



Covid-ImmunoPhenotyping - a preliminary data release, May 22, 2020

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“ – Leticia Monin

We are trying to understand at a basic level why some patients with the virus don't show any symptoms at all, while others need to be hospitalised and some sadly die.

Summary

- Our initial high throughput in-depth analysis of over 60 hospitalised Covid-19 patients and over 50 controls has revealed:
 1. An immunological signature of Covid-19 disease
 2. Several discrete components of that signature that track with severity

Potential importance

- SARS-Cov-2 virus is not the threat to global stability; that threat stems from the seriousness of the disease that the virus has the potential to cause
- Understanding the basis for serious disease symptoms may permit new approaches to diagnosis and therapy that may considerably reduce the risk that infection by SARS-CoV-2 would lead to severe, potentially fatal disease.



Scene setting

- Despite most exposures being pauci-symptomatic, Covid-19 has been the most socially-transformative infectious disease in living memory - Millions infected; hundreds of thousands dead or only gradually recovering from severe disease - Precipitous burden on health care.
- The virus has not gone away – major rises in global infections continue to be reported, and may “re-surge” in autumn. Effective anti-virals and vaccination are still in development.
- Serious symptoms have been associated with underlying morbidities, age and gender, but are there other factors?
- Serious symptoms are clinically associated with lymphopenia, CRP, and some other metrics, but what mechanisms and processes do these reflect?

Hypothesis for Covid-IP

- The immune system lies at the heart of the host-pathogen relationship and may track with and/or underpin disease course.

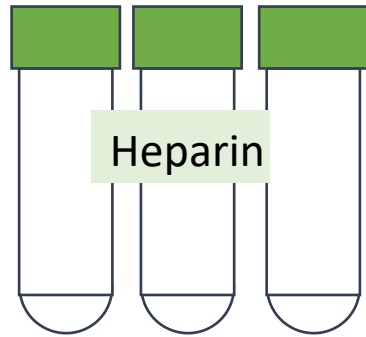
Key questions

- Can we identify biologically meaningful markers or ‘immune signatures’ that predict disease course at the front door?
- Can we identify ‘immune signatures’ in patients who do well? This will guide us toward “correlates of protection”, informing vaccine design and helping to predict the likely efficacy of current or proposed medications that can be re-purposed?
- What is the relative contribution to sustained protection of T cells versus antibody production?
- What is the nature of covid-19 lymphopenia and what are its implications - an impact on innate→adaptive switch?

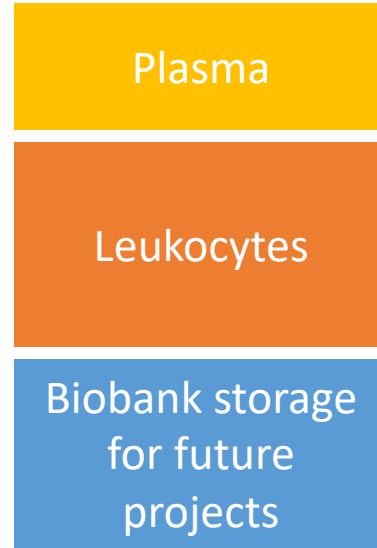
The Covid-IP Pipeline *

Day 1, 3, 9

Co-ordination with other studies



Heparin



Plasma

Leukocytes

Biobank storage
for future
projects

Serology; IgG/IgM [S/RBP/N]
ELISA- Dr Katie Doores
LIPS – Professor Pärt Peterson (Tartu)

- Legendplex assay – plasma cytokines, inc. IL-6, IL-8, IP10, IL10
- Additional analytes, including Tryptophan metabolites.
- Autoantibodies

8 panel flow cytometry on fresh specimens to measure cell types, cell status, and cell cycling

Individual CD4 and CD8 T cells are sorted and DNA extracted for T cell Receptor repertoire sequencing

Research
Nurse
Collections



PAXgene for RNA

*facilitated by the King's College Infectious Diseases Biobank

The COVID-IP cohort:

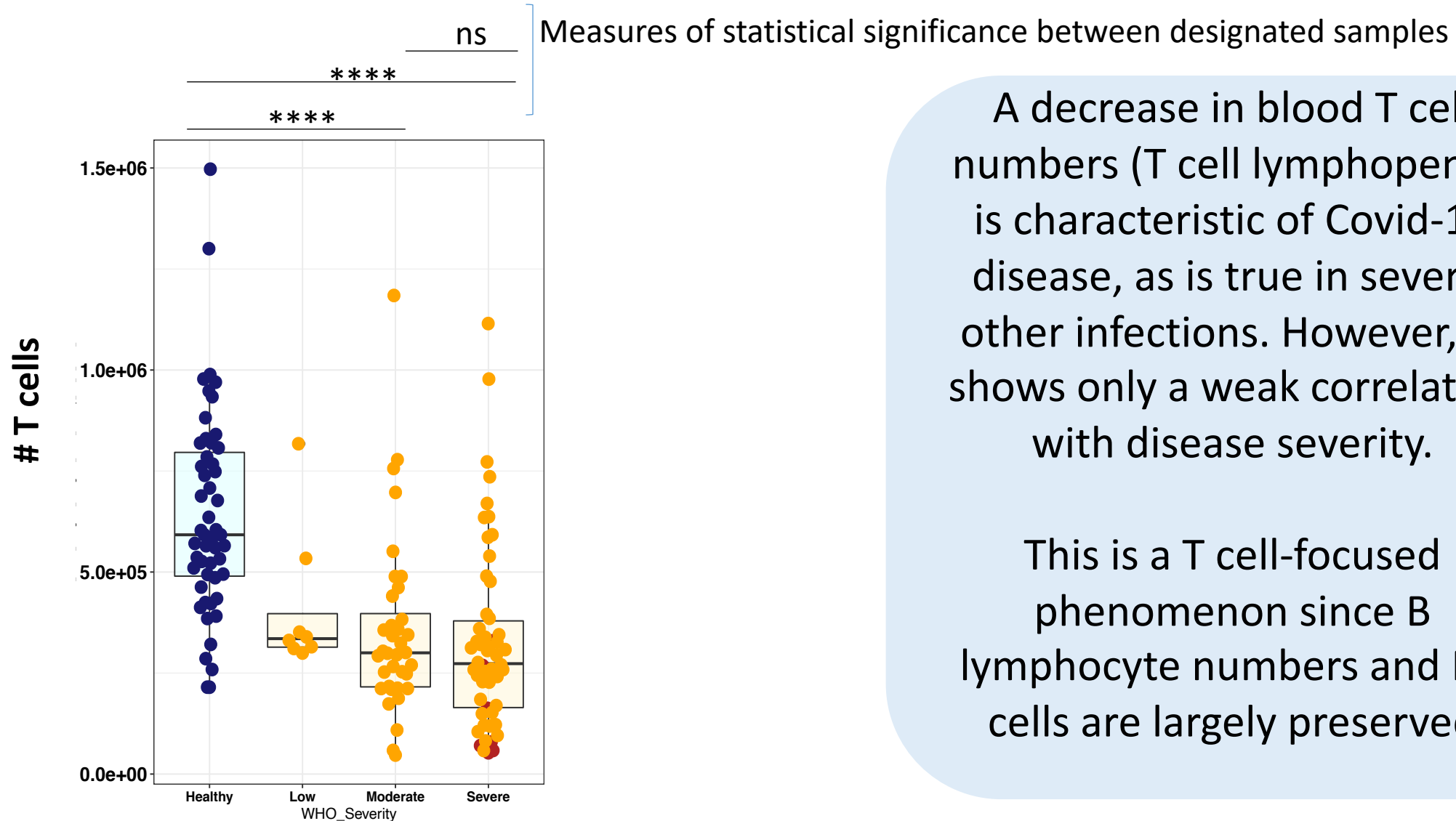
hospitalized SARS-CoV-2 infected patients, ~80% of whom recovered without intensive care, and 20% of whom declined, with ~10% declined irreversibly.

Clinical conditions were classified according to a combination of local clinical practice and WHO classifications.

What we have learned?

This is a preliminary data-set, made broadly available in the hope that it can guide and/or inform others internationally. More formal peer-reviewed data releases will follow, respectful of compliance with appropriate scientific standards.

Covid-19: highly selective diminution of T cells, associated with disease but not obviously with severity



A decrease in blood T cell numbers (T cell lymphopenia) is characteristic of Covid-19 disease, as is true in several other infections. However, it shows only a weak correlation with disease severity.

