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# COVID-19 Community Journal Club No. 14

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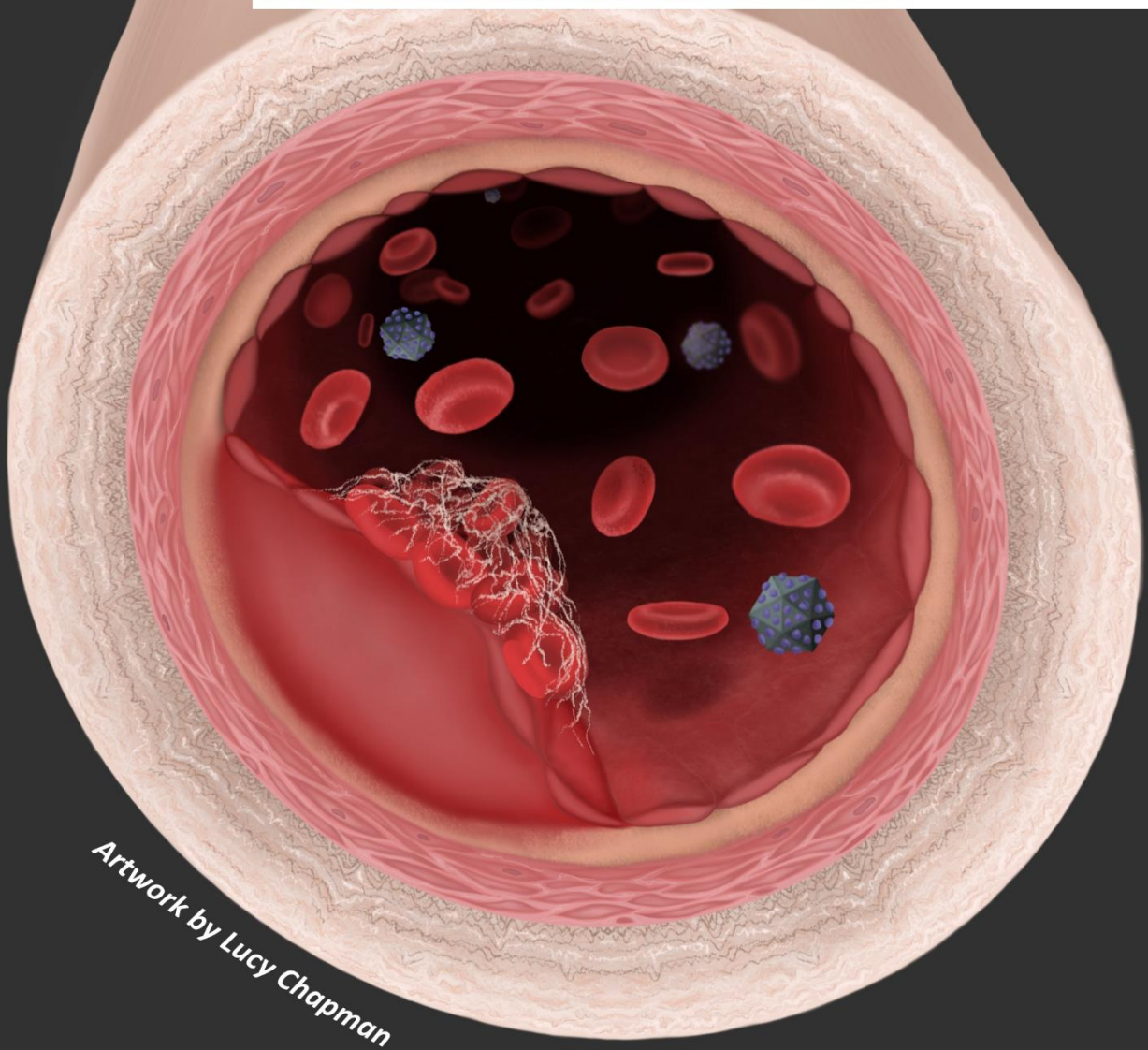

Artwork by Lucy Chapman

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

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**Please direct any comments or queries to Awen at** gallimoream@cardiff.ac.uk

**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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# Coagulation

## COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection

Al-Samkari, H. *et al*. 2020. *Blood.*

**Link:** https://doi.org/10.1182/blood.2020006520

**Summary:**

Samkari *et al*., report the rate and severity of bleeding and thrombotic complications in 400 critically ill and non-critically ill COVID-19 patients. Previous studies report associations of coagulation and inflammation with mortality in COVID-19 patients. This study evaluated markers of bleeding and coagulation by radiographic imaging and clinical observation against patients without bleeding and thrombotic complications. Elevated D-dimer was associated with risk of bleeding, thrombosis, critical illness and death. Thrombosis correlated with increased inflammatory markers. The authors advise caution against aggressive anti-coagulation therapy for COVID-19 patients and recommend further studies to address the clinical implications of anticoagulant therapy in COVID-19.

**Main Findings:**

- The thrombotic complications group had higher D-dimer, fibrinogen, C-reactive protein (CRP), ferritin, and procalcitonin. Elevated D-dimer, platelet count, CRP, and erythrocyte sedimentation rate (ESR) at initial presentation was predictive of thrombotic complications during hospitalisation and thrombocytopenia
- The bleeding complications group had higher D-dimer, procalcitonin and lower platelet counts. Elevated D-dimer at initial presentation was predictive of bleeding complications
- Elevations in D-dimer, CRP, ESR, ferritin, procalcitonin and troponin at initial presentation were predictive of critical illness during hospitalisation and elevations in D-dimer, PT (International Normalised Ratio), activated partial thromboplastin time (PTT), fibrinogen, CRP, ESR, and procalcitonin predictive of death

**Highlights:**

1. Study demonstrates elevated D-dimer at presentation predicts bleeding and thrombotic complications, critical illness and death. Beyond D-dimer, thrombosis was associated with inflammatory markers rather than coagulation parameters.
2. In concordance with previous studies, venous thromboembolism and bleeding rates were comparable with critically ill non-COVID-19 hospitalised patients.

**Impact for COVID-19 research:**

- The authors recommend further large-scale studies to address the clinical implications of anticoagulant therapy in COVID-19

- Although thrombotic events in COVID-19 patients are likely, given the observations of bleeding, the authors advise caution in the use of aggressive anti-coagulation therapy

**Methodologies:**

- Study Type: Multicentre retrospective study

**Limitations:**

- Overall, small numbers of patients with bleeding and thrombotic complications leading to wide confidence intervals in data
- Some patients unable to undergo radiography and therefore presumed thrombotic events determined by other clinical features
- No standardized imaging protocol available for diagnosis of thrombotic and bleeding disorders highlighting the possibility of misdiagnoses
- Laboratory data not collected for every patient

## COVID-19 coagulopathy, thrombosis, and bleeding

Chan, N.C. and Weitz, J.I. 2020. *Blood*

**Link:** https://doi.org/10.1182/blood.2020007335

**Summary:**

This study is based on Al-Samkari *et al's* report on the results of a multicentre retrospective study that included 400 hospitalized patients diagnosed with COVID-19 between 1 March and 5 April 2020 at 5 Partners Healthcare institutions in Massachusetts. Rates of thrombosis, bleeding, and mortality were captured, and the prognostic value of markers of inflammation (C-reactive protein, erythrocyte sedimentation rate, ferritin, and procalcitonin) and coagulation (D-dimer, fibrinogen, prothrombin time, activated partial thromboplastin time, and platelet count) measured at presentation was examined. Rates of venous thromboembolism (VTE) in critically ill COVID-19 patients in this study is lower than previously reported. With anticoagulant thromboprophylaxis, the authors report a rate of major bleeding of 5.6% in critically ill patients with COVID-19. The authors have identified baseline platelet count below 150 × $10^9$/L and D-dimer levels over 2500 ng/mL as independent predictors of a threefold increase in the risk of major bleeding.

**Main Findings:**

- In addition to thrombotic complications, bleeding is a significant cause of morbidity in patients with COVID-19.
- D-dimer elevation at admission was predictive of bleeding, thrombosis, critical illness, and death in patients with COVID-19.

