

Scientific update on COVID-19

Updated on April 19th 2021

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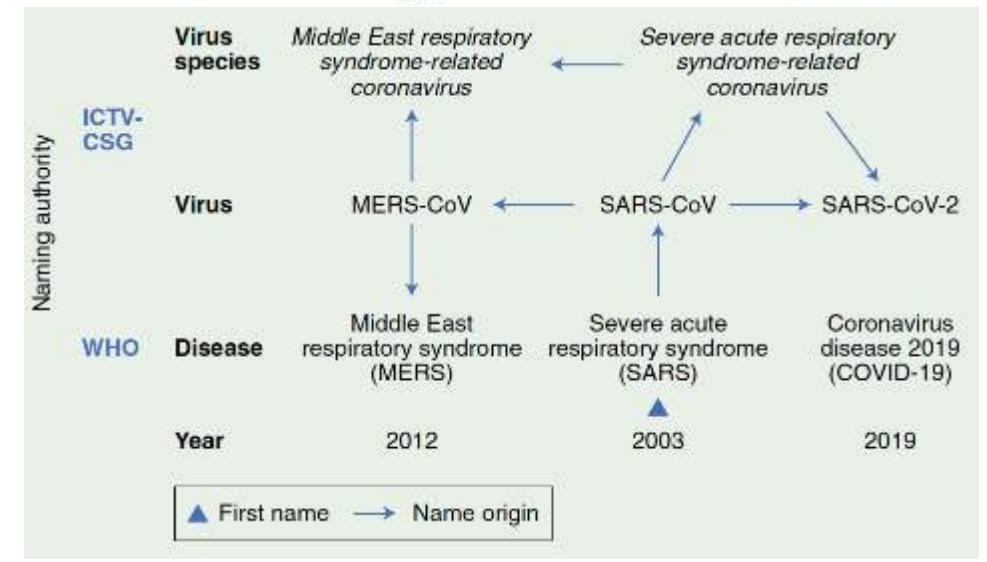
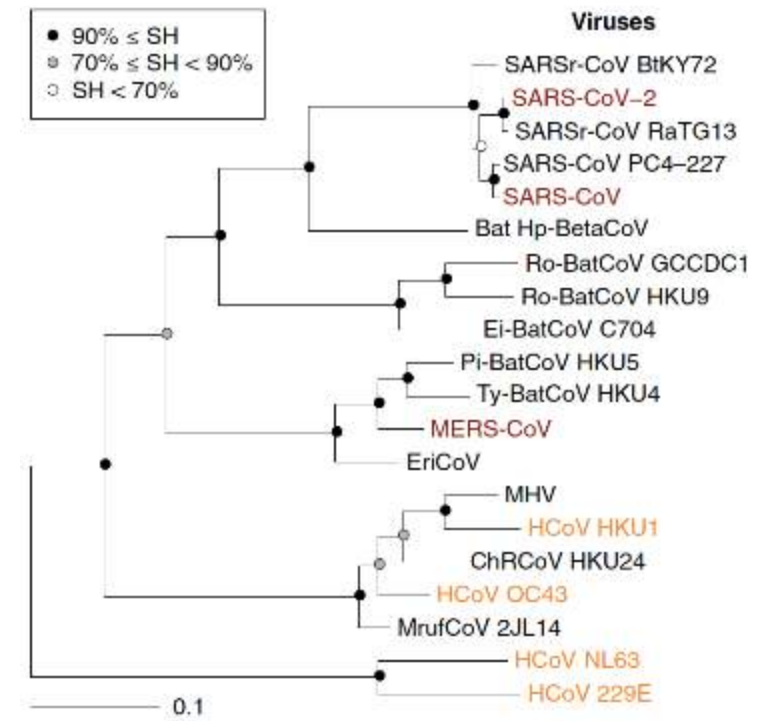
Benoit Visseaux – *Inserm, AP-HP Bichat*

Questions:

- Which type of virus is SARS-CoV-2?
- What is the stability and viability of SARS-CoV-2?
- What do we know about viral load and shedding according to different samples?
- Alternative to the nasopharyngeal swab for SARS-CoV-2 detection?
- What is the impact of mutations of SARS-CoV-2? What are the characteristics of the current Variants of Concern?

SARS-CoV-2

- Part of family of enveloped positive-strand RNA viruses (*coronaviridae*)
- Belongs to the *betacoronavirus* genus
 - 98% similarity with bat coronavirus RaTG13
 - 79% genetic similarity with SARS-CoV
- 7 coronaviruses known to infect humans
 - 4 coronavirus infect mainly the upper respiratory tract
 - HCoV HKU1 – OC43 – NL63 – 229E
 - 3 coronavirus can replicated in lower respiratory tract and cause pneumonia with high case fatality rates
 - SARS-CoV = Case Fatality Rate (CFR) of 10% (2002 – 2003)
 - MERS-CoV = CFR of 37% (2012 -)
 - SARS-CoV-2 = CFR unknown (2019 -)



Stability of SARS-CoV-2

IN VITRO

Outcome: positive viral culture

Surface stability

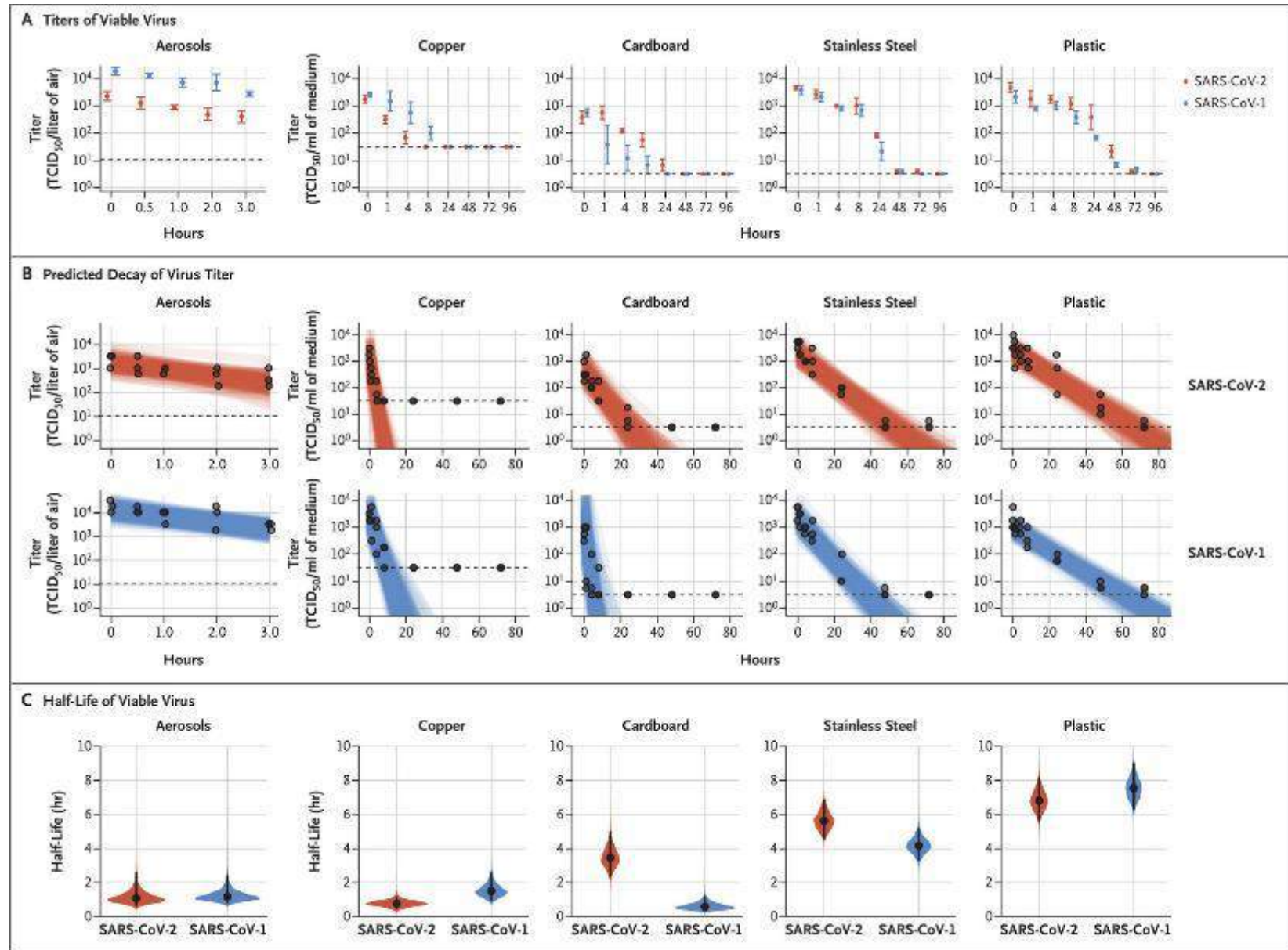
- Plastic and stainless steel: **72 hours**
- Cardboard: **24 h**
- Copper: **4 hours**

Viable in aerosol: **3 hours**

Half-life in aerosol:

- **1.1 to 1.2-h [0.64 – 2.24]**

Aerosol transmission is possible in experimental conditions



Persistence of virus RNA

49 patients with 490 specimens → 171 specimens positive for SARS-CoV-2 RNA

Frequency and duration of detectable SARS-CoV-2 RNA in body fluids?