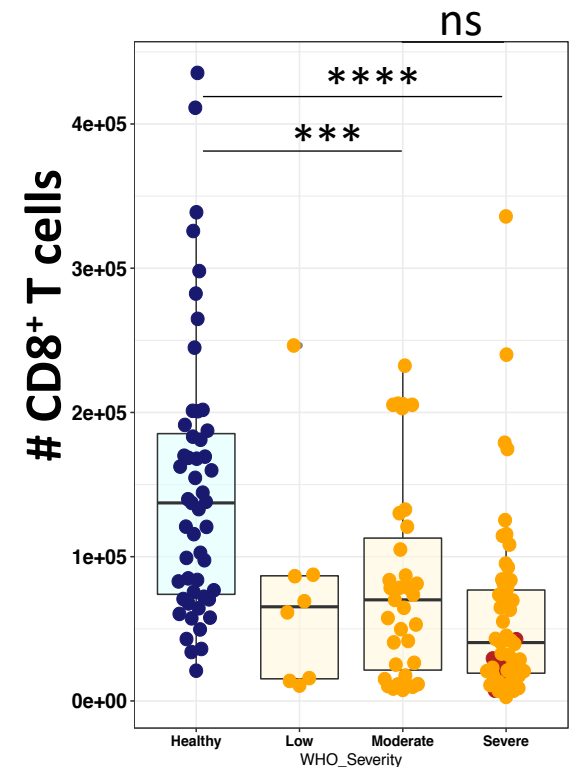
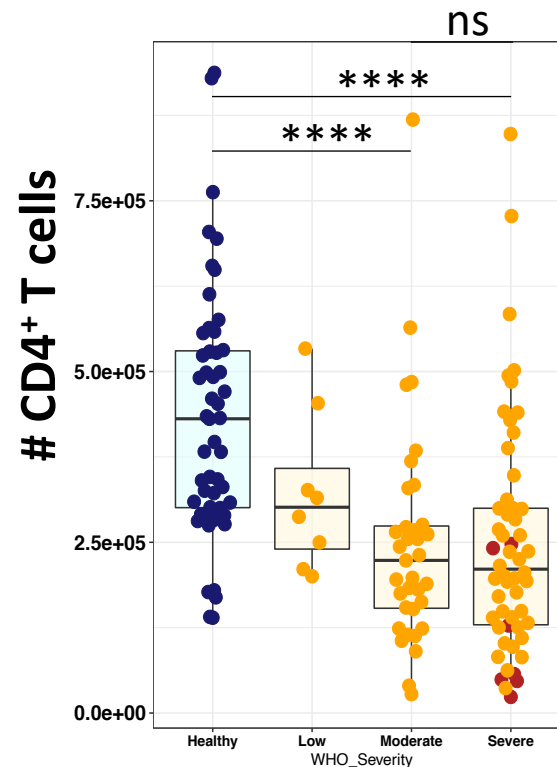
This is a T cell-focused phenomenon since B lymphocyte numbers and NK cells are largely preserved

Covid-19: both Helper and Cytotoxic T cells are diminished with disease, with some link to severity

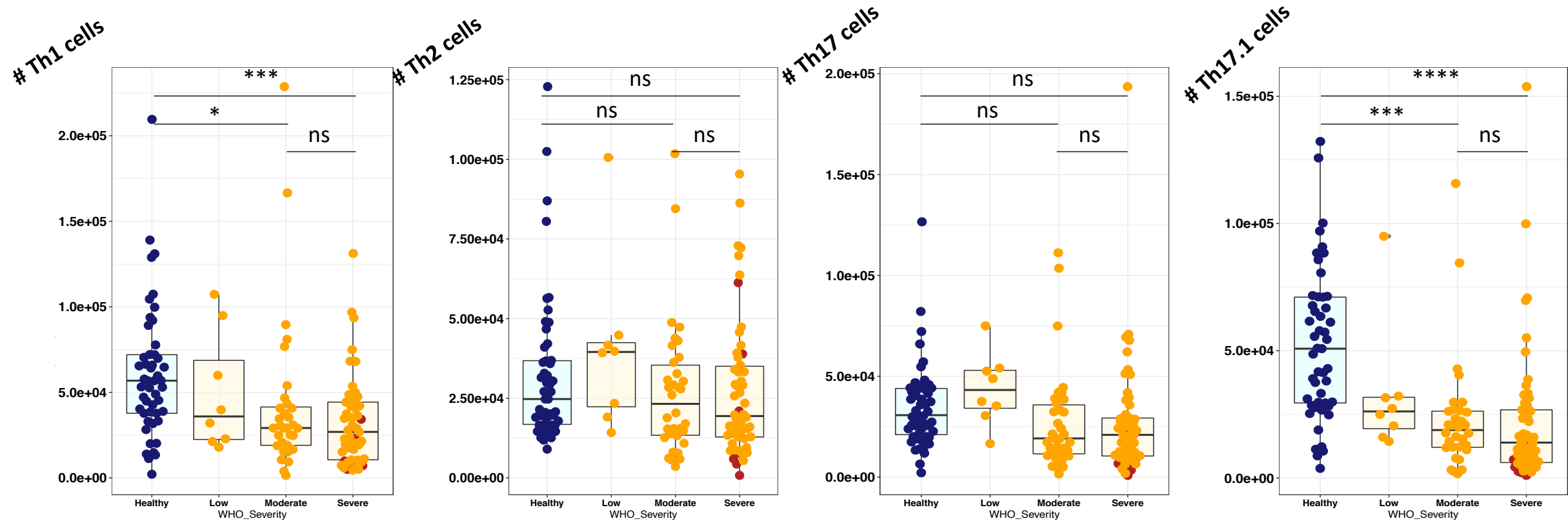
T cells are one of the major components of the **adaptive immune system**.

CD4+ Helper T (Th) cells help to shape, activate and regulate the adaptive immune response by activating other immune cells, releasing cytokines, and helping B cells produce antibodies.

CD8+ Cytotoxic T cells recognise and directly kill virus-infected cells.

