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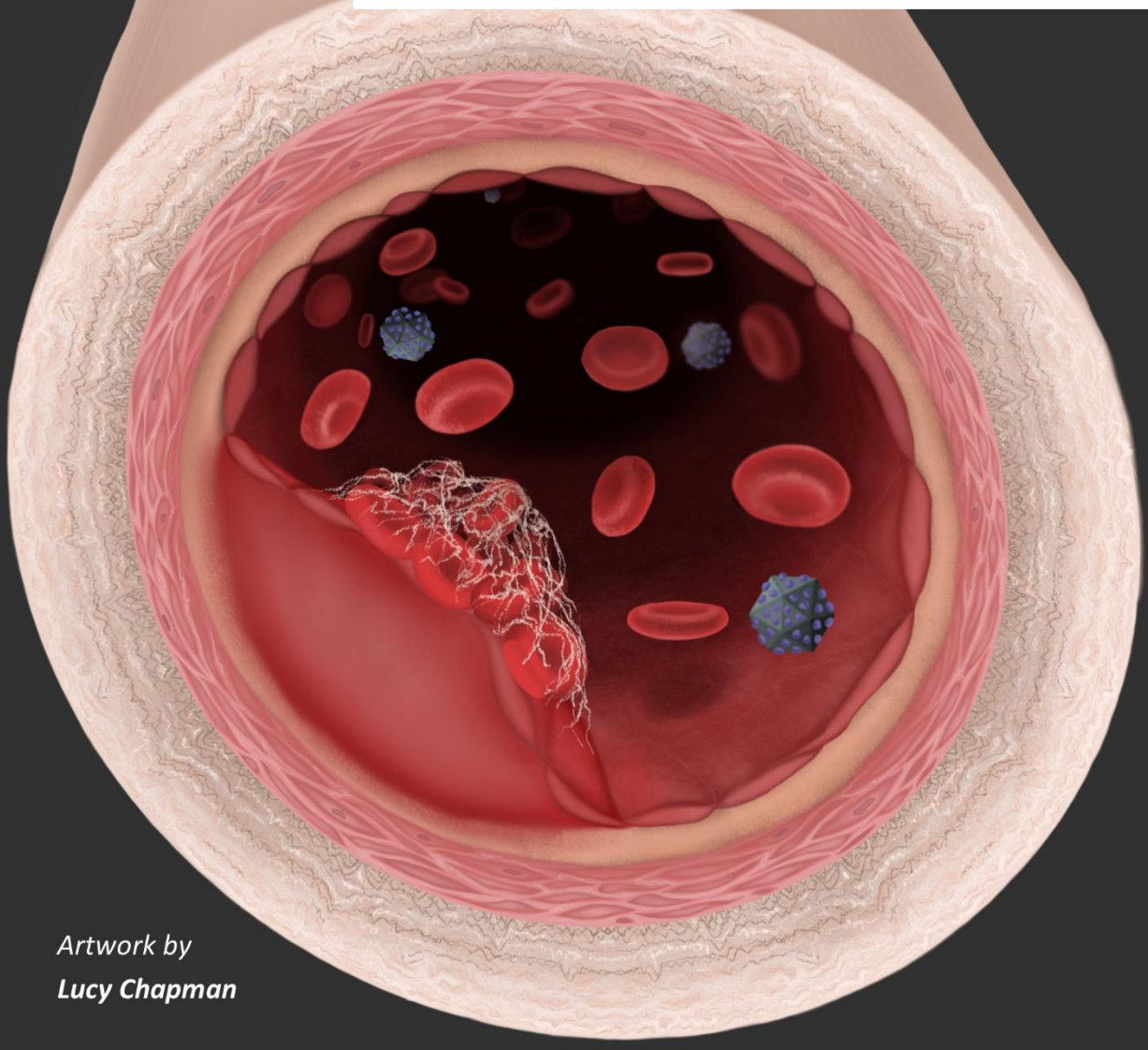

Artwork by
Lucy Chapman

These reviews are the opinions of PhD students, Post-docs and ECRs within Cardiff University and University of Oxford, who voluntarily took on this work.

