EIP
European Immunogenicity Platform

COVID influence on *in vitro* assays

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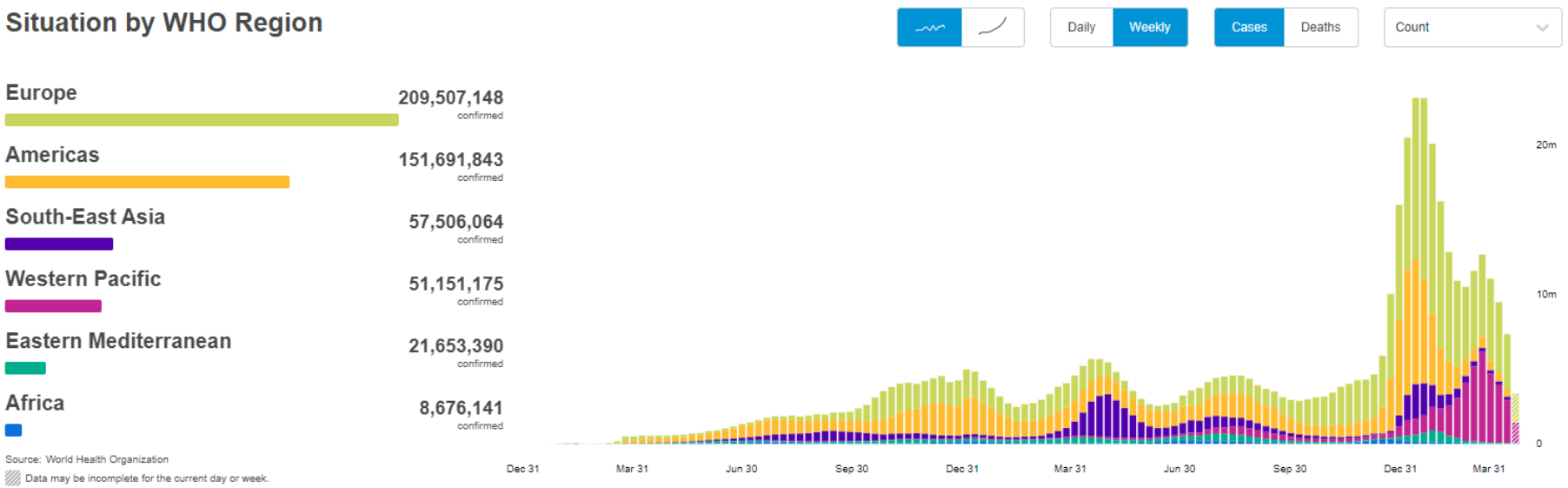
Introduction



Source : https://covid19.who.int/?mapFilter=cases

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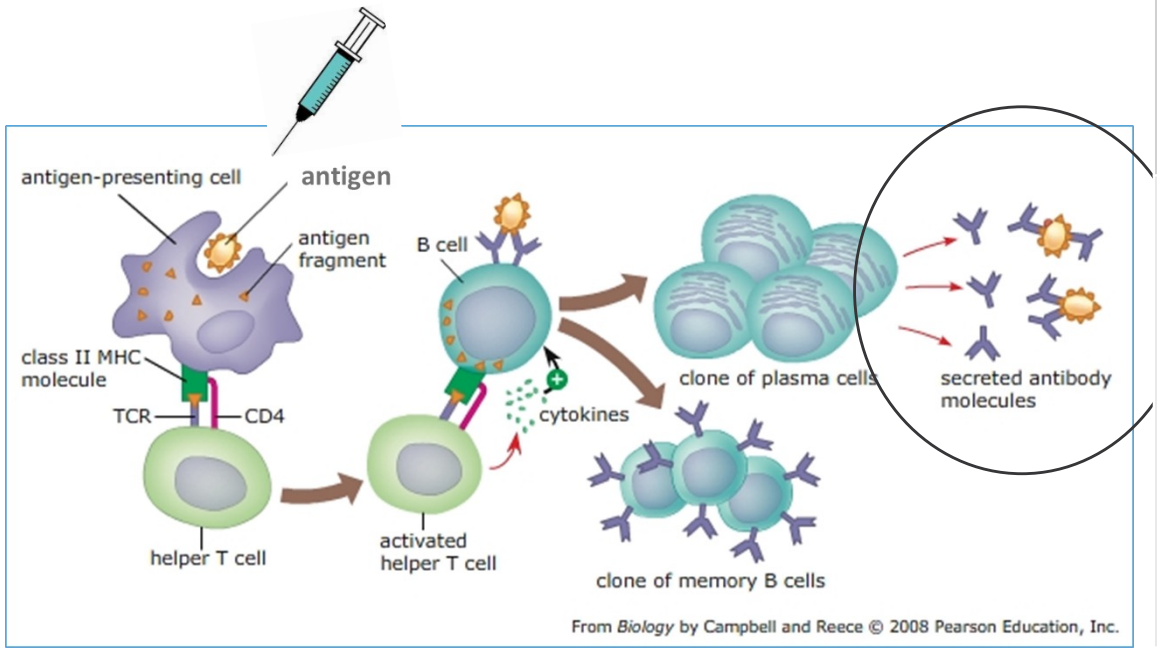
Immunogenicity

"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"



WANTED	UNWANTED	
Vaccines	**Therapeutic protein**	**Stem Cells and Gene Therapy products**
Immune response against the pathogen (virus, bacteria) aiming at protecting the organism	Production of antidrug- antibodies (ADAs) possibly neutralising the therapeutic effects of the treatment and, in rare cases, inducing adverse effects	Cellular and humoral responses Anti HLA antibodies Immune rejections Potential safety effect

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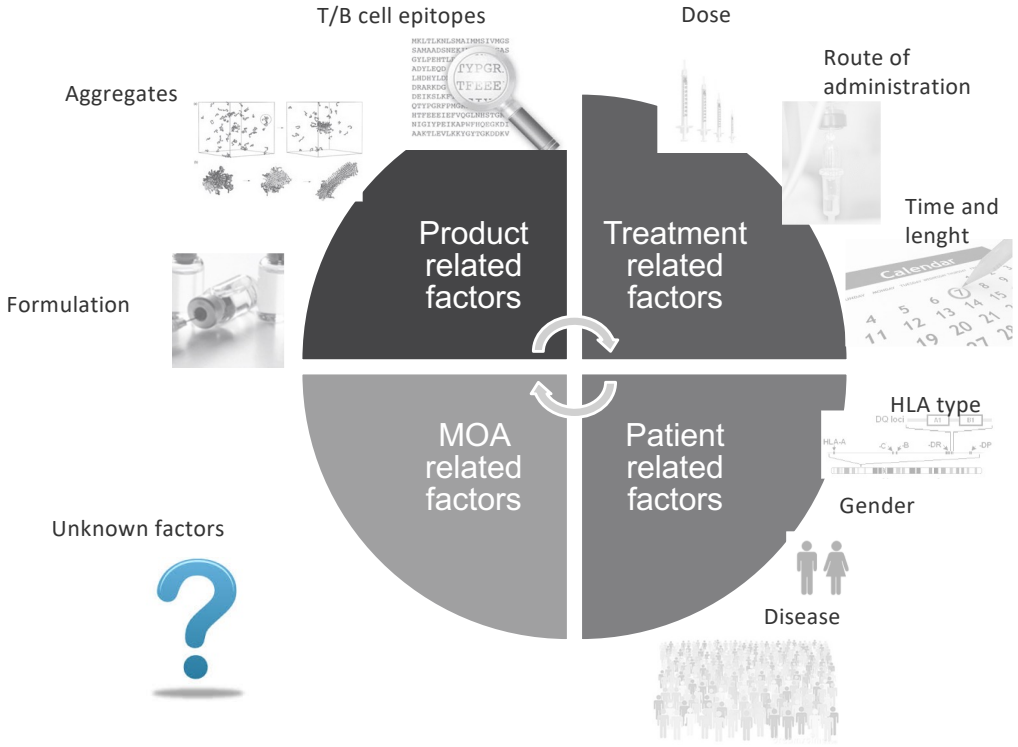
Factors impacting Immunogenicity



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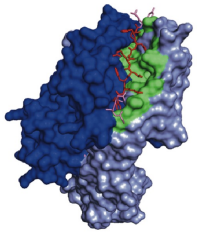
Early Immunogenicity Risk Assessment Tools



In silico Assays

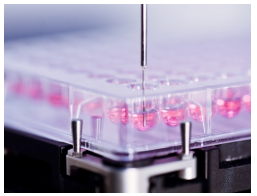
1. Computer based assessment of T cell epitopes
   De-immunization

