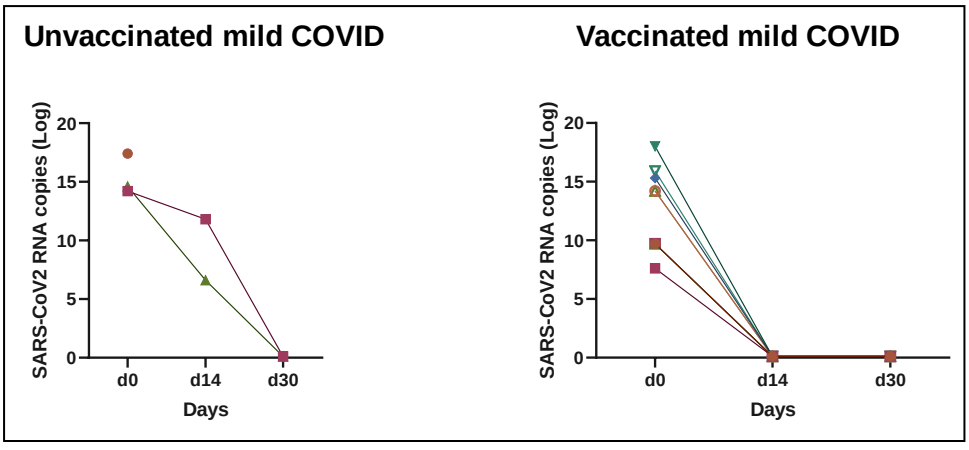
3. There were no significant differences in mean salivary cytokine levels between the UK and South Indian subjects but Post- COVID patients were found to have higher mean probing depth ($p<0.01$) than non-infected controls.(Abs 9). *Similar cytokine levels in UK and South Indian cohorts did not support the hypothesis that differences in innate immune factors were responsible for the differences in morbidity. Greater periodontal disease in the smaller cohort of Long COVID patients did support the hypothesis that pre-existing oral disease might play a role.*

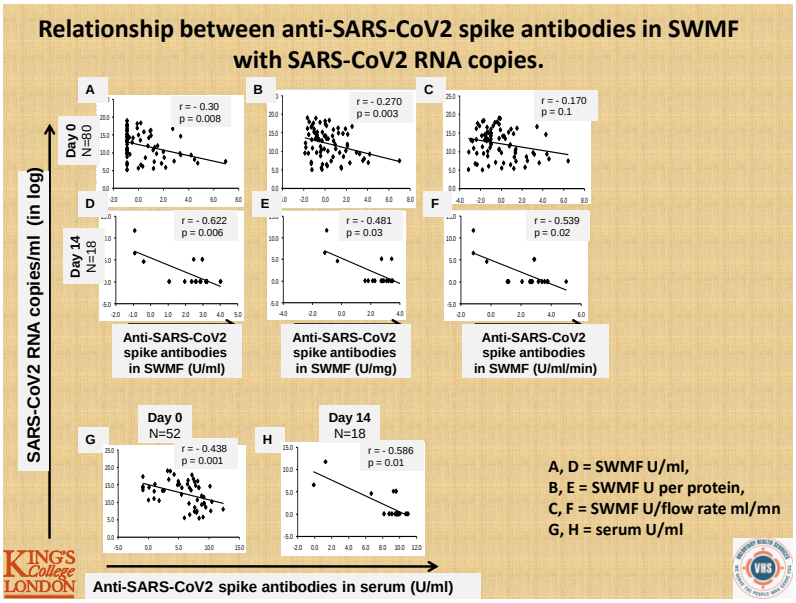


Comparisons	Serum			SWMF		
	Increased	Decreased	No change	Increased	Decreased	No change
Ind NIC vs UKSA NIC	MCP-1	IP-10, IL-1b, CCL20	MIG, IL-8, IL-6	IP-10, CCL20	MIG, MCP-1, IL-8,IL-1b, IL-1a	IL-6
Ind NIC vs UKWB NIC	MCP-1	IP-10, IL-1b, CCL20	MIG, IL-8, IL-6	CCL20	MIG, MCP-1, IL-8,IL-1b, IL-1a	IP-10, IL-6

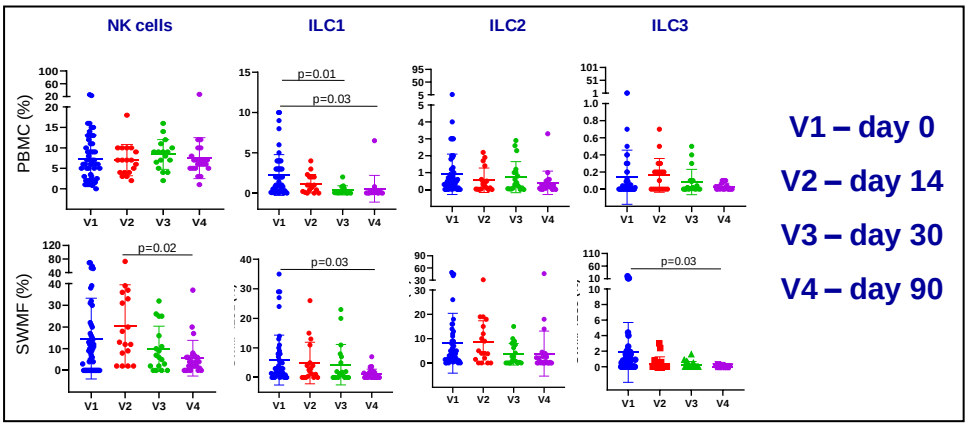


4. SARS-CoV2 clearance was achieved 14 days earlier in vaccinated than unvaccinated. The SARS-CoV2 exposed groups showed a differential expression of mucosal T cells suggesting a strong local immune response in COVID-19. (Abs 2). *Vaccination resulted in quicker clearance of SARS-CoV2, and this may have been due to an enhanced mucosal response exemplified by mucosal T cells.*