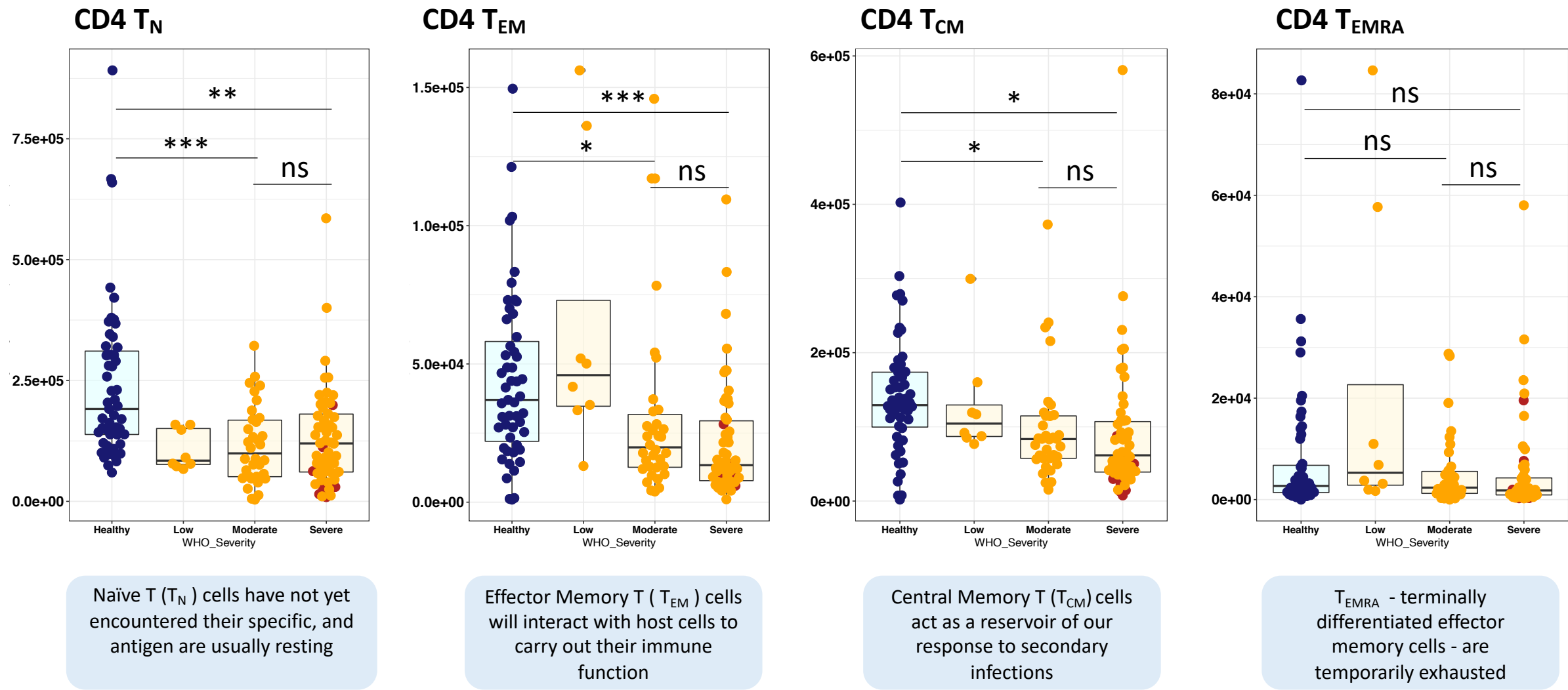


Covid-19: T cell lymphopenia is selective: T helper (Th) cell subsets are differentially affected.