In vitro early/Innate Assays

2. Monocyte Activation Assay
3. DC Activation/Maturation Assay
4. Reporter cell line Assay
5. Cytokine Release Assay

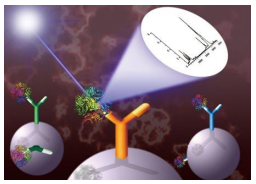
In vitro T cell Assays

6. CD8-depleted PBMC Assay
7. DC-T cell Assay
8. Peptide Assay



MAPPS Assay

9. Identification processed and presented epitopes



In vitro Assays using Primary Cells

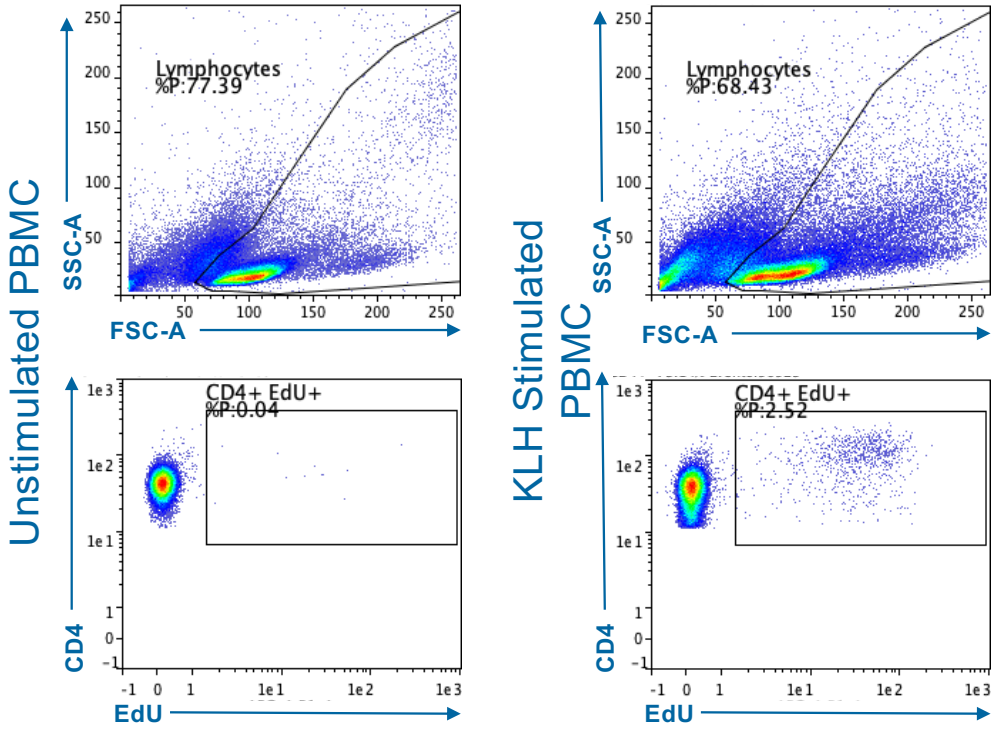
Quality of the primary cells:

- Variability and reproducibility of the results highly depends on the initial quality
- Quality = viability and functionality
- Most critical reagent
- Standardized procedures for sampling, shipping, isolation, cryopreservation, thawing, handling, …
- Need for a large number of HLA-typed donors in order to represent the wide range of responders (strong-responders versus medium-low responders)
- Plus 900 healthy donor samples (4-digit HLA typed)

Functionality Assessment

- Assessment of proliferative response towards polyclonal stimulation (anti-CD3 antibody)

- Assessment of proliferative response towards naïve antigen Keyhole Limpet Hemocyanin (KLH)



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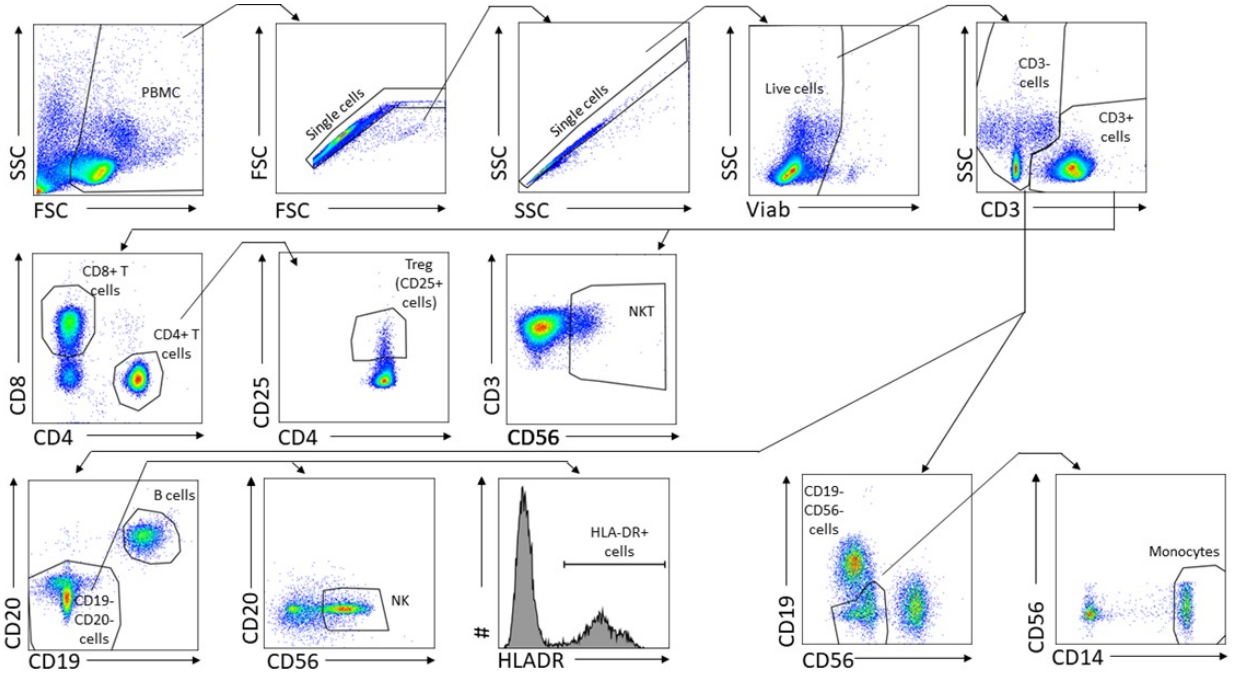
Subpopulation Analysis

Classic Surface Marker staining:

- CD14: Monocytes
- CD3: T cells
- CD4: Helper T cells
- CD8: Cytotoxic T cells

Extended:

- CD14: Monocytes
- CD3: T cells
- CD4: Helper T cells
  - PD-1^{+}
  - CD25^{+}
- CD8: Cytotoxic T cells
- CD56: NK and NKT
- CD19/20: B cells



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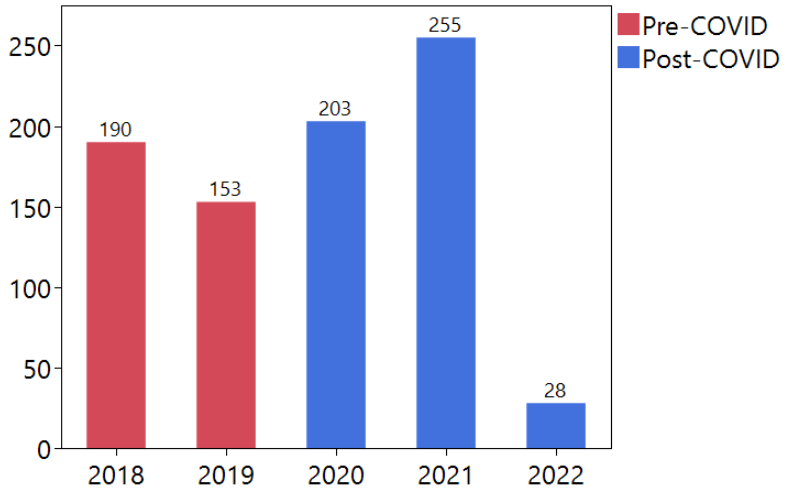
Introduction

Research question : based on observations in QC PBMC -> Is there a long-lasting difference in PBMC quality and quantity after COVID infection or vaccination or any health measure taken related to it?

Methodology

- Analysis of QC results of:
  - 828 PBMC preparations
  - 408 unique donors
  - From 03/01/2018 to 08/03/2022



- Evaluation of time trends: are there differences in outcomes after start of COVID that were stable in pre-COVID years?

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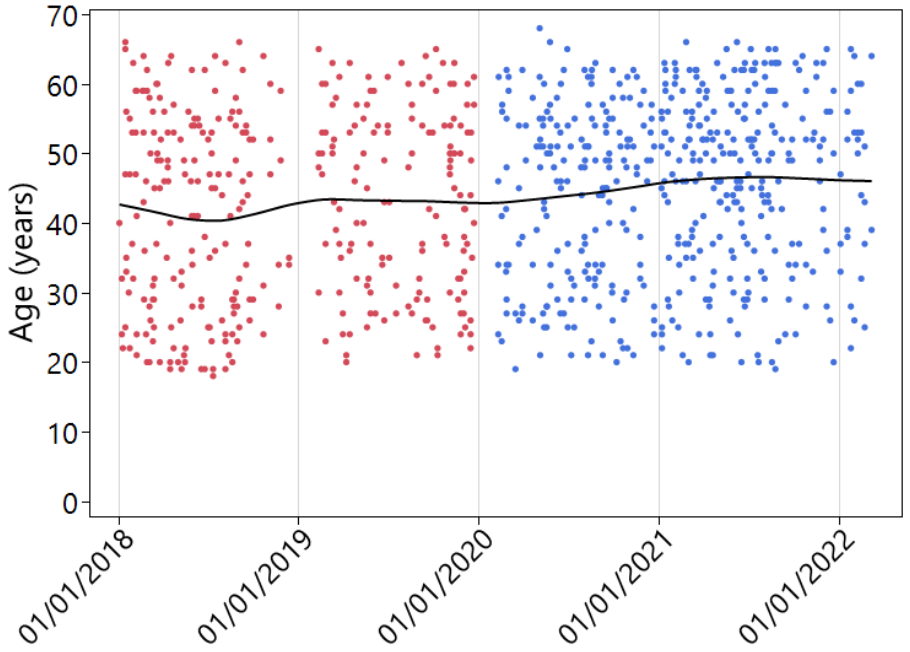
Demographics

- Age: 18-68 (median 46), stable across time period
- Gender: 74% female, 26% male
- 97% Caucasian