**Highlights:**

1. Relatively large sample size
2. Comprehensive reporting of clinical outcomes

**Impact for COVID-19 research:**

- This research will alter out view of the disease and will benefit disease management in the clinic.

**Methodologies:**

- Study Type: *Cohort study*
- Novel Techniques: *Blooding event-grading; Pulmonary embolism (PE) and deep vein thrombosis (DVT)- radiography; Coagulation based lab assays*

**Limitations:**

- Retrospective design as well as the potential for bias in case ascertainment.
- Underestimation of VTE rates because of the inability to image all critically ill patients.

## Platelet Gene Expression and Function in COVID-19 Patients

Manne, B.K. *et al*. 2020. *Blood*

**Link:** https://doi.org/10.1182/blood.2020007214

**Summary:**

This study described the identification of distinct alterations to the gene expression profile of circulating platelets as well as changes in functional responses in patients acutely ill with SARS-CoV-2 infection. Resting platelets from COVID-19 patients had increased P-selectin expression basally and upon activation. Circulating platelet neutrophil, monocyte, and T-cell aggregates were all significantly elevated in COVID-19 patients compared to healthy donors.

These findings are important as they demonstrate that SARS-CoV-2 infection is associated with platelet hyper-reactivity which may contribute to COVID-19 pathophysiology including thrombotic events which are known to commonly occur.

**Main Findings:**

- RNA sequencing on RNA isolated from highly purified platelets from 6 non-ICU and 4 ICU COVID-19 patients and 5 matched healthy donors
- 3,090 differentially expressed genes between non-ICU patients compared to healthy donors while 2,256 were differentially expressed in ICU patients compared to healthy donors (FDR<0.05)

- Only 16 genes were differentially expressed in a direct comparison between non-ICU and ICU COVID-19 patients, suggesting minimal impact of ICU status
- Ingenuity pathway analysis revealed differential gene expression changes in pathways associated with protein ubiquitination, antigen presentation and mitochondrial dysfunction
- Anti-viral immune protein (interferon induced transmembrane protein) previously found to correlate with clinically severe COVID-19 was one of top differentially expressed genes (upregulation confirmed by western blot)
- RNA-seq demonstrated no expression of ACE2 mRNA independent of ICU status
- SARS-CoV-2 N1 gene mRNA detected in 2/25 patients suggesting platelets may take-up SARS-COV-2 mRNA independent of ACE2
- Non-ICU and ICU COVID-19 patients generally had a platelet count and MPV in the normal reference range whilst plasma thrombopoietin levels were significantly elevated in all COVID-19 patients
- Transmission electron microscopy on more than 50 platelets from 4 COVID-19 patients (3 ICU/ 1 non-ICU) indicated an intact platelet ultrastructure without any identifiable virus particles
- Basal P-selectin surface expression was modestly, but significantly, increased in all COVID-19 patients independent of ICU status as was platelet-derived growth factor (PDGF)
- Activating platelets with low dose 2MeSADP and PAR1 peptide induced significantly greater surface P-selectin expression in all COVID-19 patients
- Platelet-neutrophil, platelet-monocyte, and platelet-T cell ($CD4^+$ and $CD8^+$) aggregates were all significantly elevated in all COVID-19 patients compared to healthy donors
- Platelet aggregation in response to low dose agonists (2MeSADP, thrombin, and collagen) was significantly increased in COVID-19 patients compared to healthy donors
- Platelets from non-ICU and ICU COVID-19 patients also exhibited greater adhesion and spreading on fibrinogen and collagen during clinical infection with SARS-CoV-2 suggesting circulating platelets may be primed to be hyperreactive
- Downstream MAPK signalling drives platelet aggregation and phosphorylation of ERK1/2, p38, and eIF4E was significantly upregulated in platelets from critically ill ICU COVID-19 patients, indicating increased activation of this pathway
- cPLA2 (which promotes thromboxane generation) phosphorylation was significantly increased in SARS-CoV-2 infected patients both at baseline and upon activation
- Stimulation with 2MeSADP resulted in increased thromboxane generation by platelets from COVID-19 ICU patients indicating hyperreactivity

**Highlights:**

1. SARS-CoV-2 induces robust gene expression and functional changes in platelets
2. Platelet hyperreactivity may contribute to COVID-19 pathophysiology through increased platelet-platelet and platelet-leukocyte interactions
3. Increased platelet activation (granule release and P-selectin expression) and aggregation during COVID-19 is due, at least in part, to increased MAPK pathway activation and thromboxane generation

**Impact for COVID-19 research:**

- Altered platelet gene expression profile and increased platelet reactivity during SARS-CoV-2 infection may contribute to immunothrombosis and warrants further investigation

**Methodologies:**

- Study Type: *Prospective clinical study*

**Limitations:**

- None of the COVID-19 patients enrolled in study were clinically diagnosed with thrombotic complications therefore clinical associations between platelet activation and thrombosis cannot be established
- Larger sample size required to confirm findings

## Platelet activation and platelet-monocyte aggregates formation trigger tissue factor expression in severe COVID-19 patients

Hottz, E.D. *et al*. 2020. *Blood*

**Link:** https://doi.org/10.1182/blood.2020007252

**Summary:**

This study these data shed light on new pathological mechanisms involving platelet activation and platelet-dependent monocyte tissue factor expression, which were associated with COVID-19 severity and mortality. Hypercoagulability state is a major pathologic event in COVID-19, while the mechanism is not yet completely understood. This paper exhibits the participation of platelets in this pathogenesis.

**Main Findings:**

- Increased platelet activation and platelet-dependent tissue factor in monocytes expression are related to poor prognosis and severity in COVID-19 patients.

**Highlights:**

1. Increased platelet activation was observed in severe COVID-19 cases, which was related to CRP, P-selectin and CD63 surface translocation (Inflammatory mediators).
2. Platelets form aggregates with monocytes was detected in severe COVID-19 patients, together with increased tissue factor expression.
3. Platelets from patients with COVID-19 induce monocyte TF expression through mechanisms depending on P-selectin and integrin αIIb/β3 (using related Ab can block this expression).

**Impact for COVID-19 research:**

- This study provided novel mechanisms of activation-dependent platelet induced TF expression in monocytes during COVID-19, and associated this with severity and mortality in COVID-19 patients.
- This study could provide evidence for anti-platelet therapy in COVID-19 treatment. Some potential Antibody/drugs to prevent platelet activation in ex vivo experiments, for example anti-P-selectin neutralizing antibody and abciximab works, while aspirin and clopidogrel did not. On the contrary, some inflammatory mediators can worsen this process.

**Methodologies:**

- Study Type: *Cohort study: Patients were prospectively enrolled and divided to severe or mild/asymptomatic group. Platelet and inflammatory mediators and Platelet-monocyte interaction were analysed in ex vivo experiments.*

**Limitations:**

- The experiments were carried out in vitro. Thus, the efficacy of stated anti-palate therapy in vivo is still unknown.

# Immune Responses: Clinical Implications and Possible Interventions

## Patients with immune-mediated inflammatory diseases receiving cytokine inhibitors have low prevalence of SARS-CoV-2 seroconversion

Simon, D. *et al.* 2020. *Nature Communications*

**Link:** https://doi.org/10.1038/s41467-020-17703-6

**Summary:**

Comparison of reactive IgG seroprevalence to SARS-CoV-2 spike and nucleocapsid protein between immune-mediated inflammatory disease patients not receiving (n=259) or receiving cytokine (n=534) inhibitors relative to non-healthcare (n=971) and healthcare worker (n=285) controls. Patients were receiving inhibitors for TNF (n=227), IL-6 (n=44), IL-17 (n=51), IL-23 (n=85), JAK (n=39) or other (n=88) cytokines. Patients who are being treated with cytokine inhibitors had a much lower prevalence of SARS-CoV-2 reactive IgG which cannot be explained by different exposure risk variables. This is important in the context of how elevated TNF and IL-6 levels in severe COVID-19 cases impact disease resolution.