Weibull model → time loss of SARS-CoV-2 RNA detection

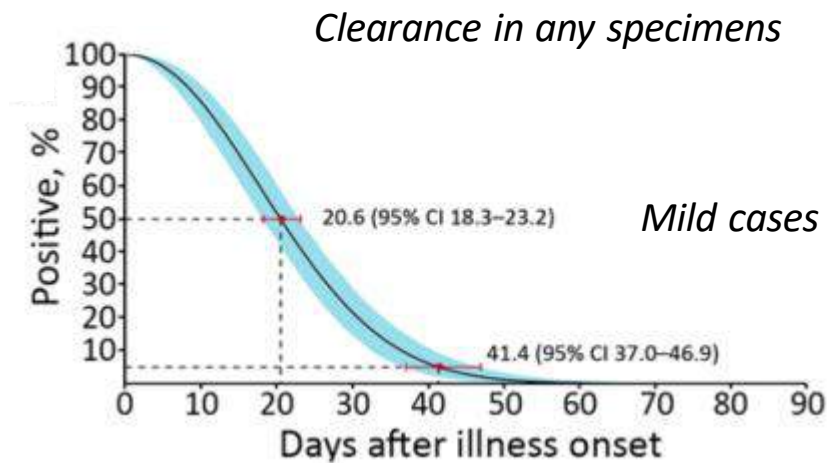
Time to loss detection

- Time to loss detection was longer for NP swabs and feces
- Significant differences for mild cases among specimens

Prolonged persistence of SARS-CoV-2 RNA detection in hospitalized patient

→ Does not imply the existence of infectious virus particles

→ Still a need for preventive measures?



Limits

- Existence of infectious particles?
- Virus isolation and tests of specimen's infectivity
- not conducted
- Unspecified concentration of SARS-CoV-2 RNA
- May not be generalized to all population

Data are presented in
days after illness
onset

Specimens	Mild cases, n = 43		Severe cases, n = 6	
	Median (95% CI)	95th percentile (95% CI)	Median (95% CI)	95th percentile (95% CI)
Throat swab	15.6 (11.8–20.7)	32.8 (25.9–42.3)	33.9 (24.2–47.3)	53.9 (39.4–81.7)
Sputum	20.0 (14.1–27.0)	43.7 (33.6–60.4)	30.9 (23.5–39.1)	44.7 (36.3–58.0)
Nasopharyngeal swab	22.7 (18.8–27.5)	46.3 (39.0–55.2)	33.5 (25.7–42.7)	49.4 (38.4–68.5)
Feces	24.5 (21.2–28.3)	45.6 (40.0–52.8)	32.5 (26.3–39.1)	48.9 (41.3–59.7)

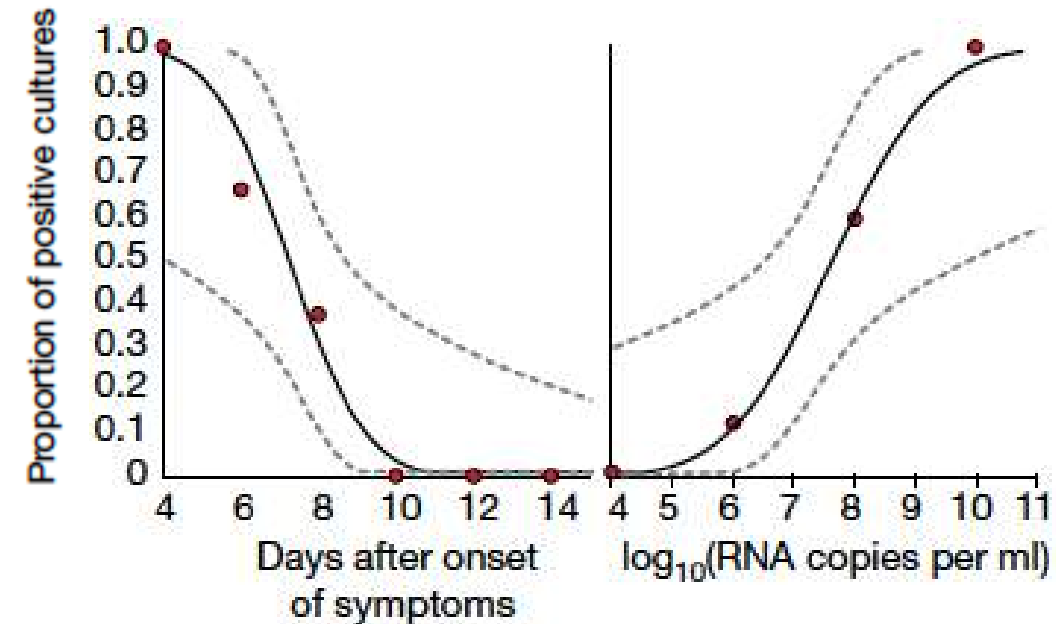
Viability

9 patients (Munich) – Virological analysis & information on virus infectivity

- Active virus replication in tissues of the upper respiratory tract
- No indications of replication in the digestive system
- Infectious virus on swab or sputum samples but not from stool samples
- None of urine and serum samples tested positive for RNA for SARS-CoV-2
- The success of virus isolation also depend of viral load

- **No isolates of the virus were obtained from samples taken after day 8 in spite of ongoing high viral loads.**