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Findings

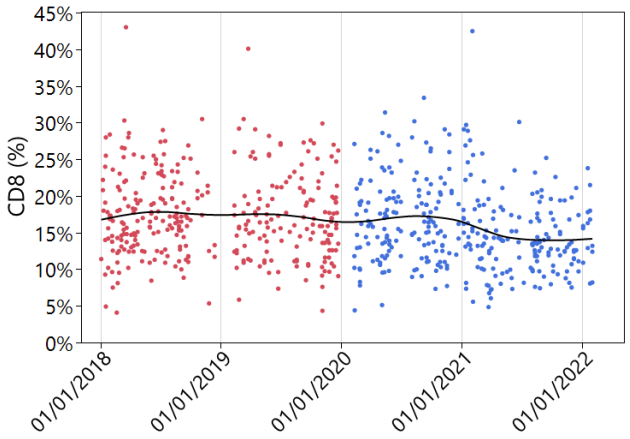
None of the outcomes show differences depending on COVID vaccination status and COVID Ab status in the tracker file

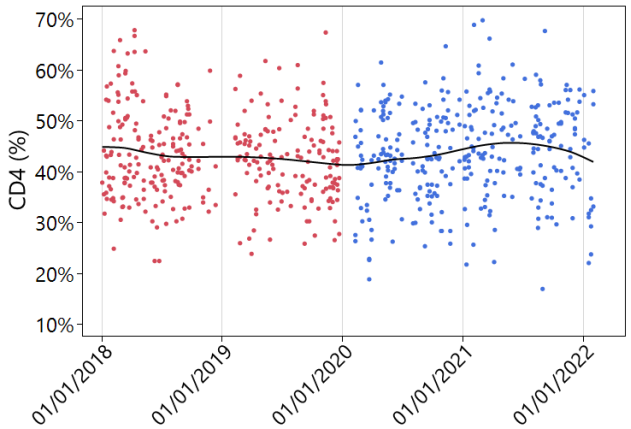
- Because these data are difficult to interpret (reliability?, for COVID vaccination no information on time after vaccination, negative for COVID Ab doesn't necessarily means no prior infection with COVID,….), data are analyzed in function of time (pre/post COVID)

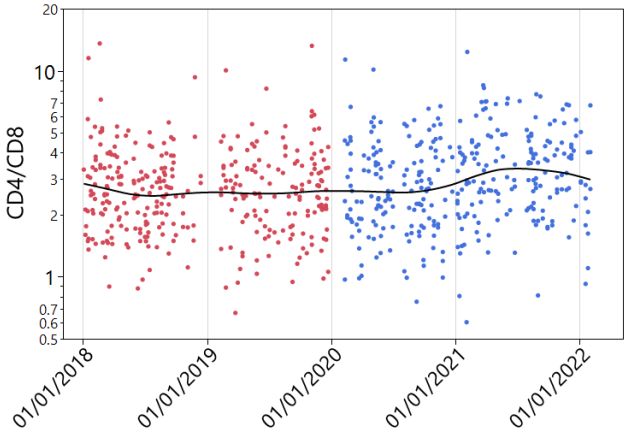
  ✓To support that changes in function of time could be related to COVID, also the data of 2 preceding years is shown, to see what is the normal year-to-year variation and if the change in COVID years is more than normal year-to-year variation

Findings CD4+ and CD8+ cells

% of CD8+ cells is relatively stable over the years, with a clear drop at the start of 2021. % CD4+ cells does not show this effect, but instead a slight trend to increase in the same time period. The ratio of CD4/CD8 thus shows a clear shift starting from the beginning of 2021.







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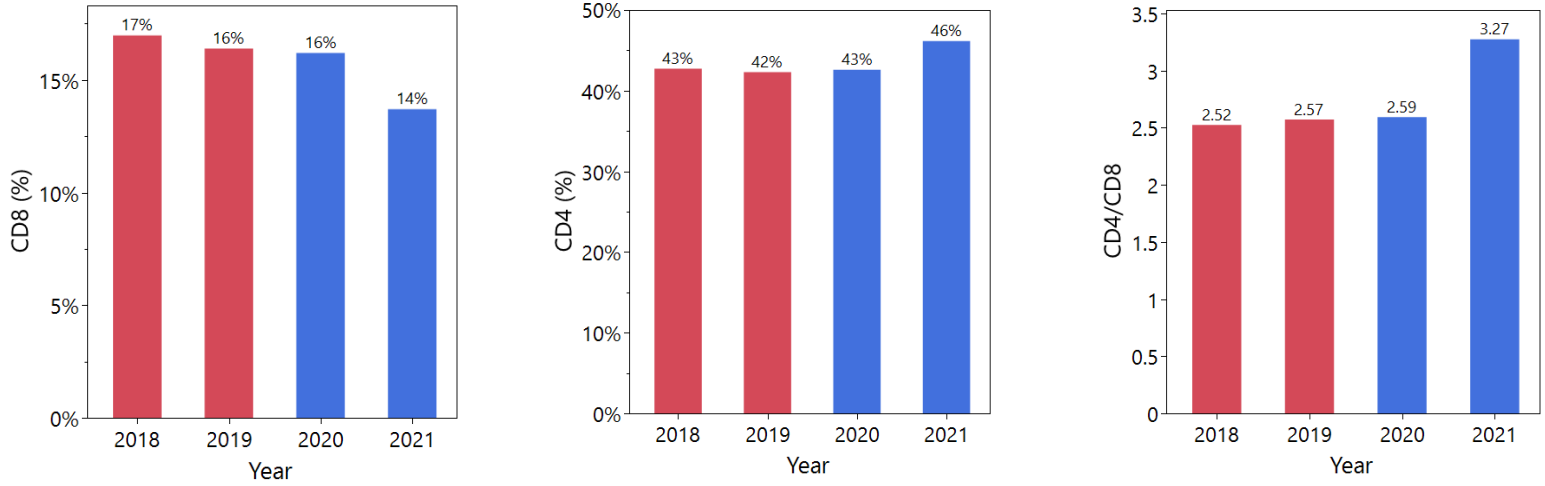
Findings CD4+ and CD8+ cells



Median % over the years, showing the stability of % CD4+ cells, CD8+ cells and the ratio of CD4/CD8 over the years 2018-2020, with a clear shift in 2021.

Wilcoxon test comparing each year to each other shows a highly significant difference ($p<0.0001$) for 2021 versus each preceding year, whereas no significant differences between any of the other years is detected.

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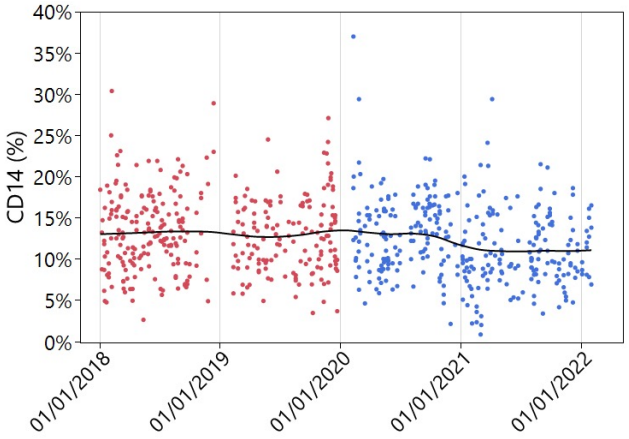
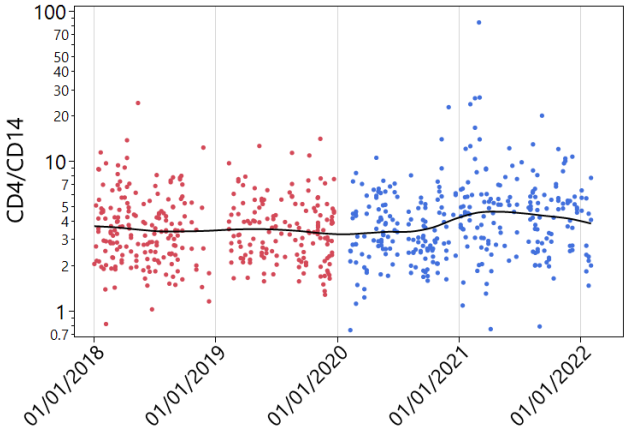
Findings CD14+ and CD4+ cells

% of CD14+ cells is relatively stable over the years, with a clear drop at the start of 2021. % CD4+ cells does not show this effect, but instead a slight trend to increase in the same time period. The ratio of CD4/CD14 thus shows a clear shift starting from the beginning of 2021.