**Main Findings:**

- Seroprevalence in each group for spike protein (S1) (and validated against nucleoprotein) were:
  - Healthcare worker control 4.21 %
  - Non-healthcare worker control 2.27 %
  - Immune mediated inflammatory disease with cytokine inhibitor 0.75 %
  - Immune mediated inflammatory disease without treatment 3.09 %
- Healthcare worker control was significantly higher than non-healthcare worker control whilst patients receiving cytokine inhibitors was significantly lower.
- The difference in exposure risk variables (i.e. contact with individuals with respiratory infections, presence at workplace, travel to risk areas) between patients receiving and not receiving cytokine inhibition for their condition was small, and not enough to explain the difference in seroprevalence.

**Highlights:**

1. Immune-mediated inflammatory disease patients who are being treated with cytokine inhibitors had a much lower prevalence of SARS-CoV-2 reactive IgG.

**Impact for COVID-19 research:**

- This study indicates that elevated cytokine levels during COVID19 may be more than just a marker and further research into the effects of these inhibitors taken i.e. in SARS-CoV mouse models.
- Patients who are currently on cytokine inhibitors as listed in this study should continue with their treatments, although no causative effect is proven in this study and care should still be taken.

**Methodologies:**

- Study Type: *cohort study, population study*
- Methodology: *S1 (spike) and nucleoprotein ELISA to test seroprevalence.*

**Limitations:**

- It is becoming increasingly apparent that the T cells play an important role in mediating recovery from severe SARS-CoV-2 as recovery co-indices with resolution of lymphocytopenia. Therefore, it would have been interesting to determine whether patients who receive cytokine inhibition had an altered T cell response especially given the reports that humoral protection wanes rapidly.
- IL-6 serum levels have been demonstrated to be elevated during SARS-CoV-2 infection and this study could have been improved by also further stratifying seroprevalence according to each cytokine inhibited.

## Comprehensive mapping of immune perturbations associated with severe COVID-19

Kuri-Cervantes, L. *et al*. 2020. *Science Immunology*

**Link:** https://doi.org/10.1126/sciimmunol.abd7114

**Summary:**

This study analysed the overall immunologic state of COVID19 patients on a larger scale (42 patients, 12 healthy donors) than previously published, investigating whole blood. COVID19 patients were stratified (7 moderate, 28 severe, 7 recovered) to discover differences associated with severity. Most changes observed in leukocyte subsets were consistent with those reported in previous studies. The authors also investigated the patients' antibody repertoire. The study offers insight into total changes in immune populations in severe COVID19 patients, suggesting immune alterations characteristic of sepsis-associated dysregulation but also those associated with viral infection.

**Main Findings:**

- Increased in patients with severe disease: neutrophils, eosinophils, neutrophil/T-cell ratio (NTR), plasmablasts and SARS-COV2 spike regulatory binding domain specific IgG and IgM antibodies
- decreased in patients with severe disease: DCs, lymphocytes
- severe cases co-localised during independent clustering driven by T cell activation in CD4+ and CD8+ T cell memory subsets and plasmablast and neutrophil frequency COVID19 patients had large, oligoclonal B cell expansions

**Highlights:**

1. Immune perturbations are observed in severe patients that are not present in moderate and recovered individuals
2. NLR and neutrophil/T-cell ratio (NTR) were strongly correlated with each other and disease severity
3. CDR3 amino acid sequences from severe COVID19 individuals contained highly variable amino acid sequences

**Impact for COVID-19 research:**

- This research could benefit clinicians if immunological perturbations observed can be used to predict disease severity during early disease stages. However this was not investigated.

**Methodologies:**

- Study Type: *cohort/in silico*

**Limitations:**

- The sample size is still small
- Some moderate and severe cases were treated with Hydroxychloroquine and/or Remdesivirc, would have been interesting to see them split into separate groups
- mild and asymptomatic cases were not included in this study

# Antibodies

## Analysis of a SARS-CoV-2-Infected Individual Reveals Development of Potent Neutralizing Antibodies with Limited Somatic Mutation

Seydoux, E. *et al*. 2020. *Immunity*

**Link:** https://doi.org/10.1016/j.immuni.2020.06.001

**Summary:**

In this study, Seydoux *et al*. isolated B cells that are specific for the spike glycoprotein (S) of SARS-CoV-2 from a SARS-CoV-2 infected individual, 21 days after the development of clinical disease. 576 S-specific B cells were isolated, from which, 45 monoclonal antibodies were generated. Little somatic mutations or clonal expansion were observed. 3 receptor-binding domain (RBD) antibodies were isolated, of which only the most potent was able to prevent binding to the ACE2 receptor and neutralize SARS-CoV-2, highlighting how only few anti-SARS-CoV-2 antibodies are neutralizing and that most bind outside the RBD.

**Main Findings:**

- Within 3 weeks of SARS-CoV-2 infection, the patient in this study developed a potently neutralizing response
- 0.65% of CD19+ B cells were specific for the S-protein in the patient compared 0.07% of B cells from a naïve donor
- In the patient, 49% of S-specific B cells were IgM+IgD+ whilst 27% were IgG+IgD-
- 0.12% of IgG+ B cells of the patient bound the RBD
- 45 paired VH and VL sequences from S-specific IgG+ B cells were isolated; these displayed little somatic hypermutation or clonal expansion
- Of these 45, only 3 bound the SARS-CoV-2 RBD; 2 were weakly neutralizing as they bound an epitope outside the RBD, whilst 1 was potently neutralizing
- Unlike the 2 weakly neutralizing antibodies, the potently neutralizing antibody was able to block the interaction between the RBD and the ACE2 receptor

**Highlights:**

1. The early antibody response to SARS-CoV-2 infection in this patient was largely non-neutralizing and displayed little somatic hypermutation or clonal expansion
2. Only a singly antibody was identified as potently neutralizing and inhibited the RBD:ACE2 interaction

**Impact for COVID-19 research:**

- Highlights how serum neutralizing antibodies during SARS-CoV-2 infection are likely directed against a small subset of epitopes
- Could contribute to the development of potently neutralizing monoclonal antibodies against SARS-CoV-2 infection

**Methodologies:**

- Study Type: *In vitro using patient sample*
- Novel Techniques: *VH and VL sequencing of S-specific IgG+ B cells*

**Limitations:**

- The analysis was limited to a single COVID-19 patient
- B cells of patient analysed only at a single time point following SARS-CoV-2 infection; longitudinal samples from patients with different degrees of clinical symptoms will be required to generalise the findings of this study

## SARS-CoV-2 infection induces robust, neutralizing antibody responses that are stable for at least three months

Wajnberg, A. *et al.* 2020. *medRxiv*

**Link:** https://doi.org/10.1101/2020.07.14.20151126

**Summary:**

The authors investigate individuals infected with mild to moderate COVID-19 to determine IgG antibody responses against the spike protein. The results show that a majority of patients demonstrate robust responses, which are stable for at least 3 months, and that the titres observed significantly correlated with neutralisation ability. This study included 19,763 positive patients – defined as detectible antibodies.

**Main Findings:**

- Robust IgG responses against the COVID-19 spike protein last for at least 3 months

**Highlights:**

1. Rate of individuals that do not seroconvert following COVID-19 infection is low and the majority of responders mount titres of 1:320 or higher
2. Neutralization titres significantly correlated (Spearman r= 0.87, p<0.0001) with spike-binding titres
3. Antibody titres remained high for up to 3 months (the authors plan to extend this experiment and follow this cohort over longer time intervals)

**Impact for COVID-19 research:**

- Information regarding how long the antibody response to COVID-19 lasts in patients could aid vaccine development

**Methodologies:**

- Study Type: *Cohort Study*

**Limitations:**

- N/A

## Lack of antibody-mediated cross-protection between SARS-CoV-2 and SARS-CoV infections

Yang, R. *et al.* 2020. *EBioMedicine*

**Link:** https://doi.org/10.1016/j.ebiom.2020.102890

**Summary:**

SARS-CoV and SARS-CoV2 share 80% whole genome and 66% Spike (S) protein identity, as well as the ACE2 host cellular receptor. The authors confirm the common entry mechanisms of these coronaviruses and reveal effective inhibition of SARS-CoV-2 viral entry using recombinant monomeric and trimeric SARS-CoV receptor binding domain (RBD) proteins. Serum from mice immunised and boosted 3 times with SARS-CoV proteins suppressed SARS-CoV-2 viral entry (pseudotyped and live) only at serum dilutions 30-fold lower than those at which homologous viral entry was inhibited. Furthermore, serum from SARS-CoV/2 convalescent patients had similarly limited cross-neutralising capacity, suggesting high specificity of protective immunity.