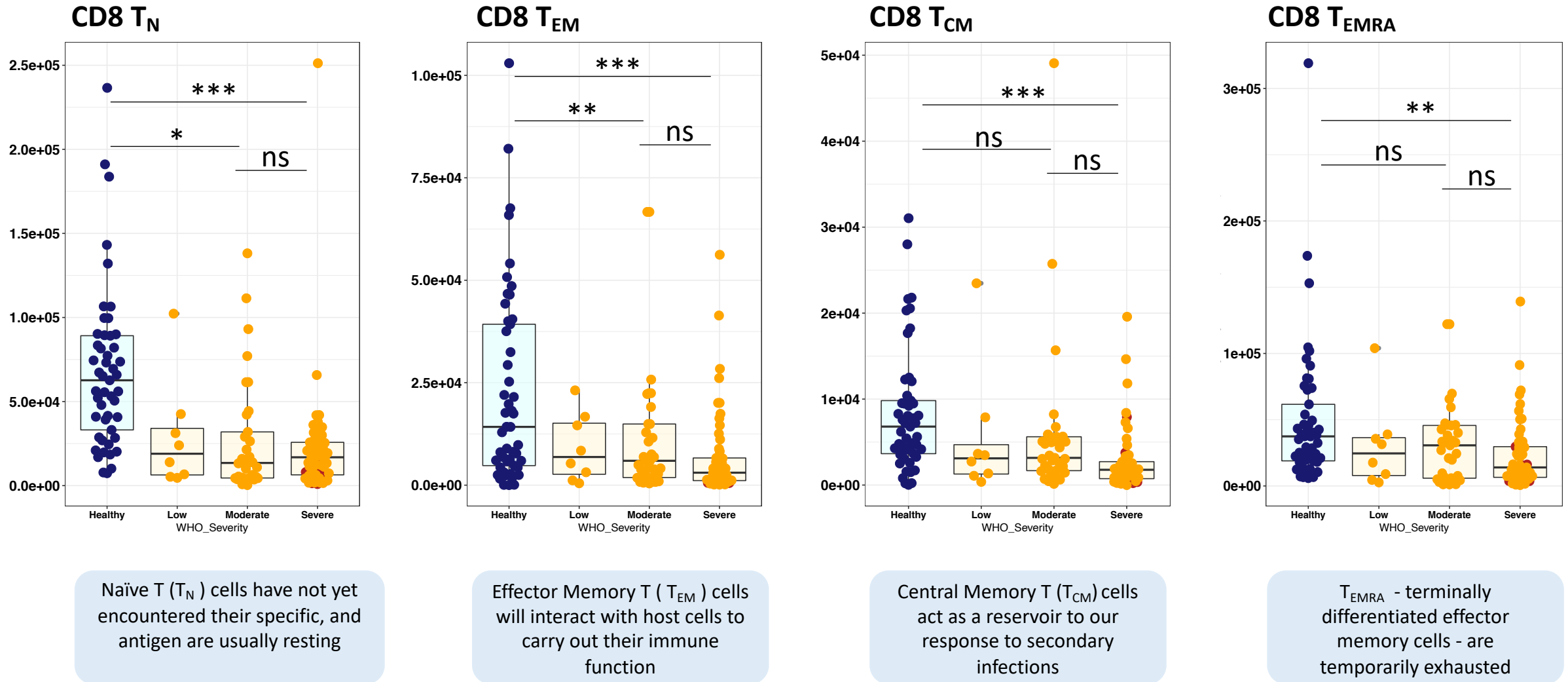
The impact on CD4+ Helper T cells is selective
Th1, Th17.1 cells are diminished, while Th2 and Th17.1 are relatively unaffected