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All previous editions of the Community Journal Club can be found at:
https://www.cardiff.ac.uk/news/view/2260179-getting-to-grips-with-covid-19/_recache

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CAUTIONARY NOTE:

SOME REVIEWS ARE OF PRE-PRINTS POSTED ONLINE
(in *arXiv, bioRxiv, medRxiv and Research Square) BEFORE* PEER REVIEW.

Innate Responses

Dynamic dysregulation of IL-6 and genes functional in NETosis, complement and coagulation in severe COVID-19 illness

Mukhopadhyay, S. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.13.20211425

Summary:

Reanalysis of 5 published transcriptome datasets suggest that complement, coagulation, and IL-6 driven NETosis pathways are up-regulated in severe COVID-19 cases.

Increased NETosis gene expression was reported in whole blood and post-mortem lung tissue, associated with a high Neutrophil-to-Lymphocyte Ratio (NLR) and inversely related to O_2 saturation in patients. IL-6 clustered with complement activation. Additionally, while IL-6 expression can be used to stratify cases by severity early on (day 0-4) these differences appear reduced over time.

Propose IL-6 triggers complement mediated NETosis, and platelet activation results in coagulation, immunothrombosis and severe disease.

Research Highlights:

1. Whole blood and post-mortem lung tissue showed up-regulation of NETosis gene set associated with disease severity.
2. Higher NETosis gene expression associated with NLR over time, both were higher early in severe disease.
3. Reciprocal relationship between NETosis gene expression and O_2 saturation in severe COVID-19 patients.
4. IL-6 and complement clustered together while the other cytokines were separate.
5. *DNASE1* expression (linked to NET clearance) was higher in healthy controls compared to non-ventilated or Acute Respiratory Distress syndrome (ARDS) patients.

Impact for COVID-19 research:

- Suggests severe COVID-19 pathology appears to be driven by NETosis, complement and coagulation rather than by cytokines alone.
- Suggests high NLR, high NETosis gene expression and high IL-6 could help stratify patients early on in disease.

Methodologies:

- Study Type: *Transcriptomic analysis of public datasets.*
- Key Techniques: *Gene-set enrichment analysis and deconvolution analysis. Data and R code at https://github.com/skm-lab/covid-19*

Limitations:

- Gene selection criteria for NETosis and cytokine gene sets poorly defined.
- Some analyses had a low number of patient samples, and would need to be validated in larger cohorts.

Proteomics identifies a type I IFN, prothrombotic hyperinflammatory circulating COVID-19 neutrophil signature distinct from non-COVID-19 ARDS

Reyes, L. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.15.20195305

Summary:

This study reports on the proteomics and metabolomics analysis of peripheral blood neutrophils from patients with COVID-19 ARDS (CA; n=3), non-COVID-19 ARDS (NA; n=3-7), moderate COVID-19 (MC; n=3), and healthy controls (HC; n=4-7). For some analyses normal density neutrophils (NDN) and low density neutrophils (LDN; isolated from the PBMC layer) were analysed separately. LDN consisted of both mature (CD16+CD10+) and immature (CD16-CD10-) neutrophils. Due to the small samples sizes most conclusions will need further validation, but the data suggest that neutrophils in COVID-19 ARDS may have increased IFN I signalling, degranulation, and platelet binding compared to healthy controls.

Research Highlights:

1. Comparing the neutrophil proteome of COVID-19 ARDS patients to healthy controls highlights an increase in the type I interferon signalling pathway and in markers of platelet degranulation.
2. Mature LDN from moderate or ARDS COVID-19 patients show increased CD41 staining, suggesting increased binding to platelets, compared to NDN from healthy controls. Immature LDN from COVID-19 patients do not show increased CD41 staining.
3. NDN from ARDS COVID-19 patients have slightly decreased content of granule proteins compared to healthy controls.
4. NDN from ARDS COVID-19 patients have increased glucose content compared to healthy controls, the cause is unclear.
5. In vitro stimulation of healthy neutrophils with a TLR7/8 agonist under hypoxic conditions leads to an activated phenotype regarding surface receptors.