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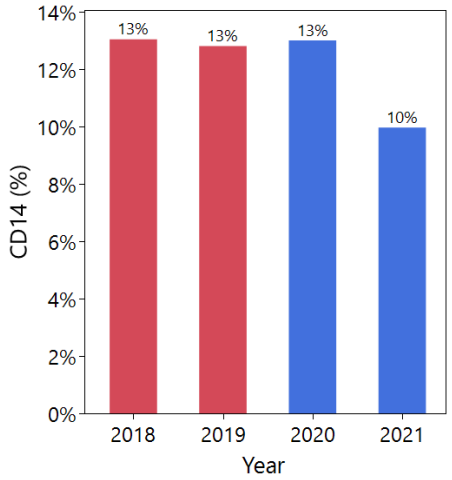
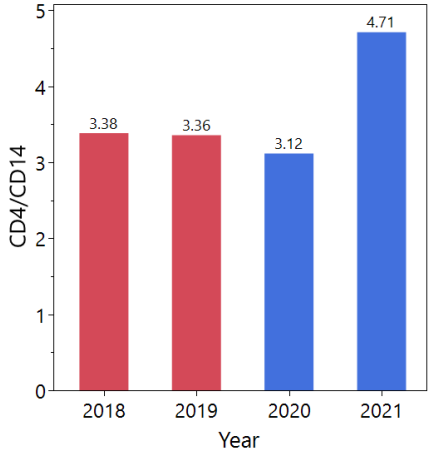
Findings CD14+ and CD4+ cells

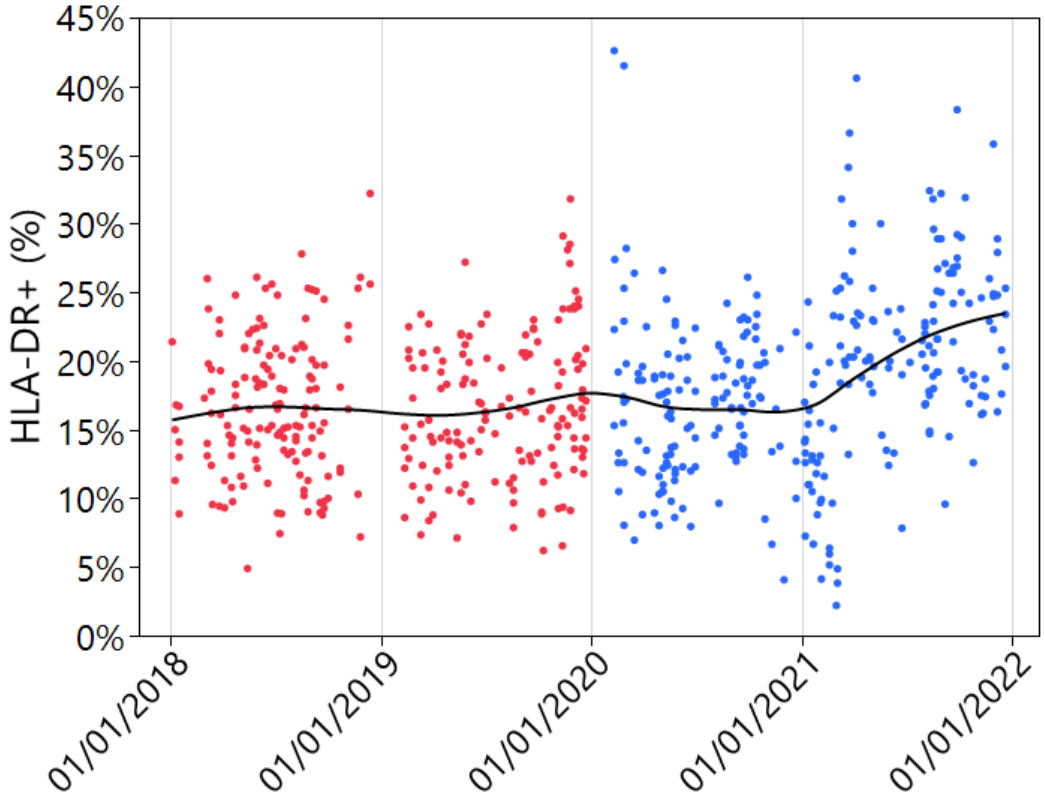




Median % over the years, showing the stability of % CD14+ cells and the ratio of CD4/CD14 over the years 2018-2020, with a clear shift in 2021.

Wilcoxon test comparing each year to each other shows a highly significant difference ($p<0.0001$) for 2021 versus each preceding year, whereas no significant differences between any of the other years is detected.

Findings HLA-DR^{+} cells



HLA-DR+ cells showing increased % starting beginning of 2021

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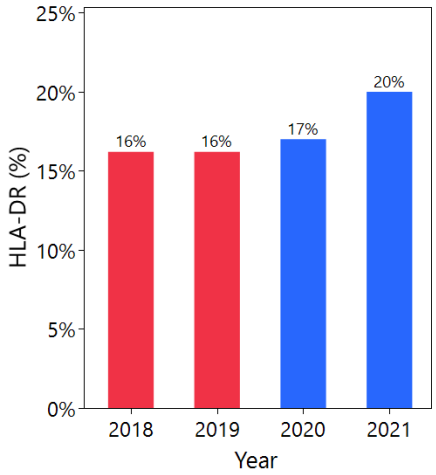
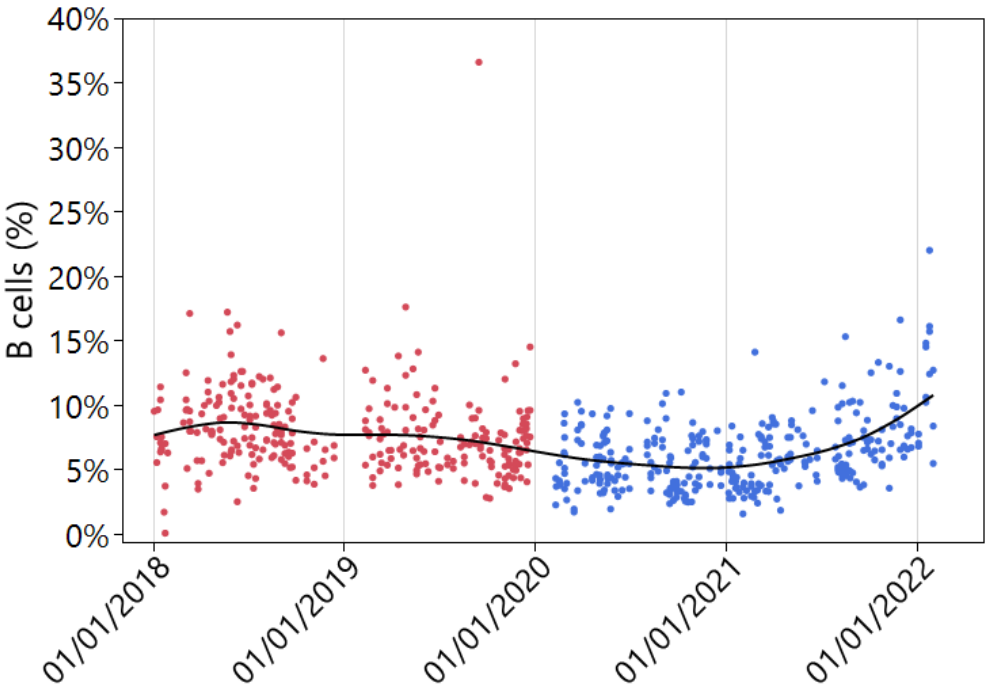
Findings HLA-DR+ cells



Median % over the years, showing the stability of % HLA-DR+ cells over the years 2018-2020, with a clear shift in 2021.

Wilcoxon test comparing each year to each other shows a highly significant difference ($p<0.0001$) for 2021 versus each preceding year, whereas no significant differences between any of the other years is detected.

Findings B cells



B cells do not show such consistent measurements over time, with only from the second half of 2021 a clear trend that is more than the normal time variation.

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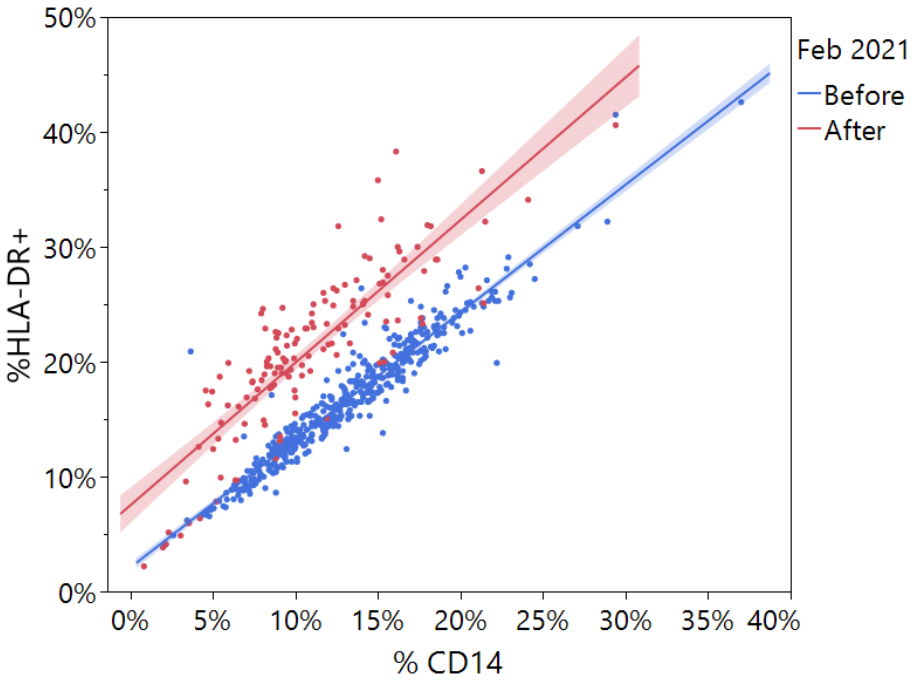
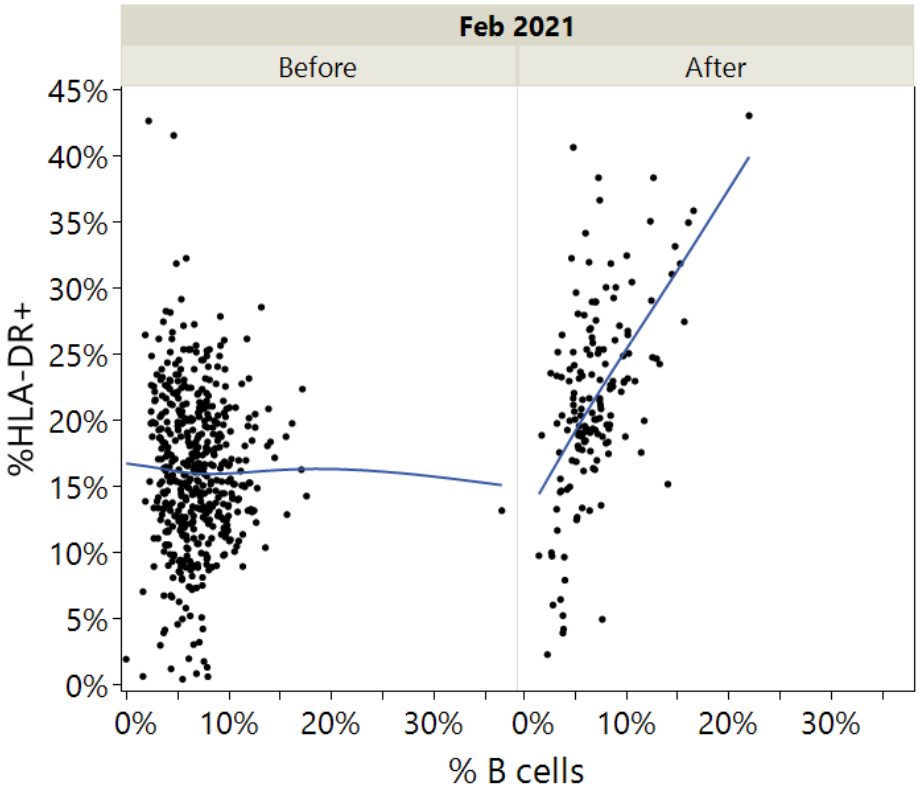
HLA-DR expression