Covid-19: Other CD4⁺T cell subsets are also differentially affected



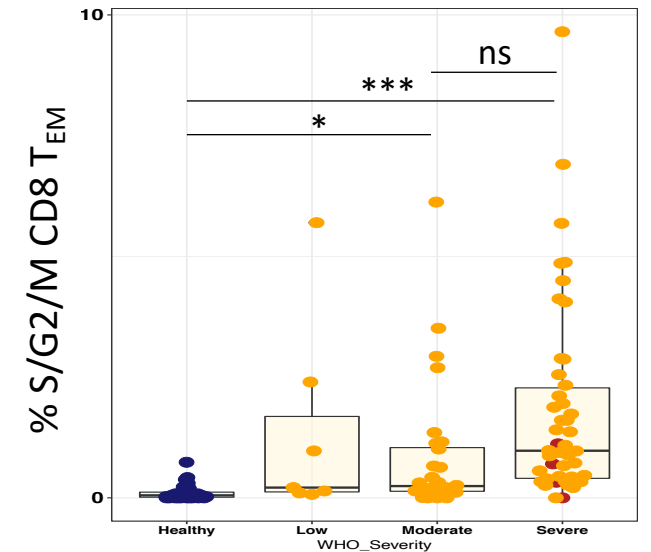
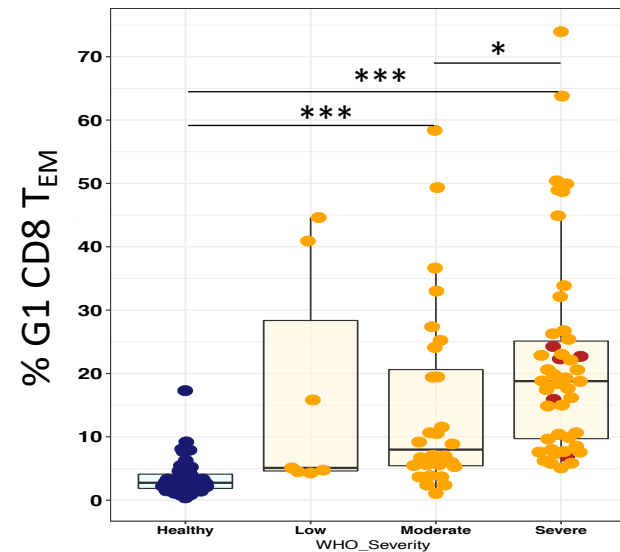
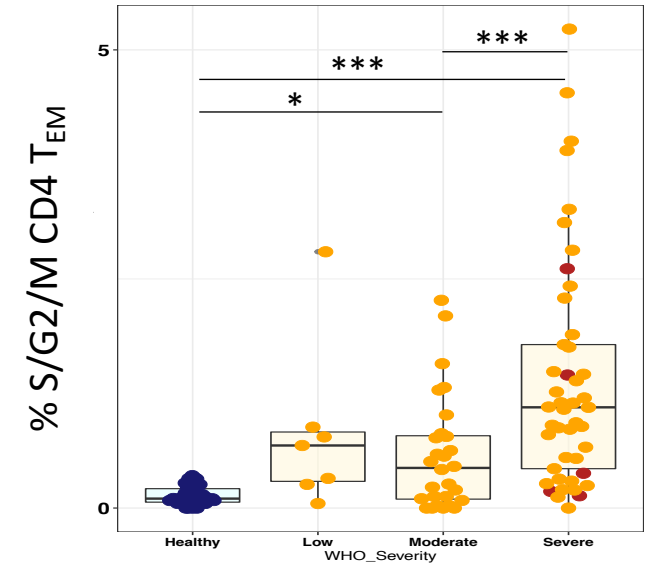
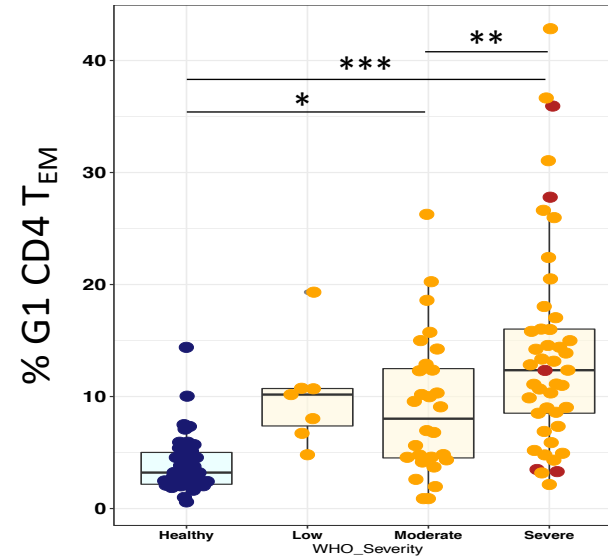
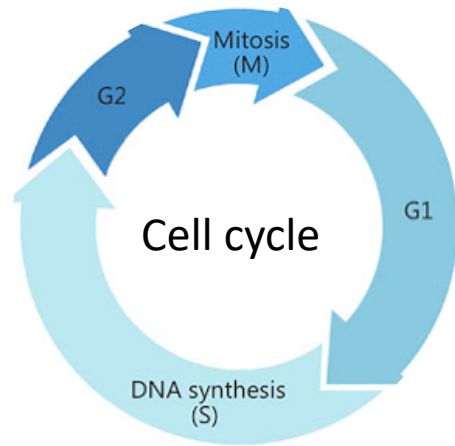
Reduced levels of CD4_{naive} and CD4_{EM} in Covid-19, while CD4_{CM}, and CD4_{EMRA} cells are largely protected