Impact for COVID-19 research:

- No direct impact because the conclusions need further validation.

Methodologies:

- Study Type: *case study, in vitro*

- Key Techniques: *flow cytometry for activation markers, proteomics, metabolomics, extracellular flux analysis*

Limitations:

- Small group sizes; n=3 for COVID-19 ARDS and n=3 for moderate COVID-19.
- Inconsistent statistical analysis including grouping and subsetting. Some comparisons appear illogical (e.g. why not compare NA directly to CA in Fig. 3?)
- Most statements in the text are based on comparisons that don't show statistically significant differences.
- Patient characteristics are only provided for ARDS patients not for moderate patients or HC. Unclear whether non-COVID-19 ARDS patients also had viral infection.

SARS-CoV-2 infection induces mixed M1/M2 phenotype in circulating monocytes and alterations in both dendritic cell and monocyte subsets

Sanja, M. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.09.332858

Summary:

In this paper Sanja *et al.* look at flow cytometry staining of whole blood from 5 healthy controls, 30 mild/moderate and 27 severe COVID-19 patients. The author found that, as previously shown, COVID-19 patients have higher neutrophils/lymphocyte ratio, as well as reduced CD19+ and NK cells. The differences are more acute in the severe patients where DC and NKs numbers were very low. Analysis of the innate cells also show a reduction in atypical CD14+ cells as well as HLA expression. The number of dendritic cells is also reduced in COVID-19 patients, especially pDCs.

Research Highlights:

1. Immune cells profile of severe COVID-19 patients is more different from healthy control than mild/moderate ones.
2. Severe COVID-19 patients show reduction in atypical monocytes
3. Severe COVID-19 patients show reduction in CD11c+ and pDC cells
4. HLA-DR expression is reduced in COVID-19 patients
5. Expression of CD23 in innate cells is upregulated in COVID-19 patients

Impact for COVID-19 research:

- This study shows that innate cells are dysregulated in COVID-19 patients

Methodologies:

- Study Type: *patient cohort*

- Key Techniques: *flow cytometry*

Limitations:

- The study does not clarify the ages of the patients (just averages) and it is not clear if the controls are pertinent, also the patients vary widely in ages. There is no information on pre-existing conditions or medications that could affect immune cells.
- Lack a clear explanation of the gating strategy
- The figures never show the variation between samples, most graph do not have error bars
- The study is mostly descriptive-but there is some statistical analysis done to measure cell numbers (see methods)

Clinical

Serum interleukin-6 is an indicator for severity in 901 patients with SARS-CoV-2 infection: A cohort study

Zhang, J. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-55909/v2

Summary:

This study gives a profile of baseline IL-6 distribution among patients with common, severe and critical COVID-19 infection, suggesting a strong correlation between IL-6 level and severity of disease. Authors suggest that the duration of IL-6 elevation, in addition to the level, may play an important role in disease severity. Several studies have indicated an importance of IL-6 in COVID infections, the results of this study are consistent with previous findings and suggests that a cut-off of serum IL-6 of 37.65 pg/ml predicted death with high sensitivity and specificity.

Research Highlights:

1. Median concentration of IL-6 at baseline was <1.5 pg/ml, 1.85 pg/ml and 21.55 pg/ml for common, severe and critical COVID-19 groups respectively ($p<0.001$)
2. IL-6 concentrations remained relatively higher in the critical subgroup, even when cured
3. Baseline IL-6 concentration was highly predictive of in-hospital death
4. No significant difference in patients receiving tocilizumab or not (15.4% deaths versus 7.5% deaths, $p=0.4$)

Impact for COVID-19 research:

- Evidence for including serum IL-6 in the diagnostic work up of COVID-19 patients to stratify disease severity.