Two subpopulations can be identified based on the correlation between HLA-DR and CD14. These subpopulations are almost perfectly separated in time (Before and After February 2021).

Before Feb 2021, HLA-DR is almost exclusively correlated with CD14 (very little spread of individual points from the regression line of HLA-DR vs CD14, donors with the same %CD14 have almost the same % HLA-DR+). After 2021, for the same amount of CD14, there is more HLA-DR expression and although there is still a clear correlation with CD14, HLA-DR expression is not explained to the same extent by CD14 (more spread of individual points from the regression line of HLA-DR vs CD14, donors with the same %CD14 can have more variation in %HLA-DR).

HLA-DR expression



Even though also after Feb 2021, %HLA-DR+ is most closely correlated with %CD14, already on the raw data it is evident that there is a positive correlation with B cells as well, that is not present before.

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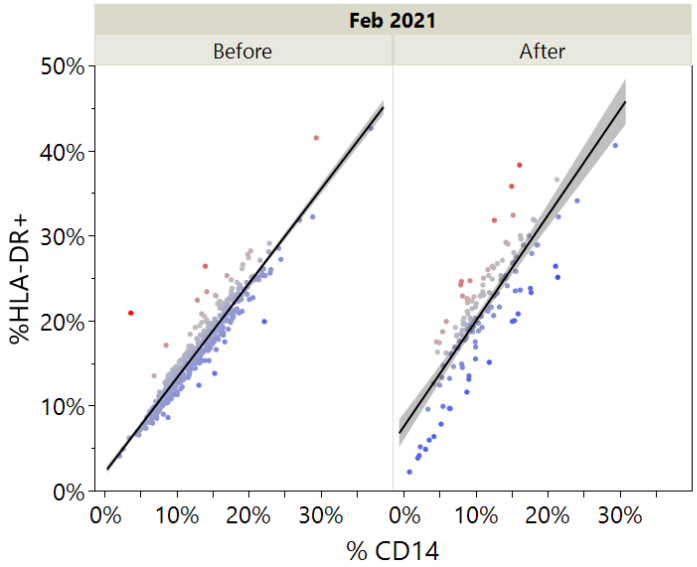
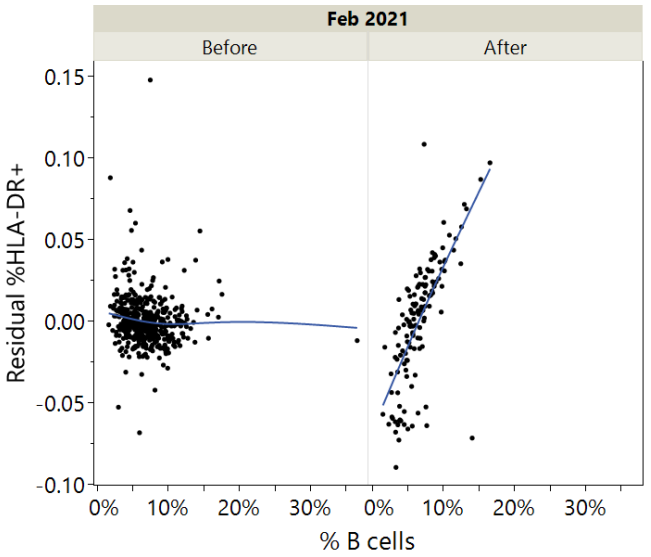
HLA-DR expression





Graphical representation of model of linear regression with HLA-DR as outcome and CD14 and B cells as predictors. Residual HLA-DR expression (color code left, y-axis right) represents the variation in HLA-DR that is not explained by CD14: higher residual HLA-DR means more HLA-DR than average per CD14 (above the regression line of HLA-DR vs CD14). Before Feb 2021 this variation is not associated with % B cells, whereas after 2021 there is a strong correlation with % B cells.

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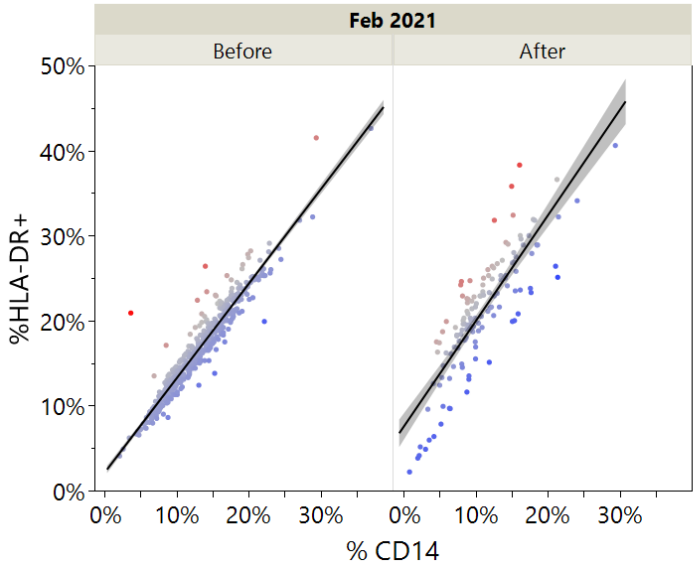
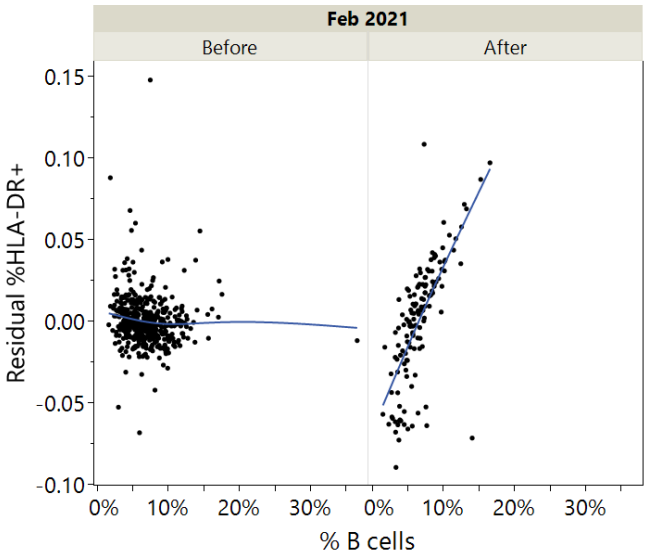
HLA-DR expression





This indicates that donors that have more %HLA-DR+ cells for the same % of CD14+ cells, are the donors that also have more B cells. B cells were excluded in the gating of HLA-DR+ cells. The higher HLA-DR+ cells are thus not B cells, but a higher % of B cells is associated with a higher % of HLA-DR+ non-B cells. This could indicate a biological phenomenon that increases both the % of B cells and HLA-DR on non-B cells.