Virus isolation success based on probit distributions



Viral load

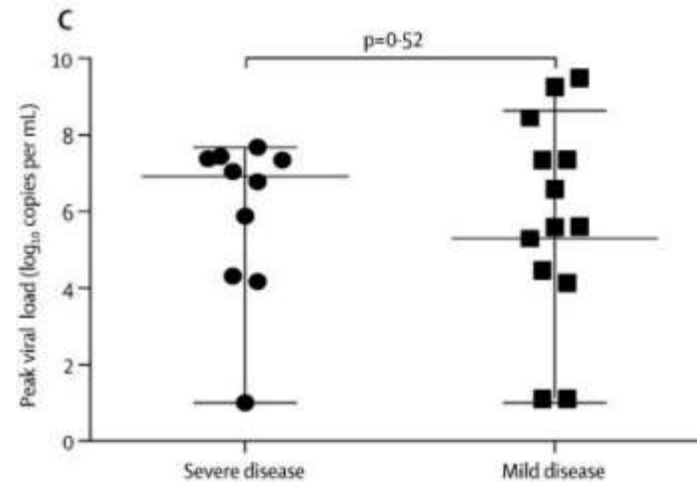
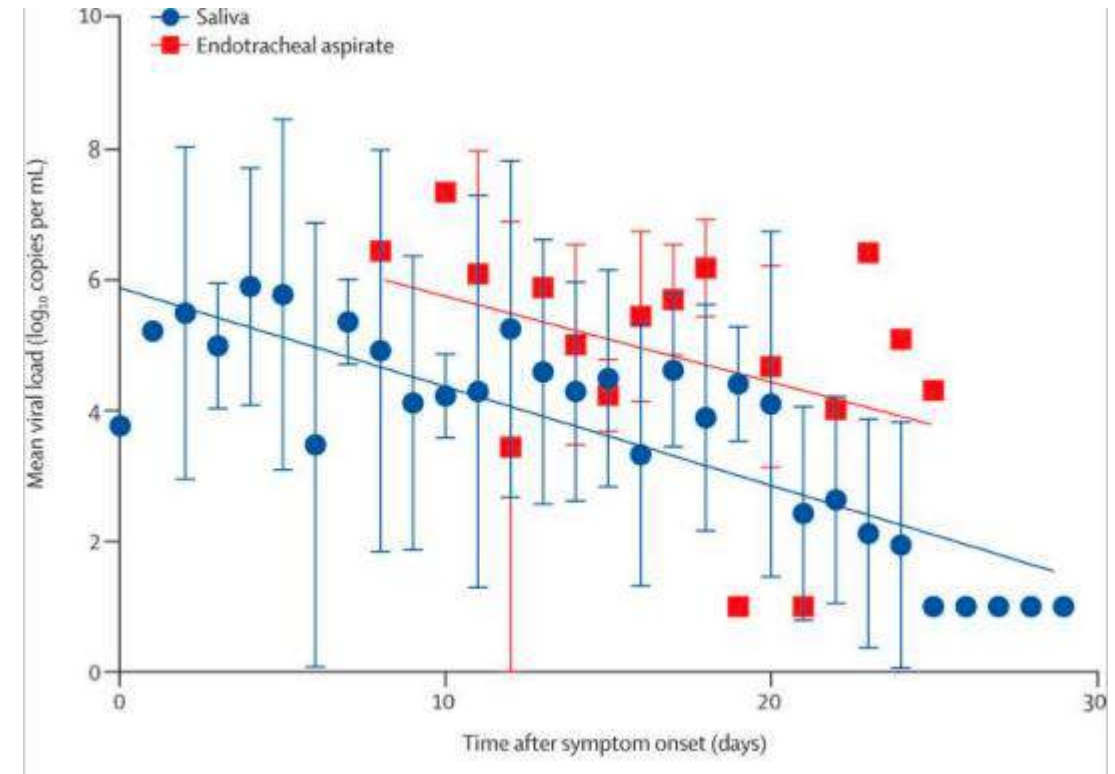
23 patients (median age: 62y) in Hong Kong → 173 respiratory specimens

- Morning saliva samples
- Endotracheal aspirate (intubated patients)

Viral load:

- Median: 5,2 log₁₀ copies per mL (IQR 4,1–7,0)
- Saliva viral load: higher during first week and declining after this point
- Endotracheal aspirate viral load: non-significant decline during the first weeks
- 7 patients had viral RNA detected 20 days after symptoms
- No association between prolonged detection and severity
- Older age was correlated with higher viral load
- No difference between mild and severe cases

Limit: low number of cases



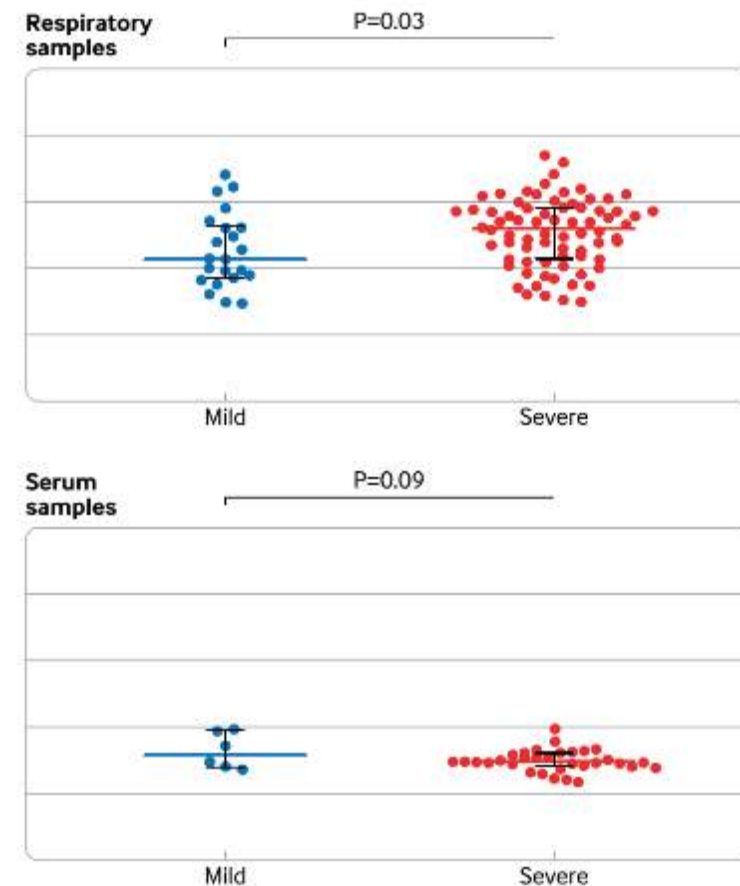
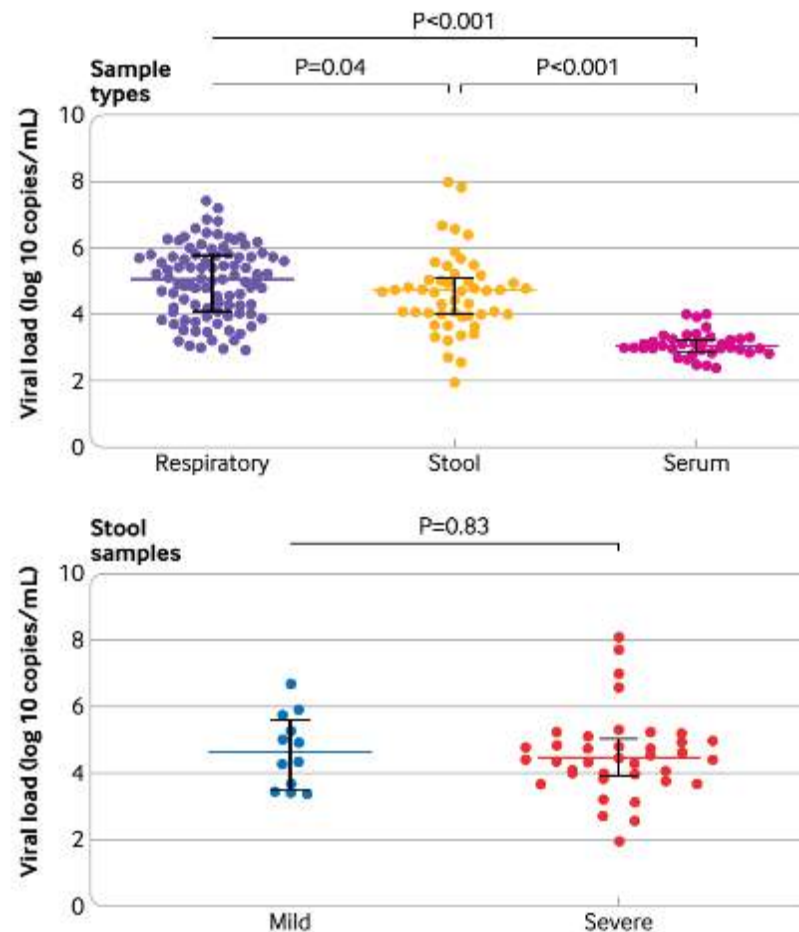
Viral load

96 patients (22 with mild disease and 74 with severe diseases) in China

Viral load:

- Duration of virus shedding in respiratory samples longer among severe patients (21 vs 14 days), also longer in patients >60 years old and male.
- 59% of patients with positive stool samples and presenting a longer viral shedding in stool than respiratory sample (22 vs 18 days).
- Viral load were slightly higher among severe cases.