Covid-19 : The impact on CD8⁺ T cells is also selective

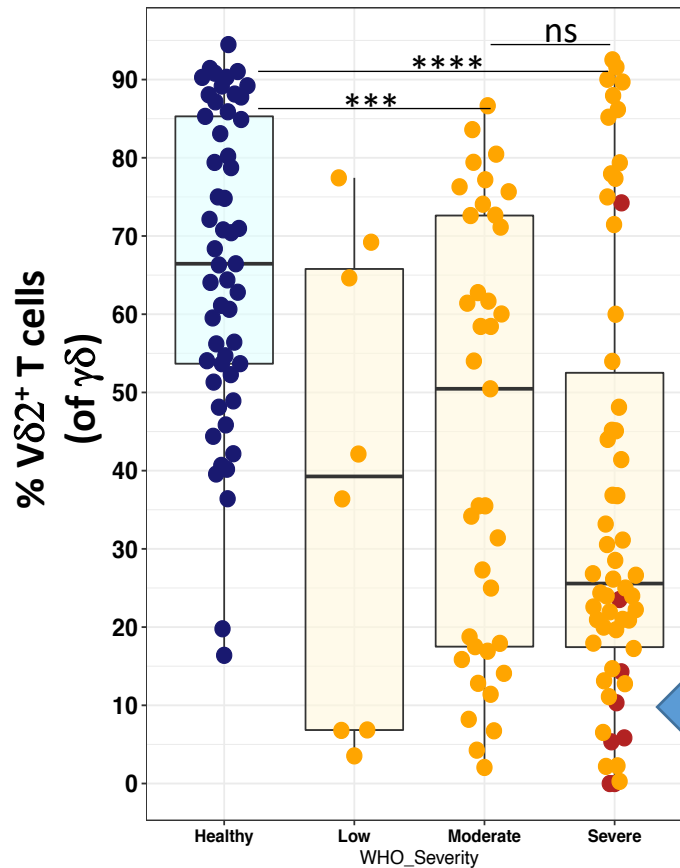
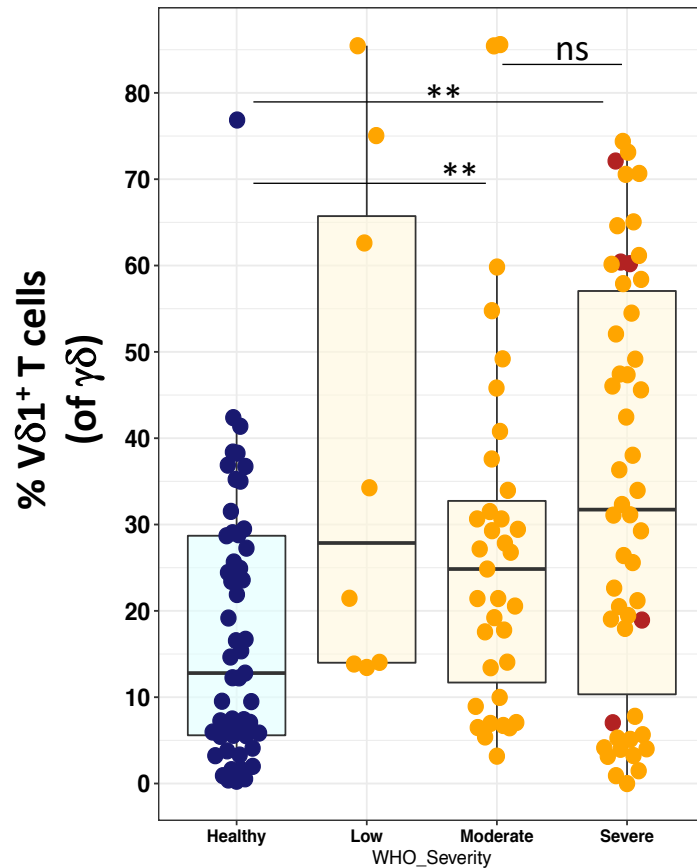


As for CD4 cells, CD8_{naive} and CD8_{EM} cells are reduced in Covid-19; but no clear link to severity

Covid-19: remaining T cells, although low in numbers, are actively traversing the cell cycle and this is linked to severity



Covid-19: a collapse of $V\delta 2$ T cells is a disease signature, that is particularly striking in some patients

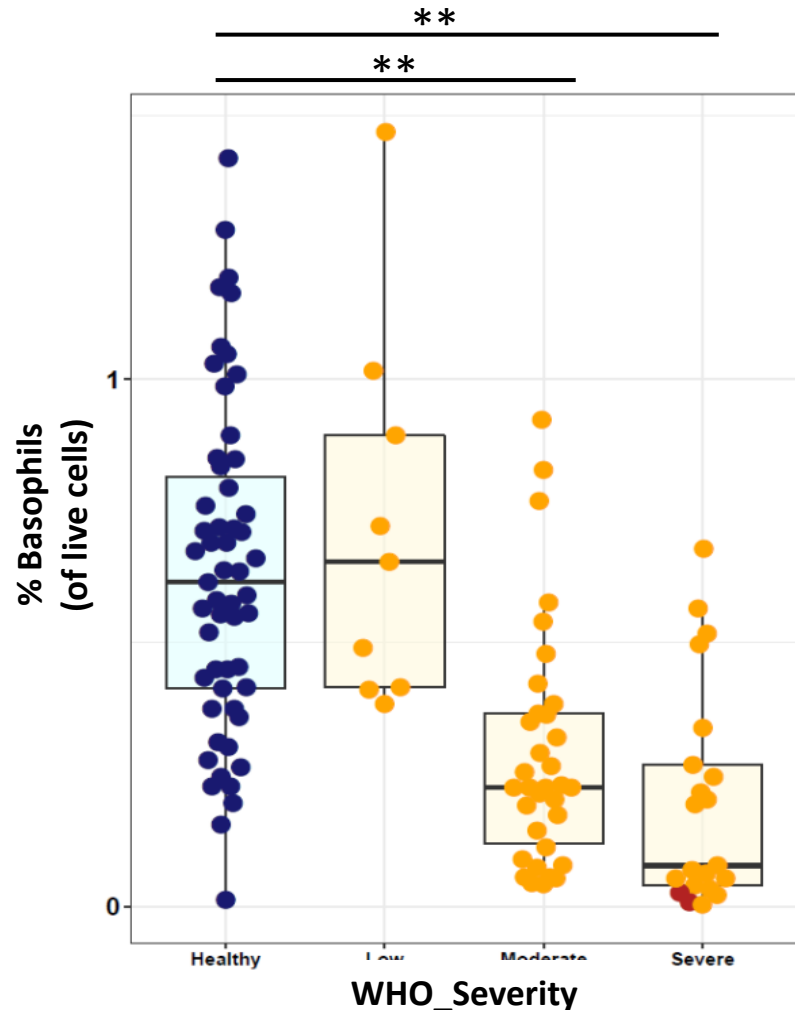


$\gamma\delta$ TCR⁺ T cells express a $\gamma\delta$ -TCR rather than the $\alpha\beta$ -TCR on the cell surface, and are a predominant lymphoid population in body surfaces such as the lung which is targeted by SARS-Cov-2.