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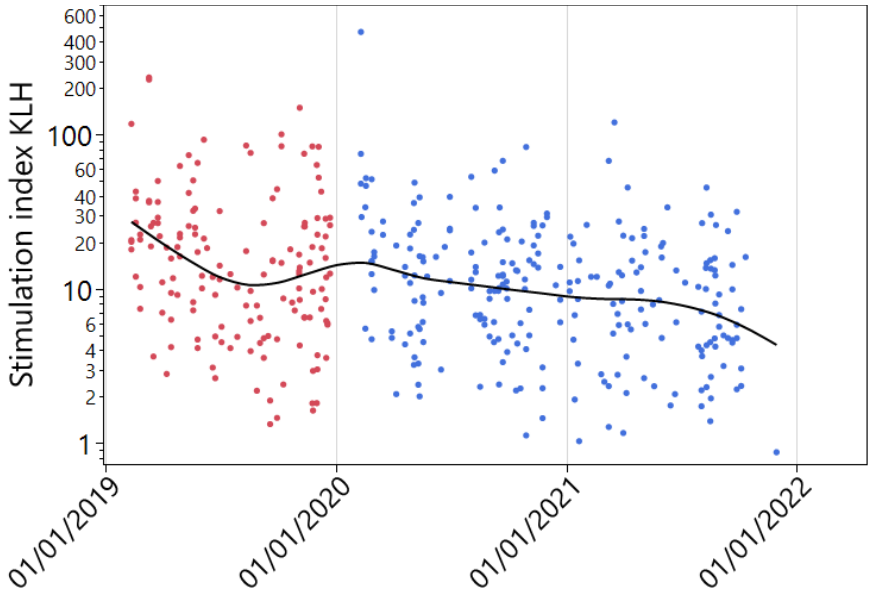
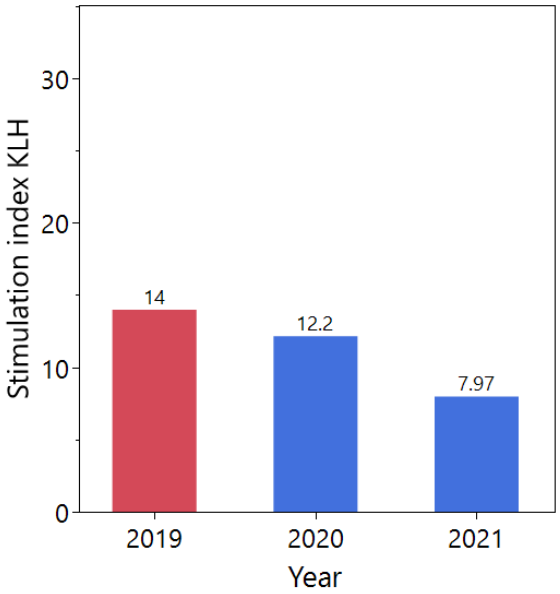
SI : KLH





In COVID years there is a lower response to KLH than previous years, but there seems to be already a similar trend in function of time.

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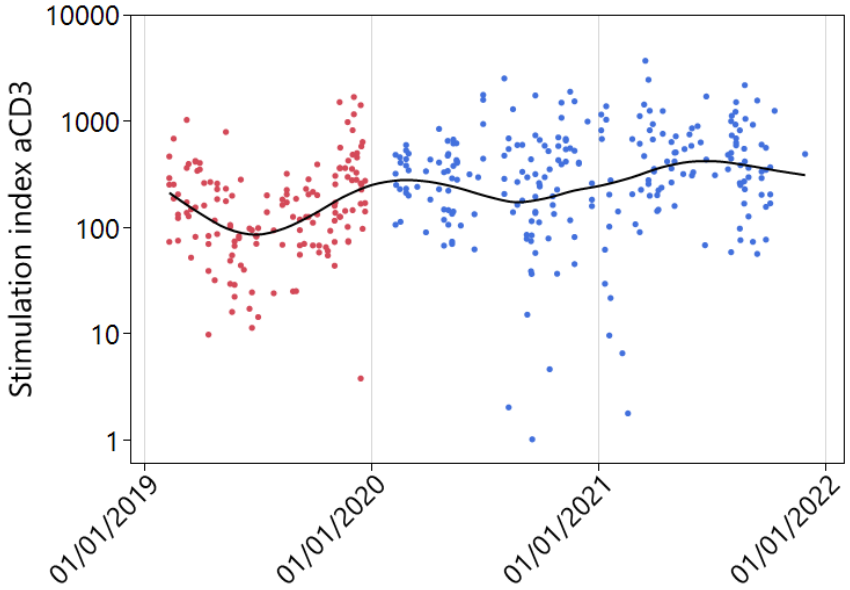
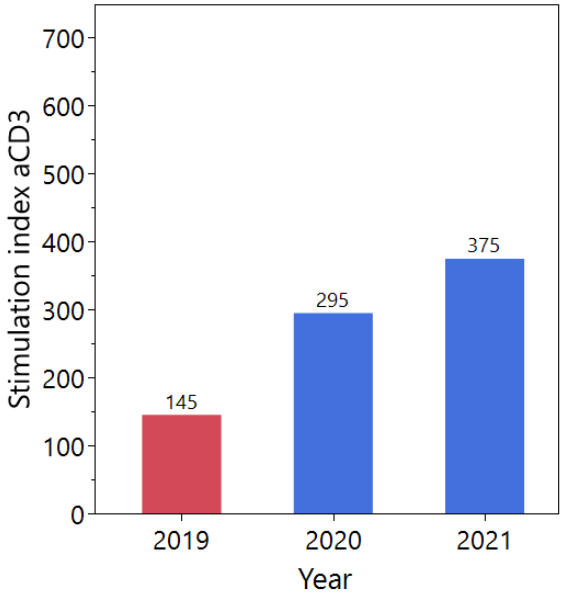
SI : anti-CD3





In COVID years there is a higher response to aCD3 than the previous year.

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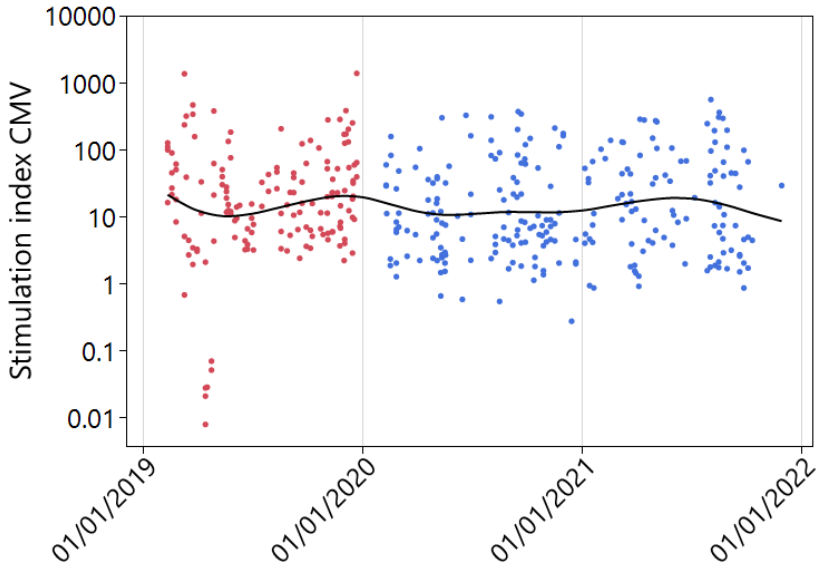
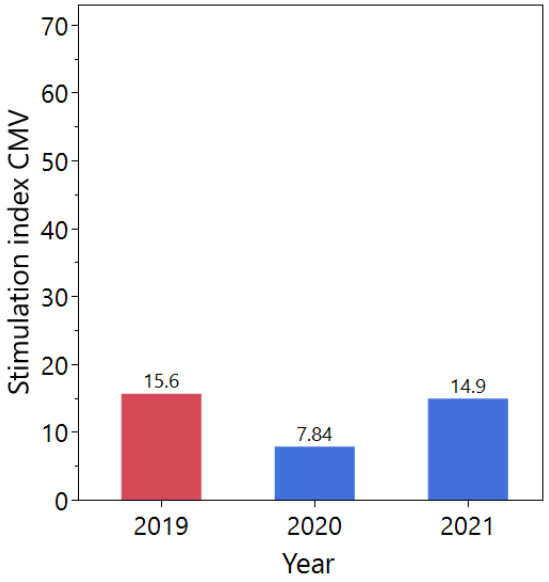
SI : CMV





Response to CMV is relatively stable with no differences between post-COVID and pre-COVID years that are greater than differences between years before COVID.

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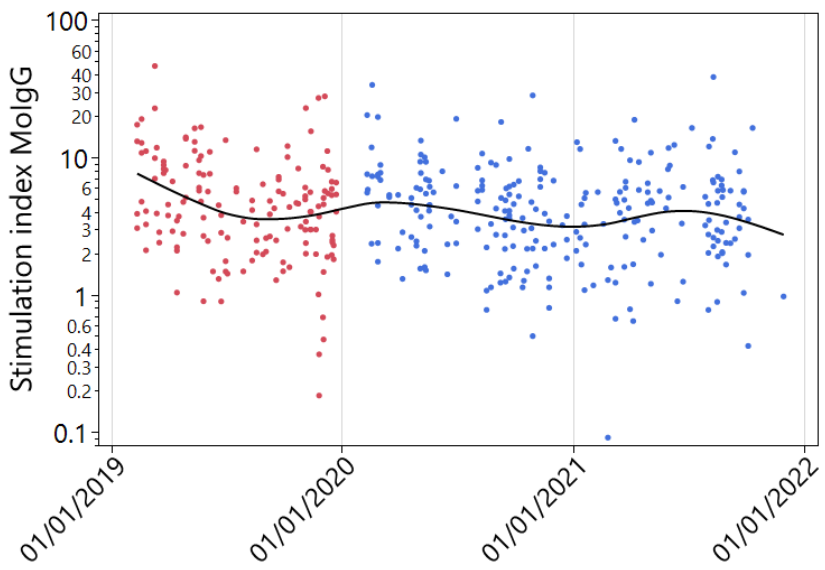
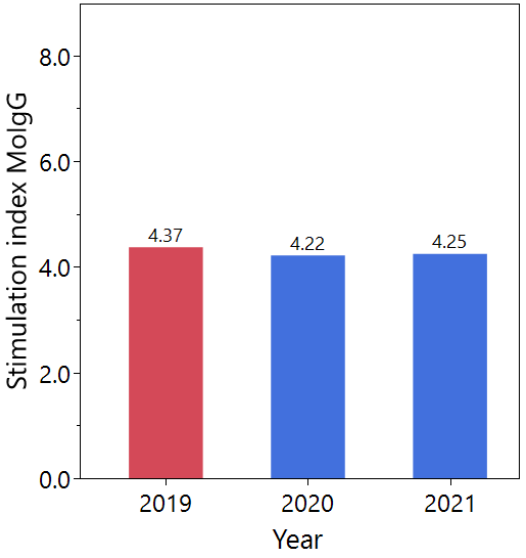
SI : mouse IgG





Response to mouse IgG is relatively stable between post-COVID and pre-COVID years.