**Main Findings:**

- SARS-CoV recombinant RBD monomers and trimers dose-dependently reduce the infection efficiency of HIV pseudotyped with SARS-CoV (ppSARS-2) and SARS-CoV-2 (ppSARS), but not MERS-CoV S proteins, demonstrating common SARS viral entry mechanisms and potential neutralising epitopes.
- Anti-sera derived from mice immunised with inactivated SARS-CoV, followed by 3 boosts with recombinant SARS-CoV S protein and SARS-CoV S1 (glycoprotein) effected an 80% neutralisation against ppSARS-2 at a 1:100 dilution. A 1:8100 dilution produced the same level of neutralisation against the homologous ppSARS. A 1:20 dilution resulted in 70% neutralisation of live SARS-CoV-2 infection in Vero cells. No other vaccination strategies produced any cross-neutralisation capacity, hence 3 boosts may be a minimal requirement to achieve sufficient antibody titres for measurable outcomes.
- Anti-sera obtained from convalescent SARS-CoV and SARS-CoV-2 patients were competent only for neutralisation of homologous and not heterologous pseudotyped and live viral infections.

**Highlights:**

1. *In vitro* neutralisation assays using serum derived from immunised mice or convalescent SARS-CoV patients do not recapitulate the effective inhibition of heterologous SARS-CoV-2 viral entry determined using recombinant RBD proteins alone.

**Impact for COVID-19 research:**

- This research conflicts with previous data reported by Pinto *et al*. (https://www.nature.com/articles/s41586-020-2349-y), who isolated an anti-S-protein antibody from a patient with SARS-CoV that had potent neutralising activity for SARS-CoV-2. The combined disparities may provide useful insights into both the prevalence and the particular characteristics of those antibodies that do have cross-neutralising capacity, facilitating effective vaccine development and clinical management.

**Methodologies:**

- No novel or bespoke methodology; *in vitro* infection and neutralisation assays; useful detail on the single mouse immunisation strategy that did result in anti-sera possessing moderate cross-neutralising activity.

**Limitations:**

- No titre or analysis of antibodies present in immunised mice or convalescent patient sera, so it is difficult to compare with other studies to draw more meaningful conclusions. For example, did the authors fail to detect cross-neutralisation because antibody titres were too low or epitope specificities were distinct from other antibodies that have been reported to have this functional capacity?
- Within the study, it would have been useful to understand the consequences of the different mouse immunisation schedules for these key antibody parameters to guide future research.
- Additionally, it is difficult to transpose the serum dilutions used in this study to other applications without any even approximate evaluation of antibody concentrations.
- The authors report >60% neutralisation of ppSARS with mouse anti-sera even at very high dilutions (1:24300). It would have been informative to extend the dilution series further to determine the functional threshold. Can the authors be sure the inhibition observed is purely antibody-dependent since uncharacterized serum is used?
- There is no information provided concerning the convalescent patient sera used – it would be helpful to know infection timescales and severity, antibody titres and viral strains (if known).
- SARS-CoV-2 neutralising antibodies may recognize complex epitopes, including quaternary epitopes (Liu *et al*., https://doi.org/10.1038/s41586-020-2571-7) and the post-fusion S glycoprotein (Pinto *et al*., https://www.nature.com/articles/s41586-020-2349-y). This study administered vaccination boosts of purified recombinant SARS-CoV S protein that may have selected against the amplification of more potent anti-SARS-CoV-2 neutralising antibodies.
- The concentrations of recombinant proteins used in the in vitro blocking experiments seem very high (<100g/ml) and difficult to relate to any *in vivo* setting.

# Convalescent Plasma

## SARS-CoV-2 viral load and antibody responses: the case for convalescent plasma therapy

Casadevall, A. *et al*. 2020. *The Journal of Clinical Investigation*

**Link:** https://doi.org/10.1172/JCI139760

**Summary:**

Wang *et al*. describes the kinetics of viral load and antibody responses of 23 individuals with COVID-19 with mild and severe disease. The viral and antibody response kinetics of these patients reinforces the case for early convalescent plasma therapy. Given that this study shows antibody is absent in the first 10 days of illness, providing an amount of antibody that can induce viral clearance during this time may overcome this early antibody deficiency. These observations provide important information on the serological response to SARS-CoV-2 of hospitalized patients with COVID-19 that can inform the use of convalescent plasma therapy.

**Main Findings:**

- Stronger antibody responses may correlate with viral burden.
- Severely ill COVID-19 patients continued to shed virus despite having neutralizing antibodies.
- IgM was more likely to be present in plasma of individuals with severe disease, which may reflect increased B cell recruitment in the setting of a more exuberant inflammatory response.
- Convalescent plasma promotes viral clearance, even in severely ill patients, even when given more than two weeks after COVID- 19 onset.
- Sera from patients with severe disease had measurable IgM, whereas that of patients with mild disease did not.

**Highlights:**

1. Individuals with mild and severe disease produced neutralizing IgG to SARS-CoV-2 10 days after disease onset
2. SARS-CoV-2 persisted longer in those with severe disease
3. There was cross-reactivity between antibodies to SARS-CoV-1 and SARS-CoV-2, but only antibodies from patients with COVID-19 neutralized SARS-CoV-2.

**Impact for COVID-19 research:**

- The observation that patients may not mount measurable antibody responses before day 10 of illness, which then peak around day 15, suggests that convalescent plasma may be most beneficial early in the course of COVID-19, before an endogenous antibody response develops

**Methodologies:**

- Study Type: *Cohort study*

**Limitations:**

- Small patient sample size
- No information on the donors: did they suffer severe disease? How much antibody was present in their blood?

## Use of Convalescent Plasma in Hospitalized Patients with Covid-19 – Case Series

Hegerova, L. *et al.* 2020. *Blood*

**Link:** https://doi.org/10.1182/blood.2020006964

**Summary:**

Preliminary study seeing if convalescent plasma (CP) from donors who have recovered from COVID-19 could be beneficial to severe/critical COVID-19 patients. 20 patients received CP in addition to other therapeutics and their temperature, C-Reactive protein and lung function were compared to 20 control patients who did not receive CP therapy. Fewer patient deaths in the CP treated group (2, compared to 5 in the control group), though other laboratory/respiratory measures did not seem to differ between groups.

**Main Findings:**

- Convalescent Plasma (CP) was from 8 donors, who had respiratory symptoms >28 days previously and were not hospitalised, 7 were SARS-CoV-2 IgG+.
- Blood-type matched CP was given to 20 severe COVID-19 patients on average 2 days after hospitalisation, alongside other therapeutics (mainly azithromycin and hydroxychloroquine) and were monitored for the following 7 days.
- Most patients had a comorbidity such as hypertension or diabetes.
- Temperature and C-Reactive Protein decreased after CP administration, by 0.3°C and 43 mg/L on average respectively.
- Several respiratory measures were used to assess function, seemed to improve for patients on mechanical ventilator, though 2 patients required intubation after CP.
- A similar improvement was seen in control patients.
- A 20 % incidence of venous thromboembolism was reported, but same percentage seen in controls so attributed to COVID-19 rather than CP intervention.
- After 7 days 5 patients were discharged, and 2 died (had been intubated for >2 weeks). Remaining patients alive at day 14.
- 20 Control patients were matched for age, comorbidities and disease severity, 10 were on Remdesivir. After a week, 7 patients were discharged and 5 died. One more patient died by day 14.