Methodologies:

- Study Type: *Observational, retrospective single-institutional study*

Limitations:

- Retrospective study
- Patients were unbalanced between those that received tocilizumab and those that did not – further study with suitable controls would be needed

Low zinc levels at clinical admission associates with poor outcomes in COVID-19

Vogel-González, M. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.07.20208645

Summary:

Zinc has a known role in immune responses and has previously shown antiviral activity against other viruses, including SARS. As zinc deficiency is more common in the elderly and those with chronic illnesses, the authors investigate the correlation between zinc deficiency and severity of COVID-19. Zinc deficiency was associated with increased mortality and time to clinical stability, while markers of inflammation negatively correlated with zinc levels in sera. *In vitro* work demonstrated that zinc deficiency promotes SARS-CoV-2 replication, however, supplementation of zinc did not potentiate the effects of antiviral chloroquine.

Research Highlights:

1. The median time to clinical stability in zinc deficient patients (< 50 ug/dl) was markedly longer than those with sufficient zinc levels (25 days vs. 8 days, p<0.001). Mortality was also significantly higher in zinc deficient individuals (21% vs 5%).
2. Markers of inflammation, including C-reactive protein and IL-6, negatively correlated with patient serum zinc content
3. Deficiency in extracellular zinc significantly increased SARS-CoV-2 RNA 48 h post infection in Vero E6 cells. Zinc supplementation did not impact RNA levels.
4. Whilst toxicity and antiviral activity of chloroquine (CQ) were unaffected by various zinc concentrations, the autophagy blocking effects of CQ were not potentiated by zinc. CQ treatment increased lysosomal levels of zinc.

Impact for COVID-19 research:

- The data obtained here is in support of other published studies which show zinc deficiency associated with poor outcomes (Jothimani *et al*. 2020) and others which suggest zinc should be considered nutritionally as a COVID-19 preventative (Mossink 2020).
- The authors suggest the use of zinc deficiency as a biomarker for COVID-19
- Findings that zinc does not potentiate CQ was found to be “contrary to what was previously suggested”

Methodologies:

- Study Type: *Retrospective observational, in vitro*
- Important cell lines/viral models used: *Vero E6 Cells, SARS-CoV-2 strain hCoV-19/Spain/ VH000001133/2020*
- Key Techniques: *Flow Cytometry and in vivo confocal imaging to determine intracellular zinc measurements, MTT viability assays, western blots*

Limitations:

1. Whilst a moderate number of patients (n=249), they were all admitted to the same hospital in Barcelona. On top of genetic variations between populations, zinc intake via diet can also vary between populations. A more diverse pool of patients would be more conclusive.

Antibodies and T-cells

Neutralizing antibodies from early cases of SARS-CoV-2 infection offer cross-protection against the SARS-CoV-2 D614G variant

Lee, C.Y-P. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.08.332544

Summary:

A new strain of SARS-CoV-2 with increased infectivity and transmission rate was identified in March 2020. The point mutation in the spike protein from aspartic acid (D) to glycine (G) at position 614 has led to this new variant known as 'D614G'. Single point mutations in coronaviruses such as SARS-CoV and MERS-CoV have induced resistance to neutralising antibodies. Therefore, to understand SARS-CoV-2 reinfection risk to the population, it is crucial to investigate whether neutralising antibodies against the D614 strain can cross-neutralise to the G614 variant strain. Antibodies were found to cross-neutralise successfully against both strains, however neutralising capacity deteriorated at the later phase of infection.

Research Highlights:

1. Plasma samples were collected from 57 SARS-CoV-2 patients at both early (31 post-symptom onset) and late (98 days post-symptom onset) recovery time-points. All patients displayed a significant waning in IgM antibodies over-time, however, IgG antibodies persisted more highly.
2. Antibodies from patients infected with the D614 strain (n=44) and the G14 strain (n=6) were tested for their neutralisation capacity against both virus strains using a pseudotyped lentivirus assay expressing the spike (S) proteins. Antibodies were able to neutralise both D614 and G614 pseudoviruses similarly, showing that the D614G point mutation does not impact humoral immunity.
3. Neutralising capacity of antibodies from SARS-CoV-2 recovered patients dropped significantly from 31 to 98 days post-symptom onset. Patients who had more severe disease also had higher levels of neutralising antibodies.

Impact for COVID-19 research:

- Understanding antibody cross-reactivity is critical for understand reinfection risks against new strain variants of SARS-CoV-2. Additionally, many antibody therapies against SARS-CoV-2 which are in clinical trials are based on the D614 strain; it is important to know if these therapies are suitable for patients infected with the G614 strain.