Conclusions

There are differences in the proportion of CD4/CD8 cells and CD4/CD14 cells in 2021 versus all previous years, whereas before 2021, these ratios were stable across the years.

- ✓ This indicates different immune cell composition in PBMC from donors collected in 2021 versus the previous years.
  - ➢ When using PBMC/whole blood in assay of samples collected after the beginning of 2021 the actual composition of immune cells is different than for PBMC/whole blood of samples collected before 2021 potentially affecting the assay
  - ➢ Unlikely that different immune cell composition is the only difference immunologically, probably also within cell types functional differences potentially affecting the assay, so even when isolating individual subsets an influence in the assay cannot be excluded.

HLA-DR expression is higher in 2021 versus all previous years, and whereas before 2021 this is almost exclusively correlated with CD14 cells, after 2021 HLA-DR expression correlates both with CD14 cells and B cells.

Functional responses to control antigens do not show clear differences between COVID and non-COVID years that are greater than between years before COVID. Only for aCD3, there seems to be an increase after COVID vs before COVID.

Because only time-trends are evident, effects that are associated with COVID years can be related to COVID infection, COVID vaccination or effects indirectly associated with COVID (eg overall reduced infections because of COVID related measures,…)

Transcriptomics analysis

- To further illustrate potential influence of COVID on immune assays performed on healthy donors sampled in time of high COVID incidence, the following publicly available dataset was analyzed:

  - GSE198256: Functional reprogramming of monocytes in acute and convalescent severe COVID-19 patients [RNA-seq].

    ✓11 healthy controls, 13 acute and 20 convalescent (3 months/ 6 months) mild to critical patients

    ✓FACS sorted CD14 cells

    Source : https://www-ncbi-nlm-nih-gov.ezproxy.u-pec.fr/geo/query/acc.cgi?acc=GSE198256

    Brauns *et al.* Functional reprogramming of monocytes in acute and convalescent severe COVID-19 patients. JCI Insight 2022 Apr 5;e154183. doi: 10.1172/jci.insight.154183.

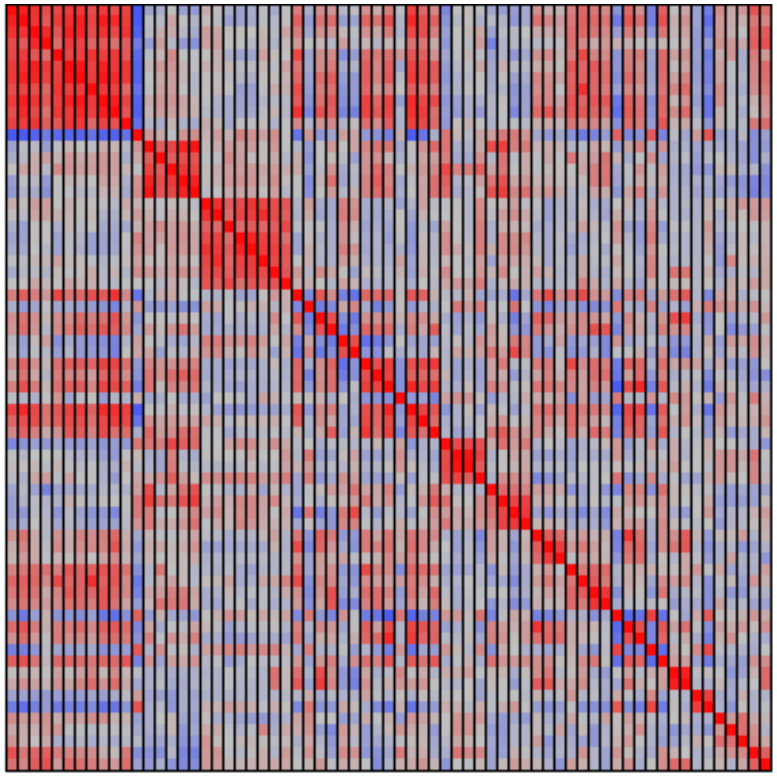
Transcriptomics analysis

- Based on the publicly available normalized counts, it was investigated if genes belonging to Antigen processing and presentation pathway were different in recovered patients versus healthy controls

  - Genes belonging to the pathway were downloaded from the Molecular Signatures Database (UC San Diego and Broad Institute, Subramanian, Tamayo, et al. (2005, PNAS) /KEGG_ANTIGEN_PROCESSING_ AND_PRESENTATION) source : https://www.gsea-msigdb.org/gsea/msigdb/

  - To evaluate an overall effect on antigen processing and presentation, principal component analysis was performed. With principal component analysis, genes that are correlated with each other are represented by a summary variable allowing to evaluate groups of similarly influenced genes instead of individual genes

Transcriptomics analysis

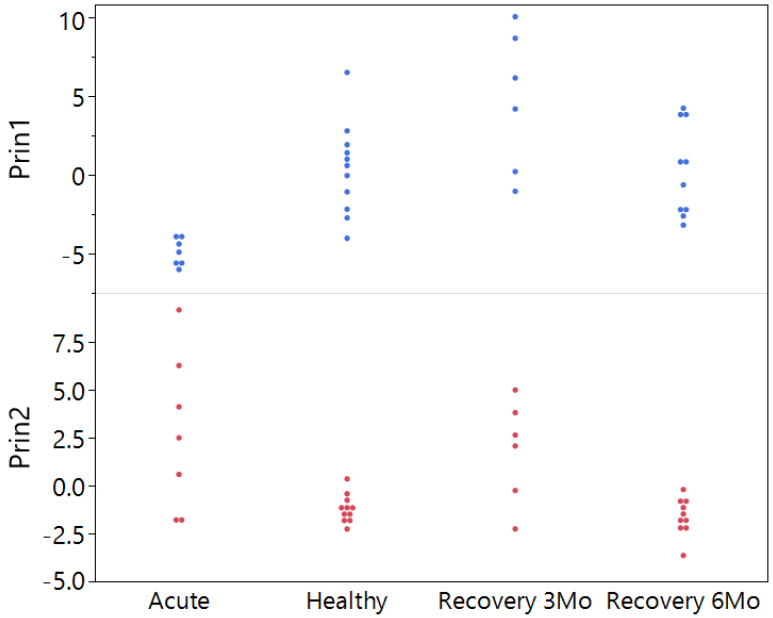


Graph showing the correlation between genes belonging to the Antigen processing and presentation pathway in the dataset under investigation.

Because of the clusters of variables that are correlated with each other, it is possible to summarize the effect of on these correlated genes by summary variables.

Transcriptomics analysis

- The 2 first principal components (summary variables explaining most of the variation present in the data), together with the top 10 genes that contribute to these summary variables are shown below per group of patients



HLA-DRA
HLA-DRB1
CD74
HLA-E
HLA-DPB1
HLA-DPA1
HSPA4
HLA-B
HLA-DQA1
HLA-DOA

Healthy vs recovery 3mo: p=0.08

PDIA3
CALR
HSPA5
PSME2
CIITA
CTSB
HSP90AB1
HLA-DMB
RFX5
HSP90AA1

Healthy vs recovery 3mo: p=0.03

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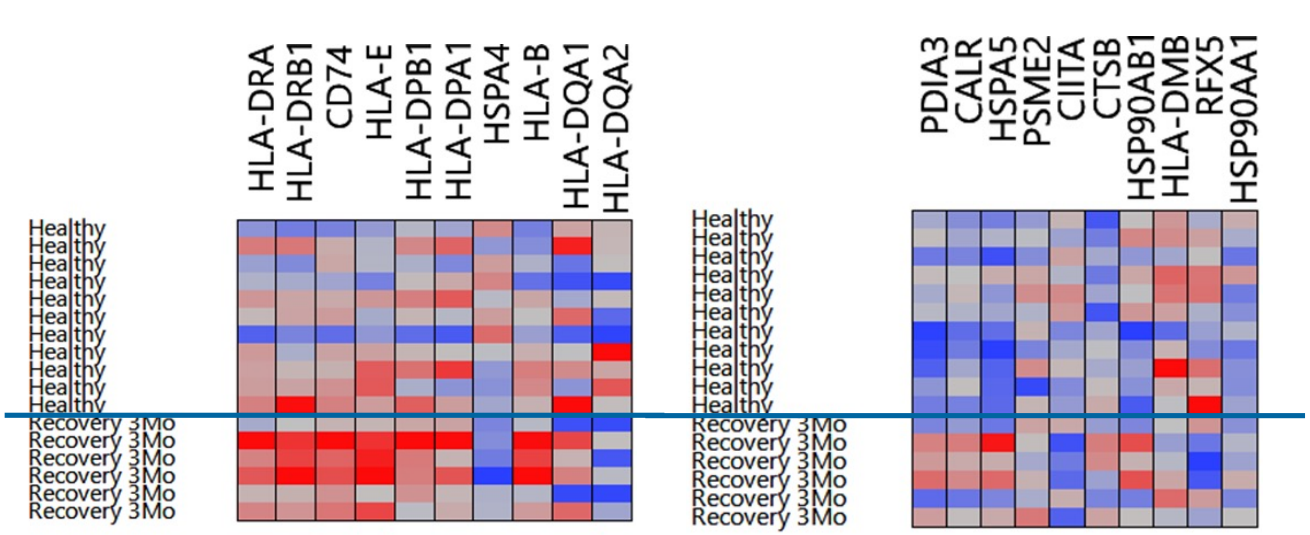
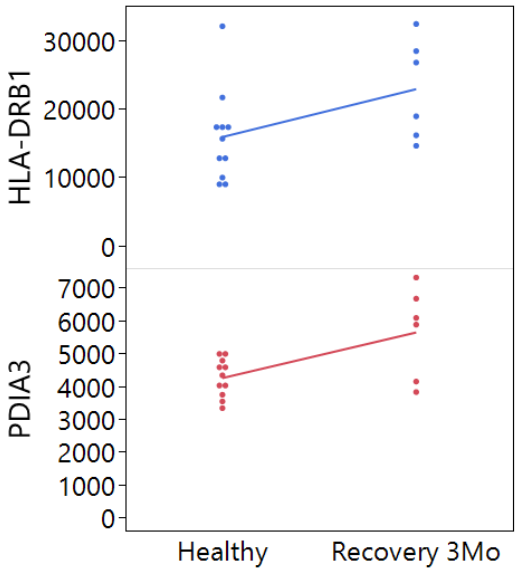
Transcriptomics analysis