- In support with other studies think CP intervention needs to happen early on.

**Highlights:**

1. Convalescent plasms may help improve outcome for patients with severe COVID-19 if given early.

**Impact for COVID-19 research**

- Low, needs to be tested in a larger population where same

**Methodologies:**

- Novel Techniques: *SARS-CoV-2 Serology determined by ABBOT Architect® and EuroImmun® antibody tests.*

**Limitations:**

- Small sample size.
- Only followed these patients for 7 days after treatment.
- All patients, control and CP, received other therapies and these therapies differed between the groups.
- Need to know how amount of neutralising antibody present in CP affects patients, and if it benefits patients who already have some neutralising antibodies.

## Improved Clinical Symptoms and Mortality on Severe/Critical COVID-19 Patients Utilizing Convalescent Plasma Transfusion

Xia, X. *et al*. 2020. *Blood*

**Link**: https://doi.org/10.1182/blood.2020007079

**Summary:**

Xia *et al*. provide a comprehensive evaluation of COVID-19 convalescent plasma (CCP) transfusion therapy for severe or critical COVID-19 patients. They analyse the clinical, radiologic and laboratory characteristics of 1568 severe or critical COVID-19 patients; out of which 138 patients received ABO-compatible CCP. Patients treated with CCP were categorised as responders, partial-responders, and non-responders. The study reports dynamic changes in viral load, SARS-CoV-2 specific antibody levels, cytokine levels and immune cell profiles before and after CCP therapy.

**Main Findings:**

- Within 2-weeks of CCP therapy, 20 out of 25 SARS-CoV-2 positive patients became virus free.

- The levels of SARS-CoV-2 spike- and receptor-binding domain specific IgG increased within 3 days post CCP treatment. In contrast, SARS-CoV-2 nucleoprotein-specific antibody levels remained unchanged.
- Percentage of lymphocytes significantly increased within 3 days of CCP treatment and were maintained at high levels even after 3-weeks. In contrast, neutrophil percentage and C-reactive protein (CRP) levels decreased post CCP therapy.
- Cytokine profiles (e.g. TNF-α, IL-10, and IL-6 levels) were not altered by CCP therapy.
- Apart from decrease in total bilirubin, there were no significant differences in cardiac, liver and/or renal functions before and after CCP treatment.
- Responders had a higher percentage of lymphocytes (20.1%) compared to partial-responders (11.9%) and non-responders (6.8%).
- Non-responders had significantly higher levels of CRP (73.1 mg/L) compared to partial-responders (22.9 mg/L) and responders (5.2 mg/L).
- Non-responders also had higher '*levels of lactate dehydrogenase, type B natriuretic peptide, urea nitrogen, procalcitonin, and glucose*' than responders' pre-CCP therapy.

**Highlights:**

1. CCP therapy within 7 weeks after symptom onset could improve clinical symptoms and mortality of severe/critical COVID-19 patients.
2. Patients with strong inflammatory reaction and/or abnormalities in metabolic functions were less responsive/insensitive to CCP therapy (e.g. CCP could not benefit extremely critical COVID-19 patients).

**Impact for COVID-19 research:**

- Analysis of large-scale clinical data helps evaluate the therapeutic potential of CCP, identify characteristics of responders and non-responders, and provides insights into timing and management of CCP therapy.

**Methodologies:**

- Study Type: *Cohort study*
- Comparison of clinical & laboratory characteristics between the standard-treated and severe/critical COVID-19 patients receiving CCP therapy. Changes before and after CCP treatment were also monitored.

**Limitations:**

- Clinical observations & data obtained from a single centre in Wuhan (some confounding factors such as biased patient allocation etc.).
- Incomplete data on neutralising antibody levels in CCP units. Additional assays are required to validate the correlation between neutralising antibody titres in donor plasma and efficacy of CCP therapy in recipients.
- Stratified analysis of severe and critical patients could not be carried out.

# Links to Influenza

## Single-cell sequencing of peripheral blood mononuclear cells reveals distinct immune response landscapes of COVID-19 and influenza patients

Zhu, L. *et al*. 2020. *Immunity (pre-print)*

**Link:** https://doi.org/10.1016/j.immuni.2020.07.009

**Summary:**

Zhu *et al.* performed single-cell RNA sequencing on PBMCs derived from healthy controls, Covid-19 patients and Influenza-A patients to characterise the immune, molecular and cellular signature that is unique to SARS-CoV-2 infection. Despite both viral infections targeting the respiratory tract and eliciting cytokine release syndrome during severe cases, they activate distinct signalling pathways in immune cells, namely STAT1 & IRF3 for Covid-19 and STAT3 & NFκB for Influenza. The authors also demonstrated genes associated with T cell apoptosis and B cell activation were upregulated in Covid-19. Furthermore, unlike Influenza, IL6R and IL-6ST gene expression was significantly increased in Covid-19. The data presented indicates genes that may provide useful for study in the pursuit of diagnostic biomarkers and potential therapeutic strategies.

**Main Findings:**

- B Cell activation genes (PRDM1, XBP1 & IRF4) were upregulated during SARS-CoV-2 infection
- T Cells & NK cells derived from Covid-19 patients had elevated expression of genes associated with a type I interferon response (ISG15, IFI44L, MX1 and XAF1), suggesting a strong anti-viral response was triggered by interferons. The patient with severe Covid-19 induced the greatest response.
- Expression of genes associated with T cell apoptosis (TNFSF10, TNFRSF10A, TNFRSF1B, FAS and FASLG) are increased in Covid-19 patients.
- SARS-CoV-2 infection activates STAT1 and IRF3 signalling pathways, whereas Influenza utilizes STAT3 and NFκB
- In comparison to influenza, SARS-CoV-2 infection induces a significant elevation in the expression of both IL-6R and IL-6ST

**Highlights:**

1. Plasma cells are significantly elevated in the blood of Covid-19 and Influenza patients.
2. A specific feature of Covid-19 patients is an elevation in the expression of genes associated with T cell apoptosis.
3. Covid-19 and Influenza infection activate distinct signalling pathways.

**Impact for COVID-19 research:**

- The data presented in this study supports previous studies, but provides details of the immune response at the single-cell level.
- This study highlights a number of genes related to the immune response to SARS-CoV-2 that warrant further study in the search for therapeutics.

**Methodologies:**

- Study Type: *ex-vivo analysis of PBMCs from healthy controls, Covid-19 and Influenza patients. Patients PBMCs were studied from symptom onset to discharge from hospital.*
- Novel Techniques: *Single-Cell RNA Sequencing of PBMCs from the cohorts of above. 15 immune cell clusters common to the three groups were identified. Differential gene expression and transcriptional profiles were analysed to identify the immune landscape present in the different groups*

**Limitations:**

- By the authors own admission, the sample sizes are small and should be increased (3 healthy controls, 2 Influenza A patients, and 5 Covid-19 patients)
- A comparable analysis of immune cells present at the site of infection (lung) is needed. This would determine if the data presented herein is an accurate representation of the genes and pathways upregulated in the immune cells at the infection site.
- Covid-19 patients with a range of disease severities is warranted to determine if there are certain biomarkers that can identify those patients that will go on to develop severe disease.

## Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19

Lee. J. S. *et al.* 2020. *Science Immunology*

**Link:** https://doi.org/10.1126/sciimmunol.abd1554

**Summary:**

'Cytokine storm' has been implicated in SARS-CoV-2 infection and is suspected to be a cause of severe disease. Peripheral blood mononuclear cells (PBMCs) from healthy donors, patients with mild or severe COVID-19 and severe influenza were collected for single-cell RNA sequencing (scRNA-seq). COVID-19 patients had hyperinflammatory signatures in PMBCs with high expression of TNF and IL-1β, not seen in severe influenza patients. Type 1 interferon (IFN-I) responses were found highly in classical monocytes of severe COVID-19 and influenza patients, but not mild COVID-19 patients. These data suggest a crucial role in type I IFNs driving inflammatory responses in severe COVID-19.