Limit: a relatively low number of cases



Viral load

205 patients (mean age: 44y) → 1070 respiratory specimens:

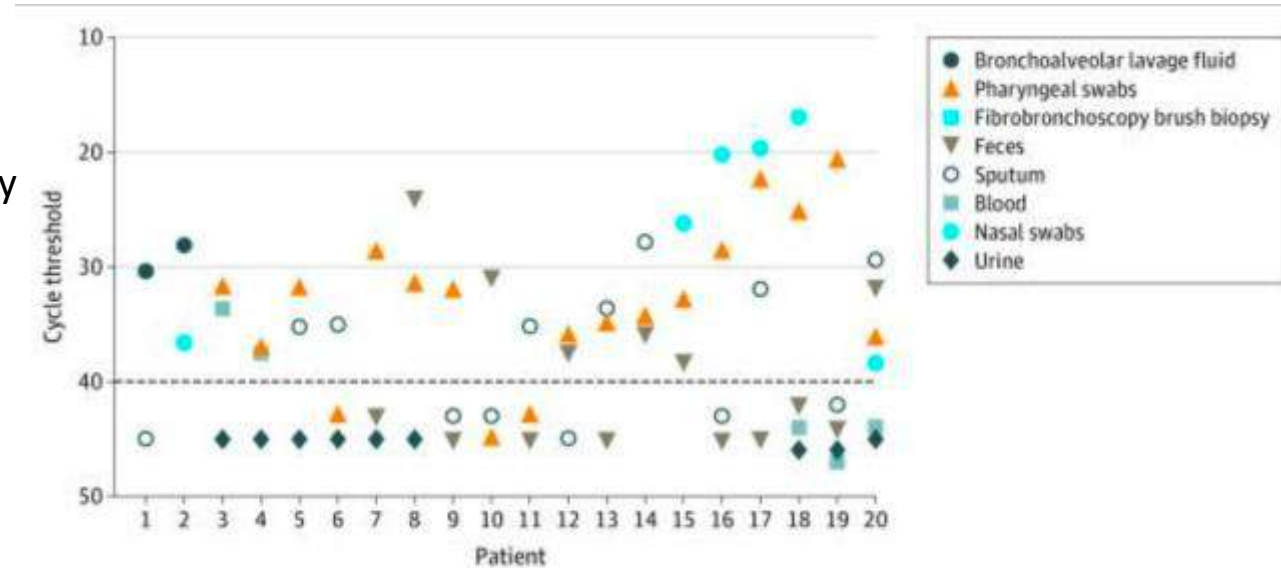
- Pharyngeal swabs, urine, sputum, blood, feces
- Bronchoalveolar lavage fluid & fibro bronchoscopy brush biopsy

Cycle threshold: indicator of the copy number of SARS-CoV-2 RNA

Cycle threshold < 40 → positive for SARS-CoV-2 RNA

Positive rates:

- Highest positive rates → bronchoalveolar fluid (93%)
- Sputum (72%) – pharyngeal swabs (32%)
- Blood showed only 1% and urine 0%
- Mean cycle threshold for nasal swabs = 24,3 → higher viral load



→ Testing of specimen from multiple sites
 ↑ sensitivity & ↓ false negative

Limit: this differ according to the typology of patients and disease stages.

Dynamic in viral shedding

94 symptomatic patients → 414 throat swabs from symptoms onset up to 32 days after

- Detection limit was Ct=40 (used to indicate negative samples)
- 50% were male
- Median age: 47 years
- No severe or critical patients

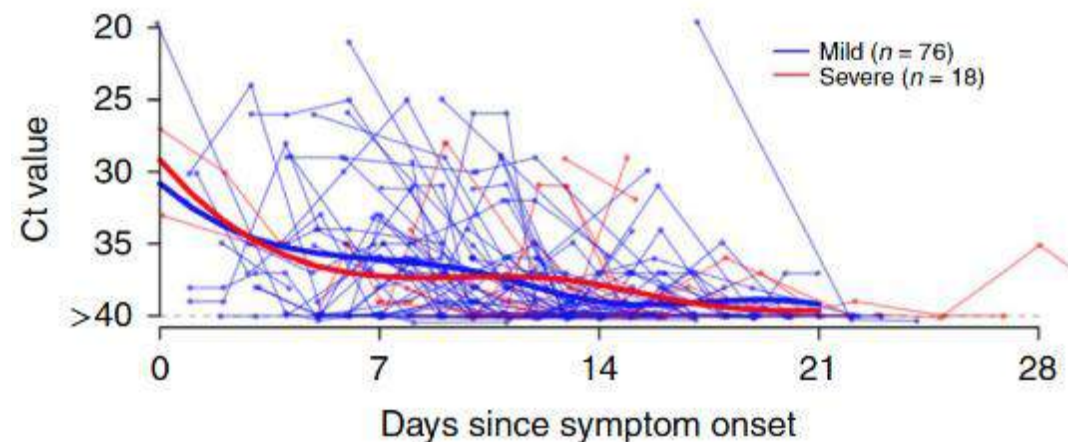
Dynamic in viral shedding

- Highest viral load soon after symptom onset
- Decreasing gradually after symptom onset
- **No difference in viral loads across sex, age groups, disease severity**

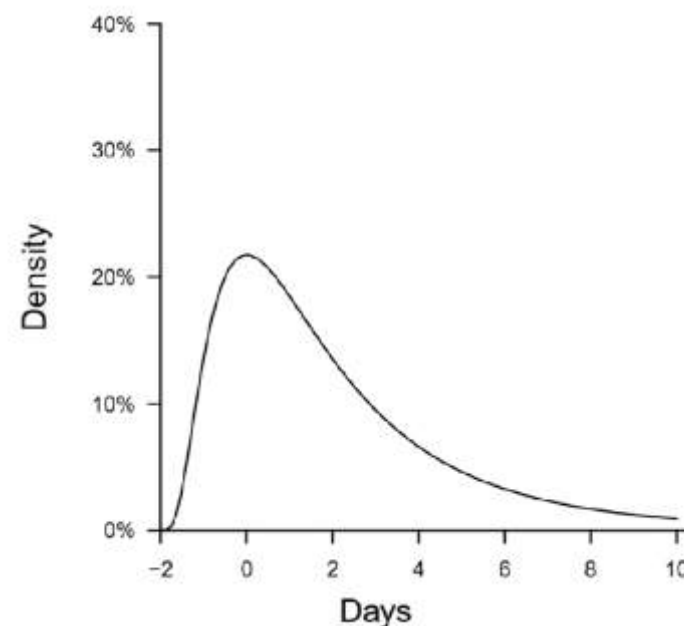
Viral shedding may begin 2 to 3 days before first symptoms

The estimated proportion of **presymptomatic transmission was 44%**

(CI_{95%} [30–57%]). Infectiousness decline quickly within 7 days



Viral load detected by RT-PCR in throat swabs from patients infected with SARS-CoV-2



Simulated serial intervals assuming infectiousness started 2 days before symptom onset

Oral & fecal viral shedding

401 patients → 1758 rectal swabs during 0 to 98 days after illness onset

- 80 patients positive for SARS-CoV-2 in the rectal swabs
 - Pediatrics: positive rate of 56,7%
 - Adults: positive rate of 16,9%
- Positive rate decreases over time

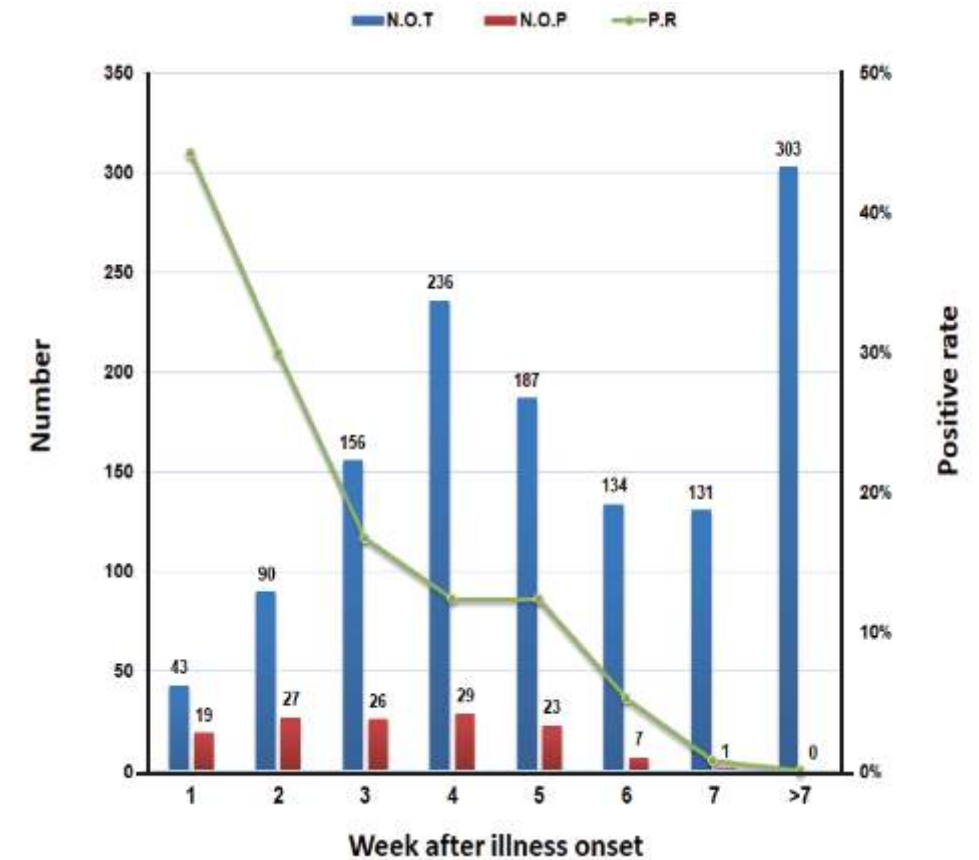
517 pairs (respiratory + rectal samples) from the 80 patients positive in rectal swabs