There is a collapse in numbers of the predominant blood $\gamma\delta$ cells type ($V\delta 2^+$). Conversely, $V\delta 1^+$ T cells show increased representation and their active cycling is severity associated (*not shown*).

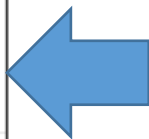
Innate immunity markers

Loss of basophils from the blood appears as a biomarker of COVID-19 severity

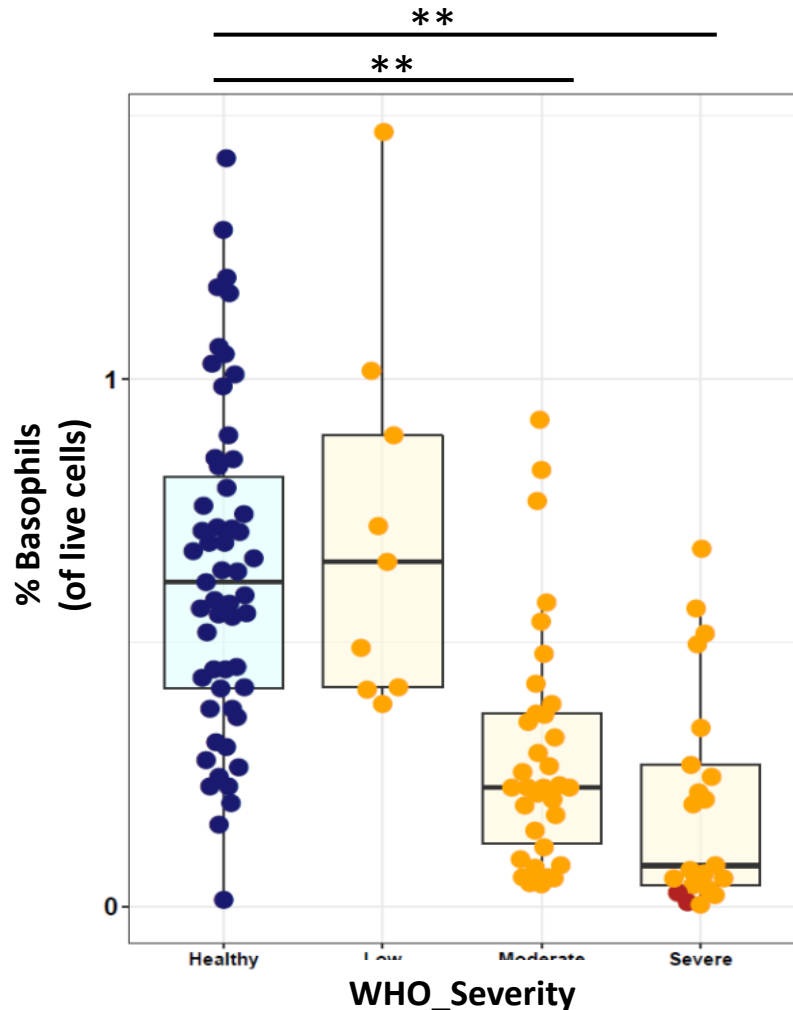


Basophils assessed via flow cytometry [gated as non-PMN based on ssc and then CD3neg, CD19neg,HLADRneg, CD14neg, CD123⁺]

Basophils are potentially disease-relevant because of their homing to the lung, wherein Covid-19 disease is manifest, and because of their implication in regulating blood coagulation



In sum, several biomarkers of COVID-19 severity are emerging



Basophils via flow cytometry

- Cell cycling of $CD4_{EM}$, $CD4_{CM}$, $CD8_{EM}$, $V\delta 1$ cells
- Depletion of specific CD4 and CD8 T cell subsets
- Basophil counts crashing
- Neutrophil counts increasing
- Crash in plasmacytoid dendritic cells
- Sustained IL6, IP10, IL10
- CRP

Other major changes, e.g. including plasmablast expansion, major changes in monocyte populations; reductions in $CD5^+$ B cells, change in DC phenotypes, and production of antibodies **compose a disease-associated signature** but are not obviously linked to severity

Take home messages

- Phenotyping → define perturbations in immunity → design a 'panel' of descriptive markers tied to severe disease → prospectively evaluated as a predictive score for severity → clinical endpoint e.g. decision aid for early ICU admission
- Phenotyping → insight into disease pathology → ongoing investigation into what drives T lymphopenia and how this impacts on memory responses → informs Vaccine design
- Data will be freely available on www.immunophenotype.org.
- Applications alongside GSTT clinical trials e.g. phenotyping before and after therapeutic interventions (e.g. IL7, anti-IL6) → mechanism of action

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