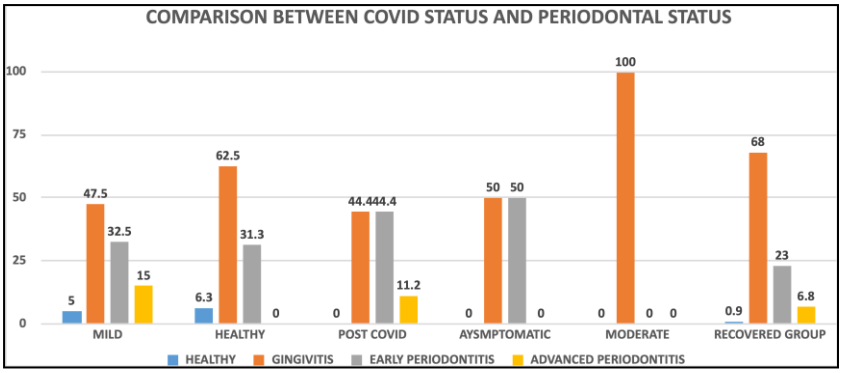




5. Innate lymphoid cells (ILC) may play an important role in innate mucosal immunity. ILC1, ILC2 and ILC3 in SWMF were elevated during active COVID and declined to normal levels upon recovery; but persist in Long COVID indicating their role in oral mucosal immunity against COVID-19 for the first time. (Abs 6). *Innate immune lymphoid cells provide the first line of defence at mucosal surfaces. This finding demonstrated for the first time their potential role in mucosal protection and that patients with long COVID show a different response pattern.*

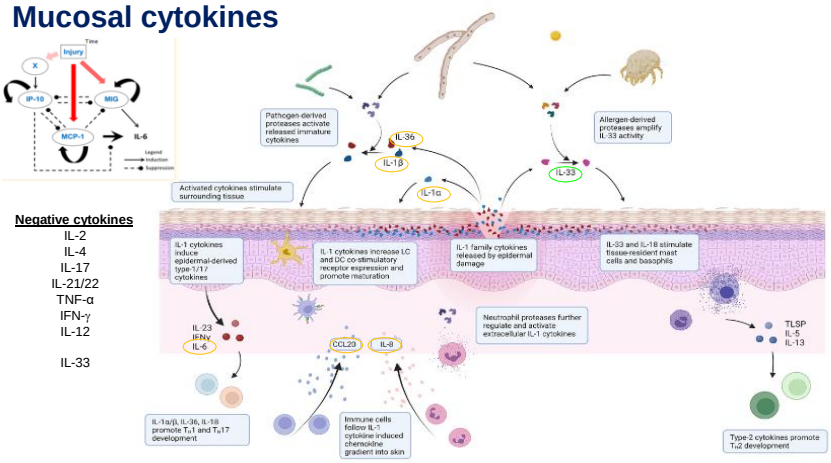


6. Periodontal health impacted by smoking and COVID severity by ethnicity, but minimal association between periodontal status and COVID severity score (Abs 7). *This finding showed differences in COVID severity between the white British and South Asian cohorts but not a direct association of periodontal status and COVID severity. Some of the difference may be related to the higher smoking prevalence in SA.*

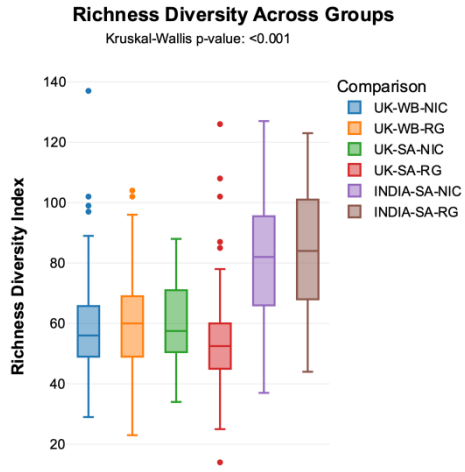


7. Mucosal innate immune factors may influence susceptibility to SARS-Cov2 infection and this study found that mean levels of salivary innate cytokines were significantly lower in those of South Asian heritage compared with those of white British heritage. (Abs 8). *Innate immunity at mucosal surfaces is the first line of defence and cytokine reflect cellular immune activity. This finding would support the hypothesis that lower cytokine production might reflect increased susceptibility to COVID.*

Comparisons	Serum			SWMF		
	Increased	Decreased	No change	Increased	Decreased	No change
UKSA NIC vs UK WB NIC	MCP-1, IP-10	-	MIG, IL-8, IL-6, IL-1b, CCL20	IL-1b	MCP-1, CCL-20	MIG, IL-8, IL-36g IL-6, IP-10



8. Comparing the UK and Indian cohorts with no active COVID-19, the non-infected and recovered Indian South Asian (SA) cohorts had the highest richness of species in the microbiome and the recovered UK-SA had the lowest. *This finding suggested that there was little difference between the microbiome in white British and South Asian populations in London, but that both showed significantly lower species diversity than South Asians in India. Further, we showed that although the microbiome is disturbed during COVID, the data suggests that it returns to normal diversity after recovery from COVID*



9. Comparison of the salivary microbiome in non-infected controls, COVID infected patients and in those recovered from COVID showed that the actively infected patients had lowest richness, while the recovered and non-infected ones were higher than infected and had similar trend. Further detailed diversity analysis, revealed that the three cohorts can be significantly separated based on their saliva microbiome composition. *This finding built on that of finding in 8 and showed that the species composition of the salivary flora was altered in COVID and that even after recovery, the species composition remained significantly different form non-infected controls.*