- 58 were double positive → coincidence rate increased during the disease progression
- 112 positive in rectal & negative in respiratory sample
- Higher viral load in rectal than respiratory samples

Factors independently associated with the duration of fecal viral shedding:

- Neutrophil level OR:1,55 IC_{95%}[1,05 – 2,40]
- Interval between antiviral treatment and illness onset OR:1,17 IC_{95%}[1,01 – 2,34]

NOT: number of tested - NOP: number of positive - PR: positive rate ¹²



→ Intestine = reservoir of SARS-CoV-2 RNA

The gastrointestinal viral reservoir is potentially a long-lasting fomite for SARS-CoV-2 transmission even for asymptomatic patients

→ **Still viable virus?**

Positivity of viral culture

Viral culture is only rarely positive for low viral load (Ct values above 25 to 30) and after 8 to 10 days after symptom onset

Viral culture is not positive for feces sample

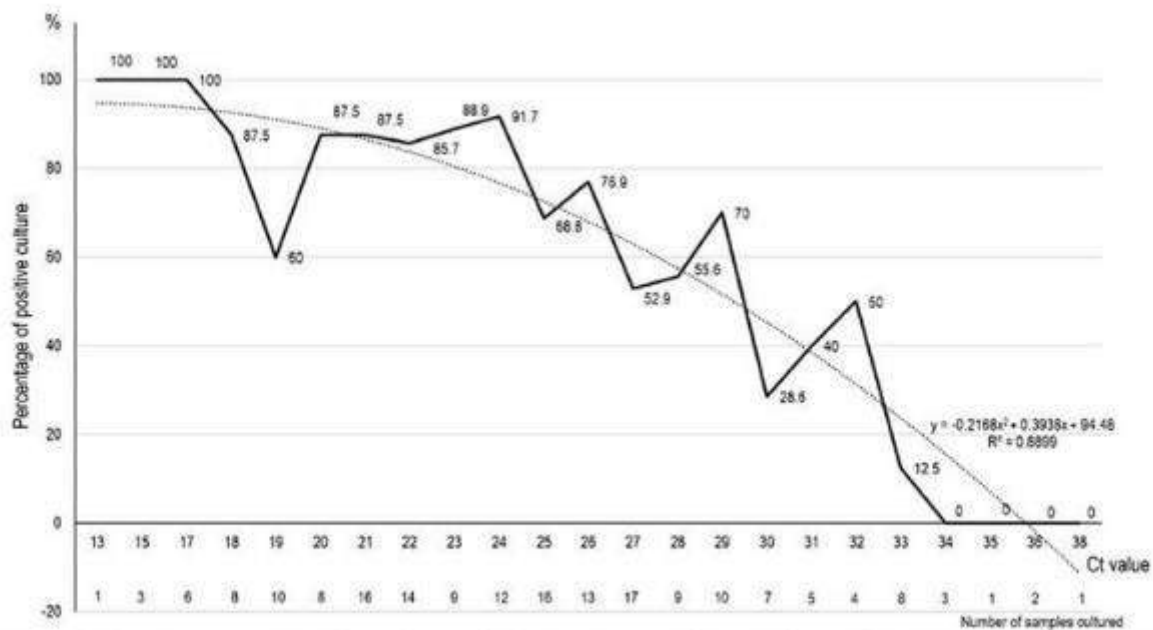
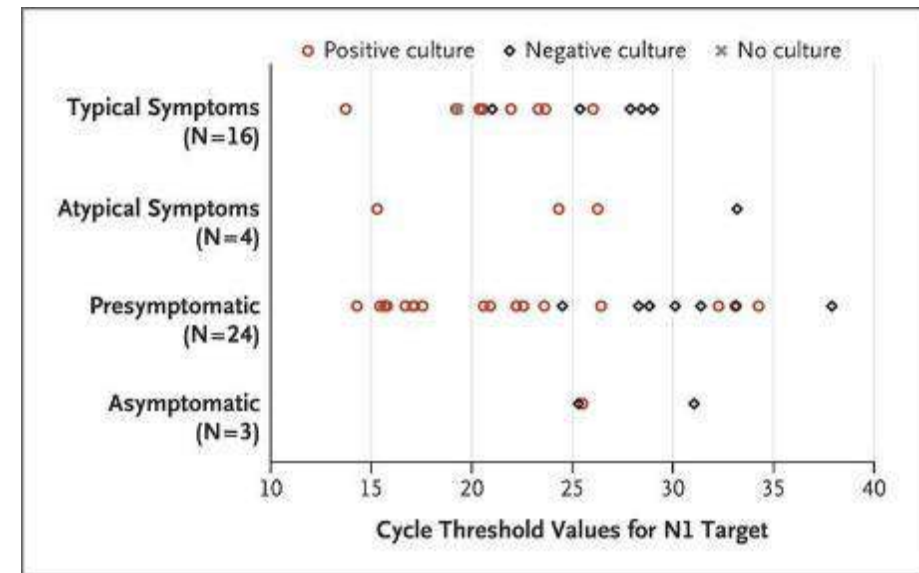
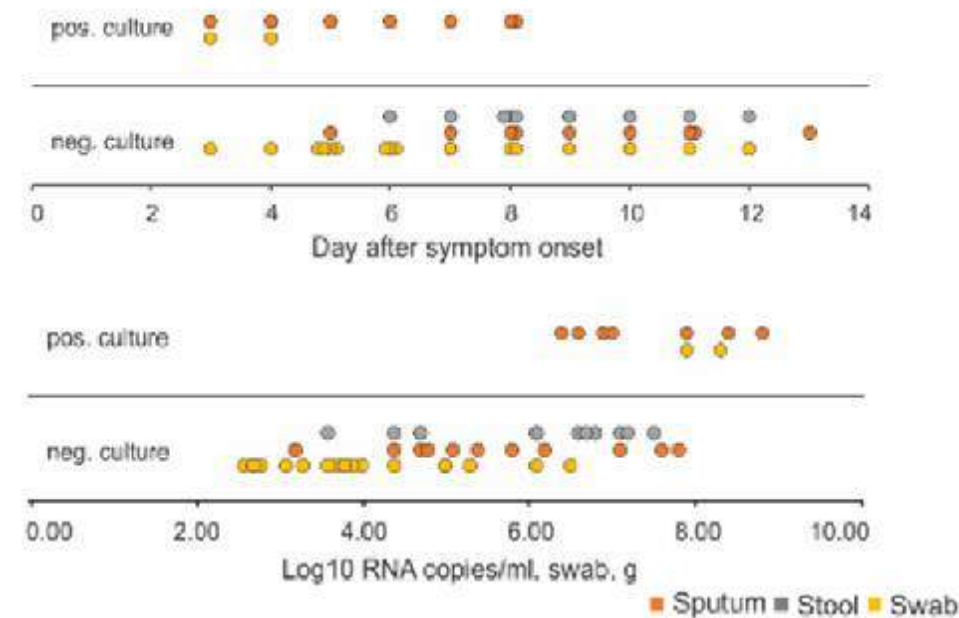
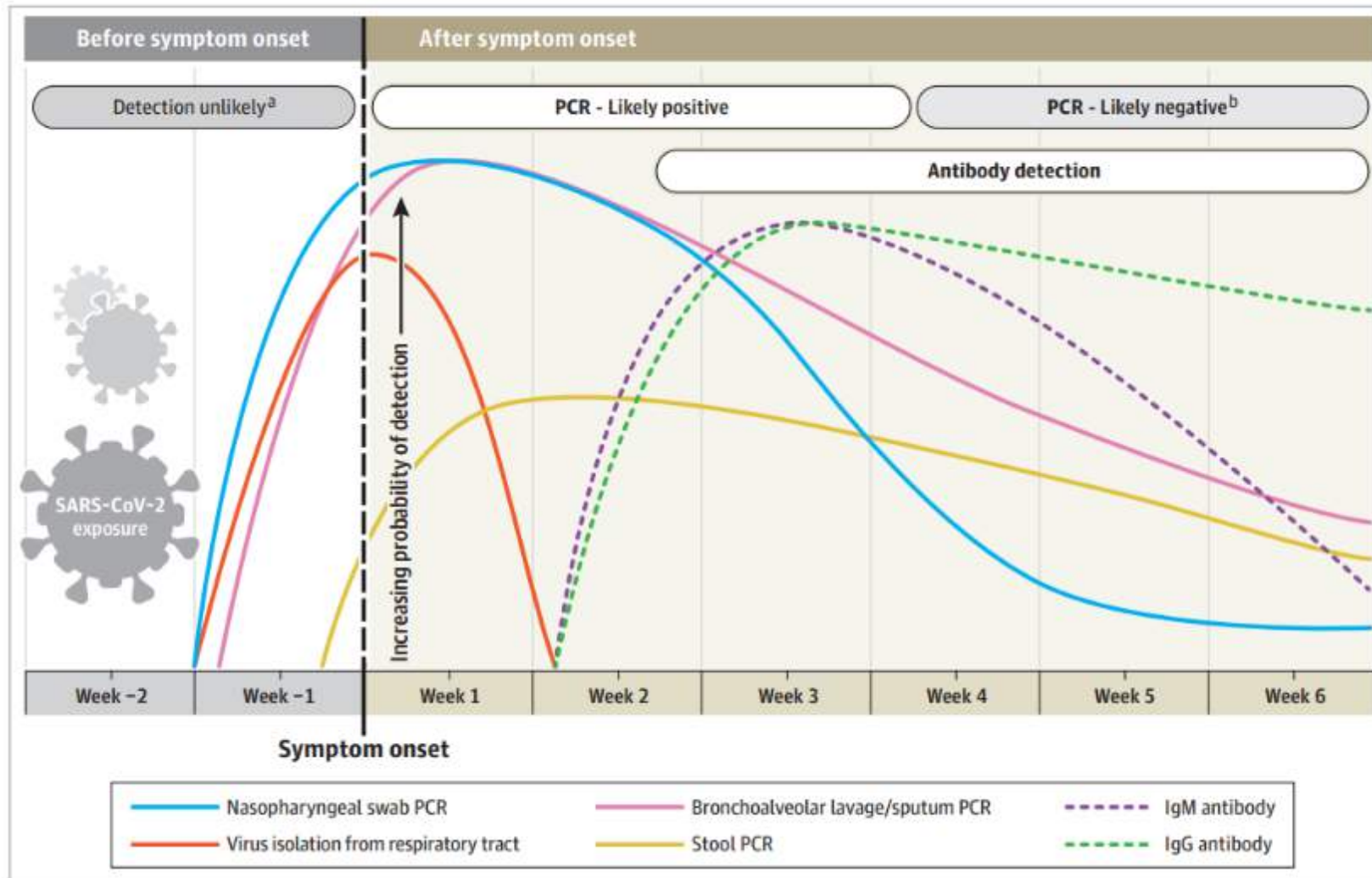