**Main Findings:**

- Using single cell RNA-sequencing (scRNA-seq) of PBMCs, severe COVID-19 and influenza patients had significantly higher proportions of classical monocytes whereas other immune cells such as dendritic cells (DCs), and effector memory $CD4^+$ T cells were significantly decreased.
- An upregulation in inflammatory response pathways were found commonly between COVID-19 and influenza, however in COVID-19 patients there were additional highly active pathways (NFKB1/2, STAT4) and underactive pathways (interferons, TCR adaptive immune responses) not found in influenza patients.
- Granzyme $B^+$ and IFN$\gamma$ cells among $CD8^+$ T cells were significantly higher in the influenza patients compared to the COVID-19 patients.
- IFN-I/-II pathways were upregulated in influenza patients $CD8^+$ T-cells, whereas pathways for TNF/IL-1$\beta$ were more upregulated in COVID-19 patients $CD8^+$ T-cells.
- Within classical monocytes, TNF was upregulated in COVID-19 patients whereas IL-1$\beta$ was upregulated in both COVID-19 and influenza.
- Using gene set enrichment analysis (GSEA), COVID-19 specific genes were found to be enriched by TNF/IL-1$\beta$ responsive genes whilst influenza-specific genes were enriched by both TNF/IL-1$\beta$ and IFN-I responsive genes. However, severe COVID-19 genes showed strong associations with both TNF/IL-1$\beta$ and IFN-I responsive genes; indicating IFN-I responses play a role in severe COVID-19.
- When comparing severe and mild COVID-19, severe patients had higher levels of TNF/IL-1-$\beta$ and IFN-I responsive genes, as well as cytokines IL-6 and IL-18.
- IFN-I and TNF/IL-1$\beta$ inflammatory responses were high in lungs of patients who died of lethal COVID-19 infection. Differentially expressed genes significantly associated with those of severe COVID-19 patients.

**Highlights:**

1. Use of scRNA-seq to analyse gene signatures.
2. Comparison of PBMC immune signatures in COVID-19 and severe influenza infection.

**Impact for COVID-19 research:**

- Implications for treatment of COVID-19 patients; understanding the inflammatory responses in mild vs severe COVID-19.

**Methodologies:**

- Study Type: *Cohort study, Bioinformatics*
- Novel Techniques: *10x Genomics Single cell RNA-sequencing*

**Limitations:**

- Small sample number.
- Using PMBCs rather than specimens from site of infection (BAL, etc).
- Differing results to a previous scRNA-seq study on PMBCs in COVID-19 patients by Wilk *et al.* (2020).

# Vaccines

## An mRNA vaccine against SARS-CoV-2 – Preliminary Report

Jackson, L.A. *et al*. 2020. *NEJM*

**Link:** https://doi.org/10.1056/NEJMoa2022483

**Summary:**

This is an interim report for a phase 1 clinical trial for the candidate vaccine mRNA-1273, which is a lipid nanoparticle-encapsulated mRNA-based vaccine that encodes the SARS-CoV-2 spike glycoprotein stabilized in its prefusion conformation. The results showed in this interim report corresponds to the evaluation of the safety and immunogenicity of mRNA-1273. In this report they show the effects of the mRNA-1273 vaccine in healthy adults. Showing the safety and effectiveness of the vaccine.

**Main Findings:**

- According to the safety and immunogenicity findings the mRNA-1273 vaccine has been approved to later-stage clinical trials

**Highlights:**

1. mRNA-1273 vaccine was immunogenic, inducing robust binding antibody responses.
2. Seroconversion was rapid for binding antibodies, occurring within 2 weeks after 1st vaccination
3. mRNA-1273 elicits CD4 and CD-8 T-cell responses.

**Impact for COVID-19 research:**

- The results obtained after this phase 1 clinical trial were satisfactory. According to this report phase 3 efficacy trial, expected to evaluate is anticipated to begin during the summer of 2020 and already a phase 2 trial in 600 healthy adults, evaluating doses of 50ug and 100ug is ongoing (check NCT04405076).

**Methodologies:**

- Study Type: *Phase 1 clinical trial*
- Important cell lines/viral models used: *mRNA-1273 vaccine; Participants: healthy adults 18-55 years.*
- Novel Techniques: *Trial design: Two injections 28 days apart at a dose of 25ug, 100ug, or 250ug. Follow up is scheduled for 7 and 14 days after each vaccination and on days 57, 11, 209, 394. Neutralizing responses were assessed by a pseudotyped lentivirus reporter single-round-of-infection neutralization assay (PsVNA) and by a live wild-type SARS-CoV-2 plaque-reduction neutralization testing (PRNT)*

**Limitations:**

- The number of participants was of 15 per group.

- For some assays results were available only for determined dates (mayoralty for days 1 and 43).
- The adverse events were graded according to a standard toxicity grading scale. Being graded as mild, moderate or severe.
- The durability of the immune response is not assessed in this interim report. Although participants are meant to be followed for 1 year.
- Participants were not screened for SARS-CoV-2 infection by serology or PCR before enrolment.

## An alphavirus-derived replicon RNA vaccine induces SARS-CoV-2 neutralizing antibody and T cell responses in mice and nonhuman primates

Erasmus, J.H. *et al*. 2020. *Science Translational Medicine*

**Link:** https://doi.org/10.1126/scitranslmed.abc9396

**Summary:**

Erasmus et al developed a vaccine candidate called repRNA-CoV2S, encoding the SARS-CoV-2 spike (S) protein. repRNA-CoV2S is an alphavirus-derived replicon RNA vaccine that was formulated with Lipid In Organic Nanoparticles (LION) to enhance vaccine stability, delivery, and immunogenicity. The vaccine candidate was tested in a prime only and a prime/boost regimen in mice and non-human primates and elicited robust production of anti-SARS-CoV-2 S protein IgG antibody as well as -in some cases- potent T cell responses. These results warrant further development of this vaccine candidate for prophylactic protection against SARS-CoV-2 infection.

**Main Findings:**

- The manufacturing process of repRNA vaccines is cell free and highly scalable. The LION formulation and repRNA can be stockpiled separately and mixed in a 1:1 ratio on site. LION is stable for at least 3 months when stored at 4 and 25°C and maintains full integrity and stability of repRNA for up to 7 days after mixing.
- Single intramuscular immunization of C57BL/6 mice with 10 or 1µg LION/repRNA-CoV2S resulted in 100% seroconversion by day 14 and induced neutralizing antibodies with an IC50 of 1:643 and 1:226, respectively. All groups responded to a boost immunization by increasing antibody concentrations. All responses were Th1 biased. Mice receiving a 10, 1, or 0.1 µg prime/boost showed robust functional T cell responses.
- 17 months old BALB/c mice exhibited significantly lower antibody responses than 2- and 8-months old mice after a single immunization but were able to approach similar levels after a boost immunization.

- LION/repRNA-CoV2S was well tolerated in pigtail macaques. Three animals received a prime-only immunization with 250µg LION/repRNA-CoV2S in 5 intramuscular injection sites and developed neutralizing antibodies with IC50 titres of 1:472, 1:108, and 1:149 by day 42. Two animals received prime/boost immunizations with 50µg in 1 intramuscular injection site and developed neutralizing antibodies with IC50 titres of 1:218 and 1:358 by day 42. These titres are comparable to those in human serum samples collected from individuals convalescing from COVID-19. The antibody responses persisted for at least 70 days. All 5 pigtail macaques developed only moderate T cell responses by day 28 post immunization. These were directed mostly towards the S1 and RBD region of the S Protein.

**Highlights:**

1. The LION/repRNA-CoV2S vaccine candidate elicited neutralising antibody titres in non-human primates that are comparable to those in serum from convalescent COVID-19 patients.
2. LION/repRNA-CoV2S induced moderate but detectable T cell responses and memory T cell responses in non-human primates.
3. LION/repRNA-CoV2S induced Th1 biased immune responses even in Th2-biased BALB/c mice (Th2 biased responses are associated with more severe COVID-19).