- Heatmap of the top 10 genes that contribute to the first 2 principal components for healthy individuals versus recovered patients, and individual data points for one example gene contributing to each of the components





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Transcriptomics analysis

- In a crucial subset of immune cells (CD14+ cells), there is evidence that a crucial pathway (antigen processing and presentation) is altered in patients 3 months after recovery of COVID infections compared to healthy controls.

Conclusion

- From the in house PBMC isolation QC, we see a shift in cell populations starting from February 2021, with an increased % of CD4+ cells, a decreased % of CD8+ cells and CD14+ cells, and an increase in % of HLA-DR+ cells, which shows a strong correlation with % of B cells (which was not observed before that timepoint).
- When assessing the functionality of the PBMCs pre- and post-COVID, there seems to be a trend for a decreased response towards KLH and an increased response towards anti-CD3.
- When looking at RNA-sequencing data from isolated CD14+ cells, there is evidence that a crucial pathway (antigen processing and presentation) is altered in patients 3 months after recovery of COVID infections compared to healthy controls.

Next steps

- From those donors where the COVID status (PCR) is known, compare different donations over time (pre- and post-COVID and at different time points post-COVID) for cell composition and immunogenic reaction.
- Analysis of correlation for those samples for which the vaccination status is known with cell composition and immunogenic reaction.
- Analysis of correlation for those samples which have a strong increase in HLA-DR+ cells with immunogenic outcome.

Acknowledgements

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Your partner in Immunology projects
"We think with You"

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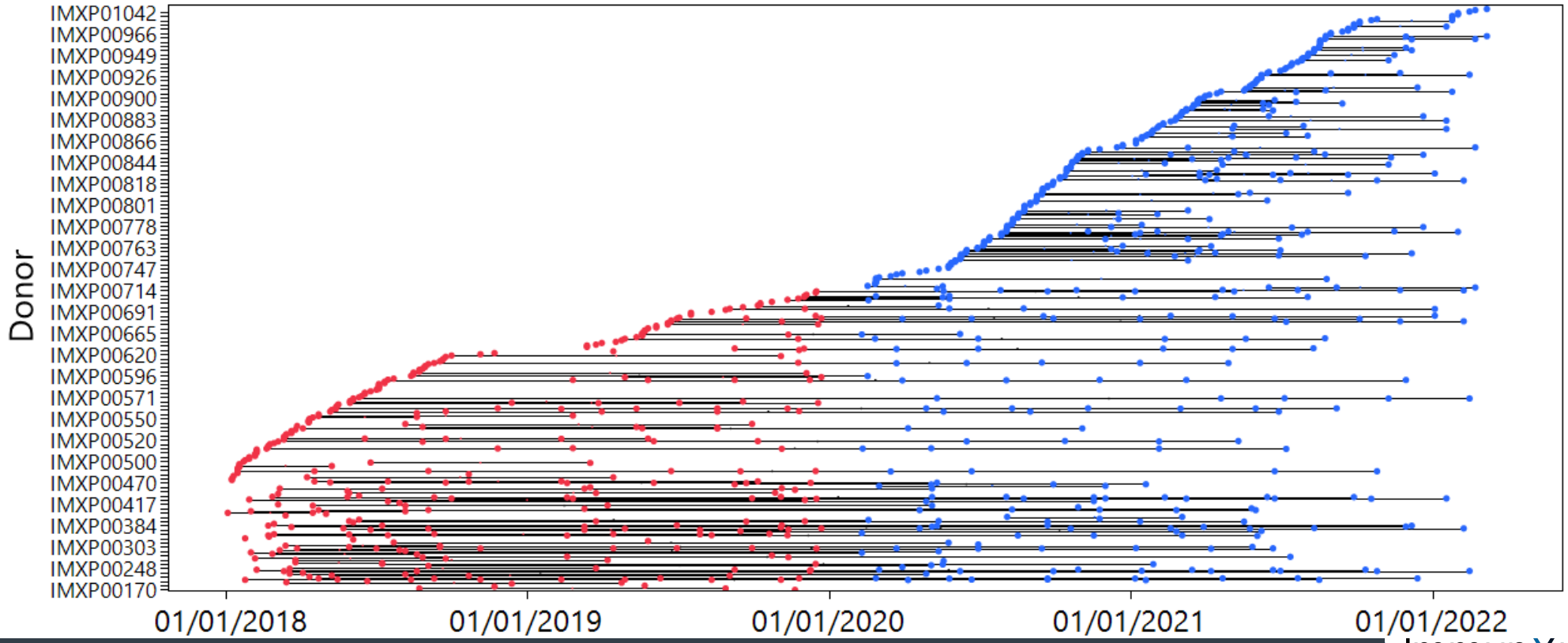
Distribution of repeated samples



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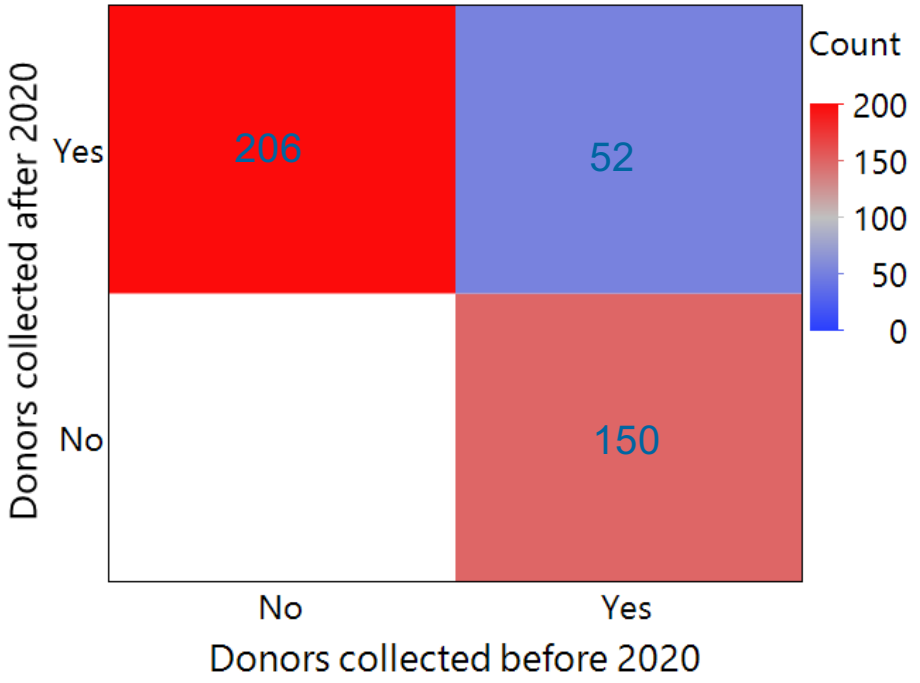
Distribution of repeated samples



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HLA-DR expression

Before Feb 2021

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0243043	0.002947	8.25	<.0001*
% CD14	1.1094082	0.016256	68.24	<.0001*
% B cells	-0.045934	0.026581	-1.73	0.0846

HLA-DR expression is explained by CD14 cells and not B cells (estimate=increase in HLA-DR per increase in B/CD14 cells: with 1% higher increase in CD14% cells on average there is 1.11% higher HLA-DR expression)

After Feb 2021

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.013648	0.007528	1.81	0.0720
% CD14	1.2051944	0.046504	25.92	<.0001*
% B cells	0.9793169	0.082118	11.93	<.0001*

HLA-DR expression is explained by CD14 cells and B cells

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0243043	0.003472	7.00	<.0001*
% CD14	1.1094082	0.019152	57.93	<.0001*
% B cells	-0.045934	0.031316	-1.47	0.1430
Feb 2021[After]	0.0715392	0.00189	37.86	<.0001*
(% CD14-0.1254)*Feb 2021[After]	0.0957862	0.038568	2.48	0.0133*
(% B cells-0.06846)*Feb 2021[After]	1.0252509	0.066896	15.33	<.0001*

Estimates before Feb 2021

Difference in estimates after Feb 2021 vs before

Combined model confirming that the effect of B cells on HLA-DR expression is significantly different before and after Feb 2021. There is also a higher increase in HLA-DR expression per increase in CD14.

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