Fig. 1 Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve



SARS-CoV-2 detection



Limit: antibody response yet to be characterized among the various patients' populations

Estimated time intervals and rates of viral detection are based on data from several published reports. Because of variability in values among studies, estimated time intervals should be considered approximations and the probability of detection of SARS-CoV-2 infection is presented qualitatively. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction.

^a Detection only occurs if patients are followed up proactively from the time of exposure.

^b More likely to register a negative than a positive result by PCR of a nasopharyngeal swab.

SARS-CoV-2 salivary detection

Rapid and accurate diagnostic tests are essential for controlling the ongoing Covid-19 pandemic

70 patients hospitalized with COVID-19 (nasopharyngeal swabs).

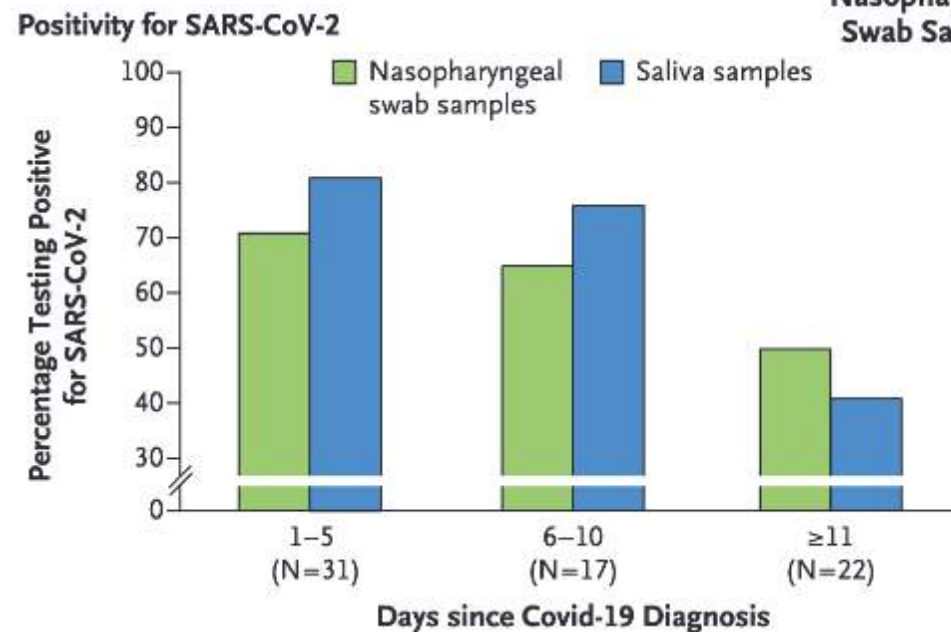
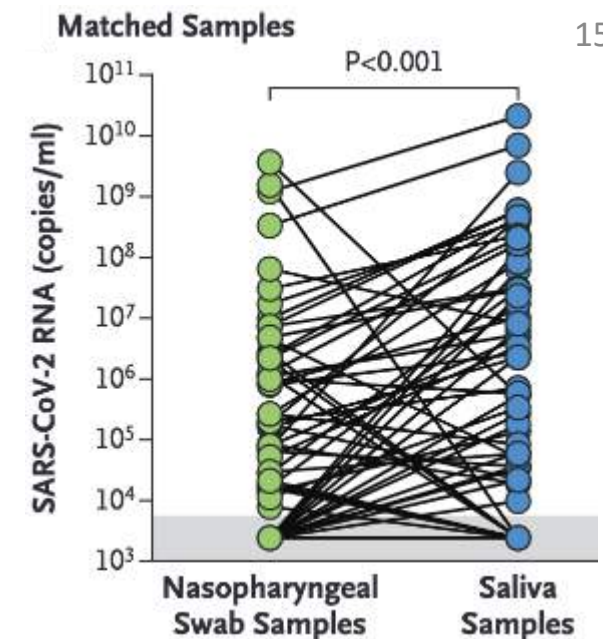
Additional samples (saliva specimens collected by the patients themselves + nasopharyngeal swabs collected by health care workers)

Detected more RNA copies in the saliva specimens than nasopharyngeal swabs (mean log copies per milliliter, 5.58 versus 4.93)

Higher percentage of saliva samples than nasopharyngeal swab samples were positive

Saliva specimens and nasopharyngeal swab specimens have at least similar sensitivity in the detection of SARS-CoV-2 during the course of hospitalization

Limits: hospitalized patients, nasopharyngeal samples presented an unusually low sensitivity ($\approx 70\%$ for earlier samples) in this study



Saliva specimens could be effective in COVID-19 diagnosis, but needs to be confirmed for outpatients

Salivary detection of SARS-CoV-2 in asymptomatic subjects

Mass screening study – 1924 asymptomatic subjects:

- Close contact with clinically confirmed COVID-19 patients (CT cohort, $n=161$)
- Asymptomatic travelers arriving at Tokyo & Kansai (AQ cohort, $n=1763$)

Saliva sample (self-collected) & NPS sample (medical officers)

Comparison between paired samples

Estimated prevalence:

- CT cohort: 29,6%, $CI_{90\%}[23,8 - 35,8\%]$
- AQ cohort: 0,3%, $CI_{90\%}[0,1 - 0,6\%]$
- The true concordance probability was:
0,998, $CI_{90\%}[0,996 - 0,999\%]$ in AQ cohort
- Viral load was equivalent between NPS and saliva samples (Kendall's coefficient of concordance = 0,87)

Diagnostic results of nasopharyngeal swab (NPS) and saliva test

Contact-tracing cohort (n=161)			Airport Quarantine cohort (n=1,763)		
NPS	saliva		NPS	saliva	
	positive	negative		positive	negative
positive	38	3	positive	4	1
negative	6	114	negative	0	1758

	Sensitivity	Specificity
NPS	86% , $CI_{90\%}[77 - 93\%]$	99,93% , $CI_{90\%}[99,77 - 99,99\%]$
Saliva	92% , $CI_{90\%}[83 - 97\%]$	99,96% , $CI_{90\%}[99,85 - 100,00\%]$

- **Equivalent utility with similar sensitivity and specificity,**
- **Self-collected saliva has significant advantages over NPS sampling,**
- **Saliva may be a reliable alternative in detecting SARS-CoV-2 in asymptomatic**
- **Limit:** the number of positive patients in the QC does not provide a strong evaluation of the saliva sensitivity in this population

Saliva vs. Nasopharyngeal swab detection of SARS-CoV-2

Systematic review of the operating characteristic of saliva NAAT for the detection of SARS-CoV-2, compared to nasopharyngeal swab NAAT

16 studies retained for data extraction (10 peer-reviewed, 6 preprints)

Analysis performed:

- Primary analysis: all papers, 5922 samples, 15.9% positive tests
- Secondary analysis: Peer-reviewed papers only
- Post-hoc meta-analysis of ambulatory settings – 4851 patients, 8.1% positive tests

Limitations:

- Limited heterogeneity of study population and timing of testing
- Comparison between two samples types often took place later in disease course
- It remains unclear if certain clinical signs warrant a specific sample type for optimal diagnostic

	Saliva NAAT		Nasopharyngeal swab	
	Pooled sensitivity % (95% CrI)	Pooled specificity % (95% CrI)	Pooled sensitivity % (95% CrI)	Pooled specificity % (95% CrI)
Primary analysis	83.2 (77.4-91.4)	99.2 (98.2-99.8)	84.8 (76.8-92.4)	98.9 (97.4-99.8)
Secondary analysis	85.6 (77.0-92.7)	99.1 (98.0-99.8)	85.7 (76.5-93.4)	98.9 (97.4-99.7)
Ambulatory settings	84.5 (73.0-95.3)	99.0 (97.7-99.7)	88.0 (77.5-95.8)	98.7 (96.2-99.8)

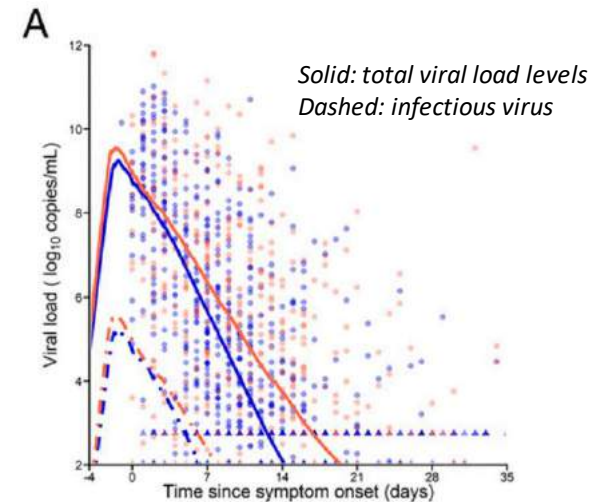
Diagnostic sensitivity of saliva NAAT is comparable to that of nasopharyngeal swab NAAT.
Testing centre should strongly consider adopting saliva as first sample choice, especially for community mass screening.

SARS-CoV-2 viral kinetics and association with mortality

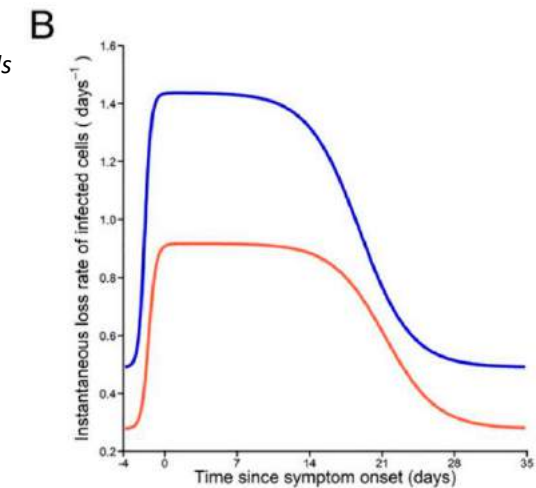
French COVID cohort: 655 hospitalized patients before 01 April 2020

- Delay between symptom onset and admission: median time of 7 days (3-9)
 - 23% admitted to ICU
 - 40% received at least one antiviral treatment (Lopinavir/Ritonavir, HCQ, Remdesivir), 20% received corticosteroid therapy
 - Median viral load at admission was 6.3 log₁₀ copies/mL
- At days 7 and 14 post symptom onset (more severe/fragile patients), high levels of viral load (≥ 6 log₁₀ copies per mL) were **significantly associated with mortality**
- Viral load peaked on average **1.1 days** before symptoms onset
- Loss rate of infected cells due to host's immune response was **age-dependent**. Half life of infected cells decreased from 50h to 13h (age <65y) and 17h (age ≥ 65 y)
- Predicted median **time to viral clearance** was 13 days (age <65y) and 16 days (age ≥ 65 y) after symptom onset

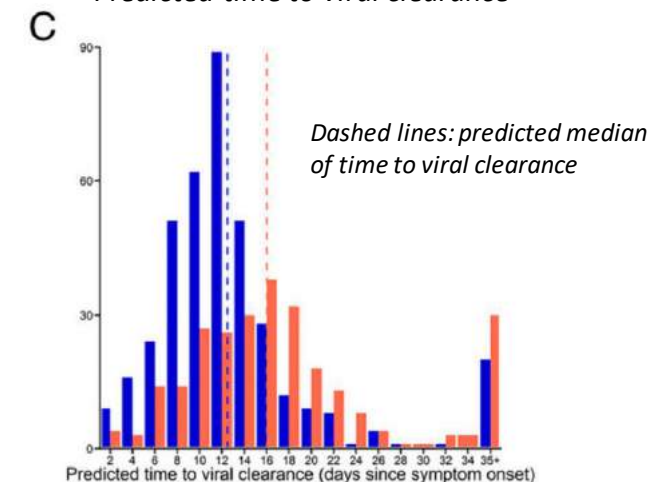
Median of the individual predicted viral load kinetics