**Impact for COVID-19 research:**

- This research does not alter our view of COVIS-19, but contributes a potential vaccine candidate.
- This could benefit clinicians in the future as prophylactic protection against SARS-CoV-2 could be achieved in the population should the vaccine candidate be successful.

**Methodologies:**

- Study Type: *in vivo study in mice and non-human primates.*
- Novel Techniques: *SARS-CoV-2 pseudovirus neutralization assay*

**Limitations:**

- The 250µg dose was administered in 5 different injection sites (50µg each). This is unpractical in a clinical setting and it has to be determined if administering this dose in a single injection site is still well tolerated.
- n numbers are very small in pigtail macaque experiments (n=3 and 2)
- Animals have not been challenged, so it is unclear if the vaccine is protective. A pigtail macaque SARS-CoV-2 infection model is under development to answer this question.
- While specific memory T cells have been detected, later time points need to be tested to determine the longevity of the response.
- Clinical evaluation of the vaccine candidate is necessary to find out if a single or prime/boost regimen is needed to induce protective immunity in an optimal and cost-effective way.
- It is unclear what concentration and volume the booster immunization was.

## Immunogenicity and safety of a recombinant adenovirustype-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial

Zhu, F-C. *et al*. 2020. *The Lancet*

**Link:** https://doi.org/10.1016/S0140-6736(20)31605-6

**Summary:**

Following positive data from a phase 1 trial, Zhu *et al*. report data from the first double-blind, placebo-controlled phase 2 trial using a replicative-deficient adenovirus type-5 (Ad5) vaccine candidate. 508 healthy adults from one centre in Wuhan were randomised to receive either vaccine at $1x10^{11}$ or $5x10^{10}$ particles per mL or placebo, given as a single dose intramuscularly. Vaccination at both doses induced rapid humoral responses by day 14 and significant antibody and T-cell responses by day 28, with limited adverse reactions. Progression to a multi-centre phase 3 trial with $5x10^{10}$ viral particles is likely.

**Main Findings:**

- Specific antibody responses to RBD were detectable from day 14 post vaccination. By day 28, geometric mean titres increased to 656.5 and 571.0 in $1x10^{11}$ and $5x10^{10}$ viral dose groups, respectively. Seroconversion rates at day 28 were 96% and 97%.
- Vaccination with both doses induced a significant neutralising antibody response to live SARS-CoV-2 28 days post vaccination.
- Seroconversion of neutralising antibodies to live SARS-CoV-2 was seen in 59% and 47% of patients, respective of dose. Seroconversion of neutralising antibodies to pseudovirus occurred in 85% and 83% of participants.
- A positive correlation was seen between both antibody titres to RBD and neutralising antibody titres to pseudovirus with neutralising antibodies to live SARS-CoV-2.
- SARS-CoV-2 spike-specific IFNγ-ELISPOT T-cell responses were significantly induced at day 28 post vaccination. Vaccine dose had no significant impact ($1x10^{11}$ = 90%, $5x10^{10}$ = 885).
- Lower antibody responses to both RBD and live virus were seen in participants with high pre-existing anti-Ad5 immunity and those over 55 years of age, irrespective of dose. However, these factors didn't affect T-cell responses.
- More than 90% of vaccinated participants displayed either seroconversion of neutralising antibodies to live SARS-CoV-2 or T-cell responses at day 28 post vaccination.
- Fatigue, fever and headache were the most commonly reported solicited adverse reactions 14 days post vaccination. >70% of vaccinated participants reported at least one adverse reaction, which was significantly higher than the 37% of individuals in the placebo group ($p<0.0001$).
- 9% of participants receiving $1x10^{11}$ viral particles had a severe adverse reaction (mostly fever) at day 14, which was significantly higher than the lower dose ($p=0.0011$) or placebo ($p=0.0004$).

- High pre-existing anti-Ad5 immunity, increasing age, and being male associated with significantly lower occurrence of fever post vaccination.
- No serious adverse events were documented up to 28 days post vaccination.

**Research Highlights:**

1. Vaccination at $5x10^{10}$ viral particles per ml is safe and immunogenic, even in older individuals (>55), who would mostly benefit from vaccination. Older individuals do have a significantly lower humoral responses than younger but higher tolerability to the vaccine. Suggestion of a second injection for a phase 2b trial in older adults.

**Impact for COVID-19 research:**

- Positive data for a safe and effective vaccine candidate to combat COVID-19.

**Methodologies:**

- Study type*: Phase 2 Clinical Trial*
- Important cell lines/viral models used*: Live SARS-CoV_2 (MT291831.1) and a vesicular stomatitis virus pseudovirus system expressing the spike glycoprotein*
- Novel Techniques*: RBD-specific ELISA and neutralisation assays for Ab responses, IFNγ-ELISPOT for T-cell responses*

**Limitations:**

- Study was conducted before data from phase 1 trial was made available, so group sizes and were assumed before power analysis could be conducted.
- Study only contains adults (no children) from one centre in Wuhan. Levels of pre-existing Ad5 immunity is usually defined by location so would be interesting if similar trends are seen in phase 3.
- Unable to assess vaccines potency against SARS-CoV-2 challenge in participants.
- Long-term data (6 months) unavailable currently.

## Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial

Folegatti, P.M. *et al*. 2020. *The Lancet*

**Link:** https://doi.org/10.1016/S0140-6736(20)31604-4

**Summary:**

Folegatti *et al*. describe a phase 1/2 controlled study comparing the ChAdOx1 nCoV-19 vaccine candidate expressing the SARS-CoV-2 spike protein, against a control MenACWY vaccine. This study is the first of its kind, showing promising preliminary evidence

that the vaccine candidate well tolerated with no serious adverse side-effects. It elicits an immune response comparable to antibody levels observed in convalescent plasma, which is a helpful comparison for understanding the magnitude of this immune response. Although both neutralising antibodies and a T-cell immune response were induced by the vaccine, more information is needed to assess whether these responses will have a protective effect against SARS-CoV-2 virus in the future.

**Main Findings:**

- Majority of adverse effects events in response to ChAdOx1 nCoV-19 were mild or moderate in severity and all self-limiting. Use of prophylactic paracetamol increased tolerability
- Antibody responses to the spike protein were maximal at day 28 in the single dose group and increased by day 35 in the 2-dose group. Neutralizing antibody responses against SARS-CoV-2 were also detected in the single and 2-dose groups.
- T-cell responses were observed as early as day 7, peaked at day 14 and maintained up to day 56. However, no boost in cellular responses was observed in the 2-dose group.

**Highlights:**

1. Preliminary results of safety, reactogenicity and immunogenicity of a viral vectored coronavirus vaccine expressing the spike protein of SARS-CoV-2.

**Impact for COVID-19 research:**

- ChAdOx1 nCoV-19 could be used to prevent infection, disease and death in the population, following further clinical studies.

**Methodologies:**

- Study Type: *Phase 1/2 randomised single-blind controlled clinical trial*
- Novel Techniques: *Total IgG ELISA against trimeric SARS-CoV-2 spike protein, three live SARS-CoV-2 neutralisation assays, pseudovirus neutralisation assay, ex-vivo IFN-γ ELISpot assay.*

**Limitations:**

- Short follow-up period reported. However, authors mention that participants will be followed up for at least 1 year.
- Although correlations with convalescent plasma are reported, it is not yet known what types and magnitudes of immune response is required for protection against SARS-CoV-2 infection.
- The trial only included participants as old as 55 years of age and the population was not ethnically diverse. More information on safety and immunogenicity in older and more diverse populations is required and is ongoing.

# Virology

## The D614G mutation in the SARS-CoV-2 spike protein increases infectivity in an ACE2 receptor dependent manner

Ogawa, J. *et al.* 2020. *bioRxiv*

**Link:** https://doi.org/10.1101/2020.07.21.214932

**Summary:**

Overtime SARS-CoV-2 has undergone positive selection resulting in the co-occurrence of a D614G mutation in the viral spike protein and within the ORF1b P314L. This aim of this paper was to determine the effect of the D614G mutation binding to Human ACE2 receptor and its effect cell fusion and infectivity, independent of the ORF1b P314L mutation. Also, the authors aimed to investigate the effect of the spike D614G mutation on infectivity.