Methodologies:

- Study Type: *Cohort study with in vitro analysis*

- Key Techniques: *In vitro pseudotyped lentivirus neutralisation assay expressing the SARS-CoV-2 S protein tagged with a luciferase reporter.*

Limitations:

- Low number of patient samples, especially for those with the G614 variant. Would also be interesting to see more time-points.
- Only investigated IgG and IgM; not IgA.

Healthcare workers with mild / asymptomatic SARS-CoV-2 infection show T cell responses and neutralising antibodies after the first wave

Reynolds, C.J. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.13.20211763

Summary:

Reynolds *et al*. conducted a longitudinal study of healthcare workers (HCW) who were regularly monitored for COVID-19. They assessed those who had mild or asymptomatic COVID-19 and found that a large majority had both a memory T cell response and antibody response at 4 months post infection. A minority had either one or the other and only 1/70 HCW studied had no detectable T cell response and an antibody titre below the threshold likely to prevent reinfection. This is important because most previous studies have focused on those with moderate/severe disease. In addition, antibody titres appear more persistent in this study than in some other reports.

Research Highlights :

1. The majority of HCW with mild or asymptomatic SARS-CoV-2 infection had neutralizing antibodies and T cell responses to a range of for at least 4 months after infection
2. Neutralising antibodies were present in 90% at 16-18 weeks (titres likely to correlate with functional protection were present in 66%). The 10% who lack detectable antibodies had fewer T cells directed against Spike but had T cells targeting other SARS-CoV-2 epitopes.
3. T cell and antibody responses were discordant: T cell responses tended to be lower in asymptomatic infected HCW than those reporting symptoms of COVID-19, while antibody titres were independent of symptoms.
4. 69-87% had a detectable T cell response, depending on the assay used. T cell responses correlated with age and sex but not ethnicity

Impact for COVID-19 research:

- Further follow up of this and other cohorts with mild/asymptomatic infection should be conducted in order to understand the trajectories of antibody and memory T cell responses and the implications for protection from re-infection

Methodologies:

- Study Type: *cohort*
- Key Techniques: *longitudinal follow up*

Limitations:

- NA

Major role of IgM in the neutralizing activity of convalescent plasma against SARS-CoV-2

Gasser, R. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.09.333278

Summary:

The authors examine neutralising capacities of different Spike-specific antibody isotypes from convalescent donors, suggesting an important role for IgM in virus neutralisation.

Research Highlights:

1. Depletion of either antibody isotype significantly decreased neutralisation ability to pseudoviral particles expressing SARS-CoV-2 S-protein compared to non-depleted plasma
2. IgM and IgG but not IgA depleted plasma showed decreased neutralising ability against fully infections SARS-CoV-2 particles, with IgM depletion having the strongest effect (4 fold reduction for IgM compared to 2.9 fold for IgG)

Impact for COVID-19 research:

- Medium: as other studies have reported that IgM and IgA antibodies decline rapidly within the first three months after infection while IgG antibodies are maintained, the importance of IgM could explain why people have been observed to get re-infected.
- The results also have implications for the use of convalescent plasma as a therapeutic which should be taken early after disease recovery to ensure the presence of IgM antibodies.

Methodologies:

- Study Type: *In vitro*

- Key Techniques: *collection of serum from convalescent donors, selective depletion of IgG, IgM and IgA via absorption on isotype specific ligands immobilised on agarose beads, neutralisation assays with pseudovirus and SARS-CoV-2*

Limitations:

- Low number of participants, biased toward male gender (21male, 4female)
- Would be interesting to take into a mouse model to investigate whether loss of one isotype enables re-infection with the virus

Long-Term Immunity

Genomic evidence for reinfection with SARS-CoV-2: a case study

Tillett, R.L. *et* al. 2020. *The Lancet Infectious Diseases*

Link: https://doi.org/10.1016/S1473-3099(20)30764-7

Summary:

In this case report they present the first North American case of reinfection with SARS-CoV-2. The 25-year-old man who tested positive for SARS-CoV-2 in April, after 6 weeks tested positive. According to the data obtained from the genomic analysis the two viral agents were genetically distinct. According to what has been reported this wouldn't be the first case of reinfection as according to this report there are at least other 4 cases of reinfection worldwide. Which means that previous exposure to SARS-CoV-2 does not necessarily translate to guaranteed total immunity. This report proves there are more at least two SARS-CoV-2 agents and being exposed to one of them do not mean you are protected. Thus, all individuals must take identical precautions to prevent infection

Research Highlights:

1. Both specimens were member of clade 20C and presented same SNVs mutations. Although Specimen B had 7 SNVs absent in specimen A

Impact for COVID-19 research:

- As only one patient has been considered and might need to be better studied, what it shows here is that what we know as Covid-19 disease it can be caused by more than one viral agent. This should be considered for vaccination

Methodologies:

- Study Type: *Clinical report*
- Key Techniques: *To compare both specimens they previously generated SARS-CoV-2-enriched sequencing libraries to be sequenced with an Illumina NextSeq. Two different bioinformatics analysis were done: first using the CLC Genomics Workbench version 20.0.4; Second using Trimmomatic version 0.39.*

Limitations:

- No assessment of the immune response to the first episode of SARS-CoV-2 infection
- There is no data about the effectiveness of the immune response
- This is a clinical report in which only one patient has been considered. It would be preferable to count with more than one subject as the reasons why this patient being symptomatic after the second infection can be multiple i.e viral dose.

Vaccine Design

Design of SARS-CoV-2 RBD mRNA Vaccine Using Novel Ionizable Lipids

Elia, U. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.15.341537

Summary:

Herein they propose an ionizable lipid as carrier molecule for successful mRNA delivery so it will be protected from degradation and facilitate cellular uptake. Also, the candidates selected for this study demonstrated to have develop a specific humoral and cellular response against the antigen, as well as neutralizing antibodies. There is an urgent necessity to develop a vaccine. Although they need a carrier mRNA vaccine is one of the approaches. In this study they present a design of a lipid nanoparticle (LNP) as a possible carrier for (in particular) a receptor binding binding domain (RBD) mRNA vaccine. Although a deep study off how this LNP-encapsulated RBD mRNA vaccine works, this could be a possible vaccine or a good method to transport this mRNA that could be considered in the future for vaccine development

Research Highlights:

1. The humoral response was rather limited after a single immunization whether there was a substantial cellular response.
2. Mice immunized with LNP RBD mRNA developed substantial anti-spike IgG titers and high levels of neutralizing antibodies after boost administration (i.m and i.d)
3. A specific and statistically significant secretion of IFNγ and IL-2 was observed in vaccinated mice compared to vehicle treatment before and after boost administration.
4. The immune response might differ depending on the vaccination route

Impact for COVID-19 research:

- What they present in this study is a LNPs-encapsulated mRNA as a vaccine platform which according to their results might be a promising candidate to protect people against Covid-19

Methodologies:

- Study Type: *in vitro and in vivo*
- Important cell lines/viral models used: *Female BALB/c mice (6-8 weeks old). To express the recombinant SARS-CoV-2 spike glycoprotein pcDNA3.1^{+} plasmid was used.*
- Key Techniques: *As an expression system they used ExpiCHO^{TM}. LNPs were synthesized by mixing ionizable lipid, DSPC, Cholesterol and DMG-PEG (40:10.5:47.5:2 mol ratio) in ethanol and three volumes of mRNA in acetate buffer. Other techniques were ELISA,*

ELISpot and PRNT for the immune analysis the immunized mouse sera or an IVIS Spectrum imaging system for the bioluminescence imaging.

Limitations:

- The number of mice used in this study might low as it's only 10 per group.
- They study the immune response in mice. Although it's a good model might be worth to test the LNP-RBD mRNA in another different model.
- For the immunization of mice with LNPs-RBD mRNA they also show the response for the rRBD immunization although in that case the administration route is s.c instead i.m or i.d which might have been a good idea to also administrate it i.m or i.d to compare the results