**Main Findings:**

- The D614G variant spike has a >1/2 log10 increased infectivity in human cells expressing human ACE2 without the contribution of ORF1b P314L.
- Observe an increase in binding and fusion activity in cells co-expressing spike D614G and ACE2.

**Highlights:**

1. A mutation in the SARS-CoV-2 spike protein (D614G) results in increased infectivity of ACE2 expressing cells.
2. The D614G mutant is able to increase membrane fusion in ACE2 expressing cells.

**Impact for COVID-19 research:**

- Informs us that SARS-CoV-2 is capable of undergoing positive selection and has potential to become more infectious over time.
- This research does not have any direct impact in a clinical setting.

**Methodologies:**

- Study Type: *in vitro*
- Important cell lines/viral models used: *293T cells, pseudotyped lentiviral vectors expressing wildtype and mutant SARS-CoV-2 spike proteins.*
- Novel Techniques: *Cell fusion assay via Flow cytometry*

**Limitations:**

- Fairly preliminary data reported, potential to expand further.
- Fusion data only showed modest increase in fusion.
- Pseudotyped viruses used, not shown in the context of whole virus, therefore limits data interpretation.

- Unable to explain why they observed increased infectivity as mutation is not predicted to affect ACE2 binding.

## Kinetics of viral load and antibody response in relation to COVID-19 severity

Wang, Y. *et al*. 2020. The Journal of Clinical Investigation

**Link:** https://doi.org/10.1172/JCI138759

**Summary:**

Wang *et al*. showed diverse viral shedding of SARS-CoV-2 in patients with mild and severe COVID-19. Viral RNA was found in multiple tissue sites in patients with severe disease up to 40 days post the onset of disease, however in patients with mild COVID-19 viral RNA was restricted to the lungs and undetectable after 10 days. Furthermore, the authors found anti-SARS-CoV-2 IgG antibodies in most patients with COVID-19 10-15 days after disease onset which persisted for a minimum of 6 weeks (the duration of this study). In line with other reports, SARS-CoV-2 antibodies isolated from COVID-19 patients were found to cross-react on other coronaviruses such as SARS-CoV. Finally, patients with severe COVID-19 had higher titres of SARS-CoV-2 neutralising antibodies compared to those with mild disease.

**Highlights:**

1. Different patterns of viral shedding between patients with severe and mild COVID-19. Patients with severe disease had viral shedding in multiple tissue sites outside of the respiratory tract which lasted up to 40 days post infection. Patients with mild COVID-19 had viral shedding restricted to their respiratory tract and viral RNA was undetectable after 10 days.
2. Lower IgM responses in patients with mild COVID-19 compared to those with severe disease, in most mild cases (8/11) insufficient IgM was produced therefore IgM monitoring is not efficient for diagnosis of mild COVID-19 disease
3. IgG was detectable after 10-15 days in most patients regardless of disease severity and remained for the duration of the study (minimum of 6 weeks). No SARS-CoV-2 antibodies were detected in healthy donors.
4. Cross reactivity of IgG antibodies from severe and mild patients against SARS-CoV and other coronaviruses such as HCoV-299E, NL63, HKU1 and OC43. But not to the other pathogenic coronavirus MERS-CoV.
5. Patients with severe COVID-19 had higher titres of neutralising antibodies which could

**Impact for COVID-19 research:**

- The difference in the length of time viral RNA is detectable in patients is dependent on the severity of disease. The extent of viral shedding is much greater in patients with

severe COVID-19 compared to patients with mild disease. Both of these findings could have an impact on the treatment of COVID-19 patients.

- This study is in line with previous studies showing that patients with COVID-19 produce neutralising antibodies to SARS-CoV-2. It also complements research indicating cross reactivity of immune responses to SARS-CoV-2 and other coronaviruses such as SARS-CoV, and uses a large cohort of healthy control (n=96)
- This study adds to the current knowledge surrounding the immune response to SARS-CoV-2 and its relationship with clinical outcomes in COVID-19 patients. The study corroborates with other research papers indicating that the level of antibody response is correlated to disease severity and that antibodies produced can cross react on other coronavirus subtypes. Furthermore, this study shows that viral shedding is more widespread in patients with severe disease and can last up to 40 days post infection. This is important in terms of the diagnosis and prognosis of patients admitted to hospital with COVID-19.

**Methodologies:**

- RT-PCR to confirm SARS-CoV-2 infection
- IgG and IgM ELISA to compare antibody responses to different SARS-CoV-2 proteins
- Luciferase reporter based pseudotype neutralisation assay to assess neutralisation activity of patients plasma
- Focus reduction neutralisation test to assess neutralisation effect of patients antibodies on SARS-CoV-2 virus

**Limitations:**

- Low number of patients enrolled in the study (N=12 severely ill patients and n=11 mildly ill patients)

# Epidemiology

## Dynamics of SARS-CoV-2 with Waning Immunity in the UK Population

Crellen, T. *et al.* 2020. *medRxiv*

**Link:** https://doi.org/10.1101/2020.07.24.20157982

**Summary:**

Crellen and colleagues used a mathematical model to explore different potential immunity dynamics to SARS-CoV-2. Under their worst-case scenario (immunity lasting an average of three months for non-hospitalised individuals, one year for hospitalised individuals and an effective reproduction number ($R_t$) post-lockdown of 1.2), a secondary peak is likely to occur in winter 2020. The authors suggest that longitudinal serological surveys to determine if immunity is waning should be done from the end of lockdown until autumn 2020. This study suggests that strategies designed to achieve herd immunity may lead to repeated waves of infection if immunity to re-infection is not permanent.

**Main Findings:**

- Secondary peak is expected in spring 2021, if $R_t = 1.1$, or winter 2020, if $R_t = 1.2$;
- Height of the secondary peak is dependent of the rate at which immunity is lost;
- Timing of secondary peak in autumn 2020 is synchronised in all immunological scenarios analysed;
- Longitudinal serological surveys to assess waning immunity should be most informative when conducted between June and September 2020**;**
- If $R_t > 1$ after lockdown, transmission of the virus becomes unsustainable and virus reaches extinction between April and November 2021;
- If $R_t = 1$ after lockdown, if immunity is permanent, epidemic becomes extinct in May 2022. If immunity wanes, there is no secondary peak, but infections persist at a low level;
- If $R_t < 1$ after lockdown, there will be subsequent peaks of infection over the next 5 years until it reaches a steady state.

**Highlights:**

1. Waning immunity impacts on the height of the secondary peak and, in the absence of future interventions, establishes the virus at levels of endemic equilibrium that could overwhelm contact tracing services and ICU capacity.
2. Higher immunity among individuals of working age has the effect of slowing the epidemic when immunity is permanent. When immunity wanes, previously infected individuals of working age re-join the susceptible pool, leading to a high growth rate and a larger secondary peak of infected cases.
3. The projected trajectory of the epidemic after lockdown is highly sensitive to $R_t$ values, showing the importance of timely and accurate estimates of $R_t$ to inform control strategies, and ensuring widespread community testing and contact tracing is in place.

**Impact for COVID-19 research:**

- This study presents considerations for policy makers on the longer-term dynamics of SARS-CoV-2 and suggests that strategies designed to achieve herd immunity may lead to repeated waves of infection if immunity to re-infection is not permanent.

**Methodologies:**

- Study Type: *In silico study*
- Novel Techniques: *Mathematical model that has into account key features of the UK epidemic, contact matrices from a comprehensive study of contact patterns in the UK, and demographic data from the Office for National Statistics. Code is available online.*

**Limitations:**

- Article has not been peer-reviewed yet.
- The model does not consider regional differences in transmission rates and it does not include deaths. It also does not include transmission in settings like hospitals or care homes.