Median of the predicted instantaneous loss rate of productively infected cells



Predicted time to viral clearance

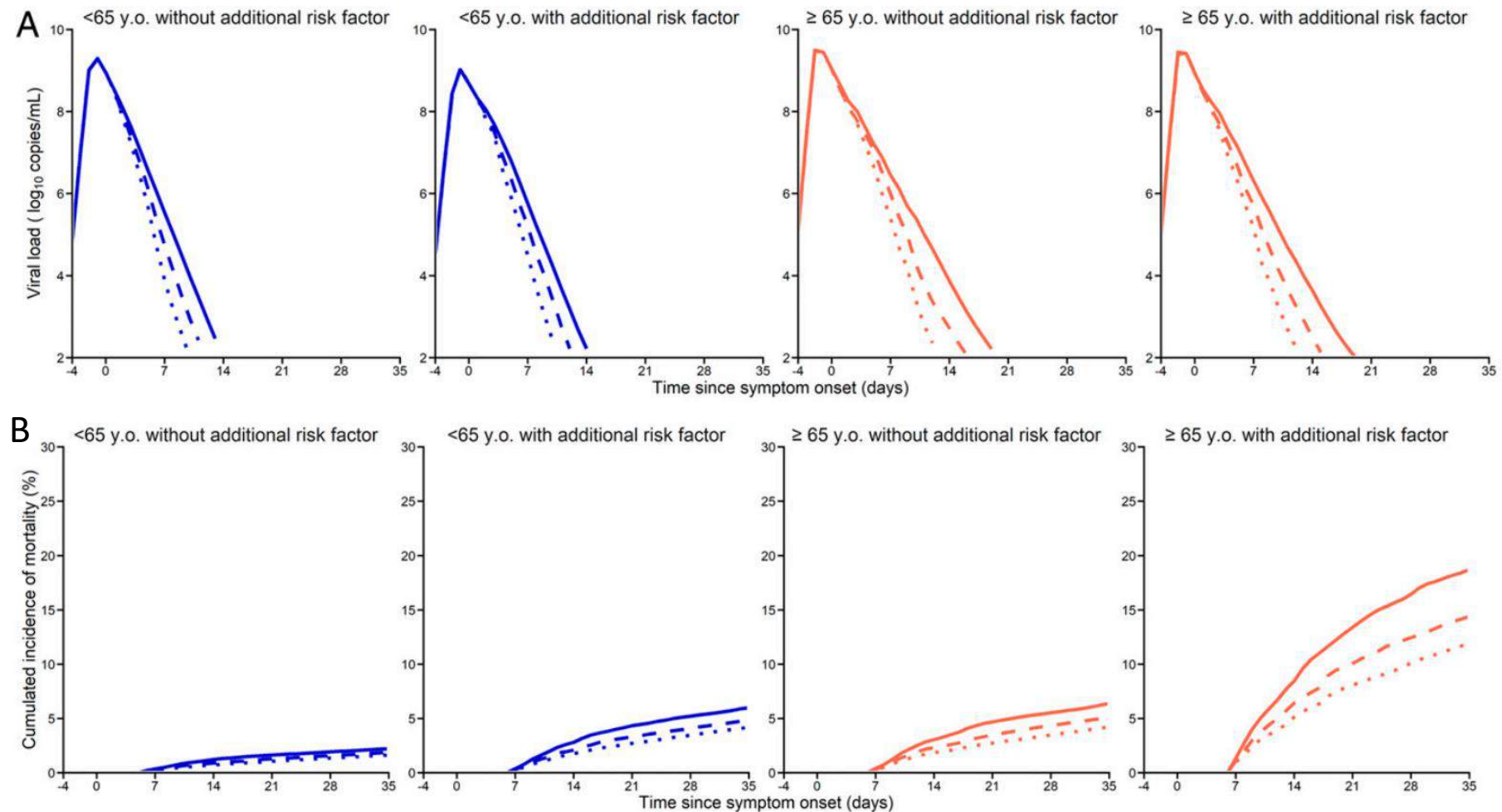


Blue, patients aged <65 y; Orange, patients aged ≥ 65 y

SARS-CoV-2 viral kinetics and association with mortality

Prediction model based on 74 individuals who died within 35 days from symptoms onset

- Viral load was significantly associated with survival (hazard ratio = 1.31, $P > 0.001$)
 - ➔ **independent factor of death**
- Hazard ratios of risk factors
 - Age ≥ 65 y = 2.58
 - Male gender = 2.55
 - Chronic pulmonary diseases = 2.31



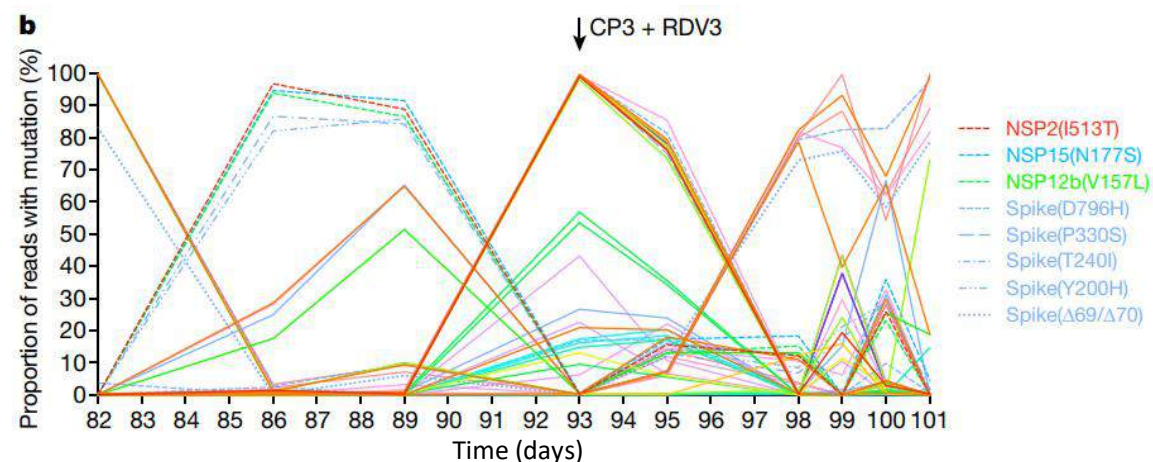
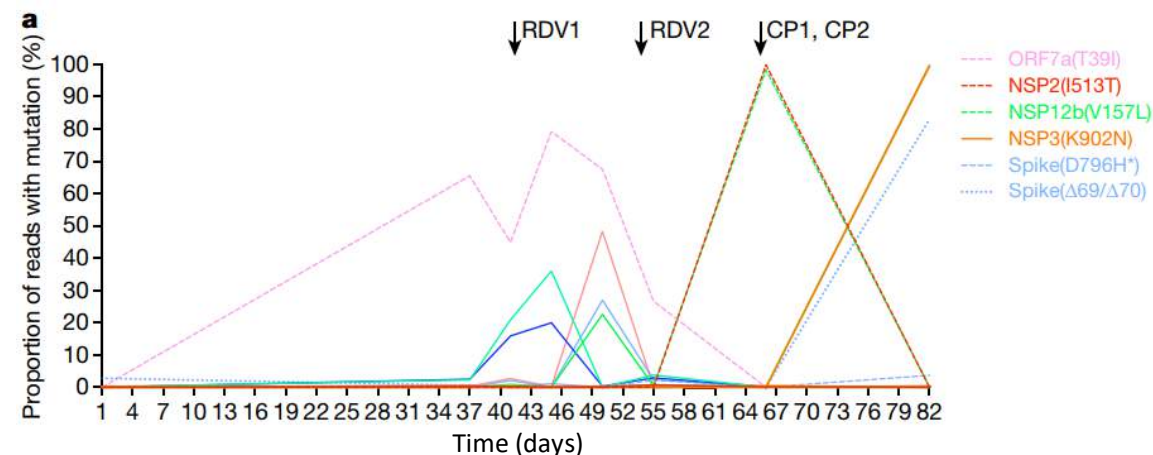
A. Median of the individual predicted viral load; B. Median of the predicted death rate.

Solid lines, predicted profile without treatment; dashed lines, treatment with 90% efficacy; dotted lines, treatment with 99% efficacy; blue, patients aged <65 y; orange, patients aged ≥ 65 y.

SARS-CoV-2 evolution during treatment of chronic infection

The evolutionary response by SARS-CoV-2 in the presence of antibody therapy in an immunocompromised host with persistent infection

- After Remdesivir at day 41
 - transient amino acid changes <50% abundance in ORF1b, 3a and spike, T39I substitution in *orf7a* reaching 79% on day 45
 - I513T substitution in NSP2 and a V157L substitution in RdRp emerged day 54 to reach almost 100% frequency on day 66
- After 2 convalescent plasma (CP) administrations
 - Shift in population, variant with S mutations S796H and $\Delta 69/70$ became dominant at day 82
- Day 86-89
 - Samples characterised by spike (Y200H,T240I) double mutant, and accompanied by I513T, V157L and N177S at high frequency
 - Mutant that was dominant on day 82 decreased to <10%
- On day 93
 - P330S on RBD, W64G in S1 domain at close to 100% abundance
 - Previously frequent variants <2%, suggesting competition between populations
- After Remdesivir on day 93 and CP on day 95
 - Re-emergence of spike(D796H, $\Delta 69/70$) population, probably under positive selection renewed by CP

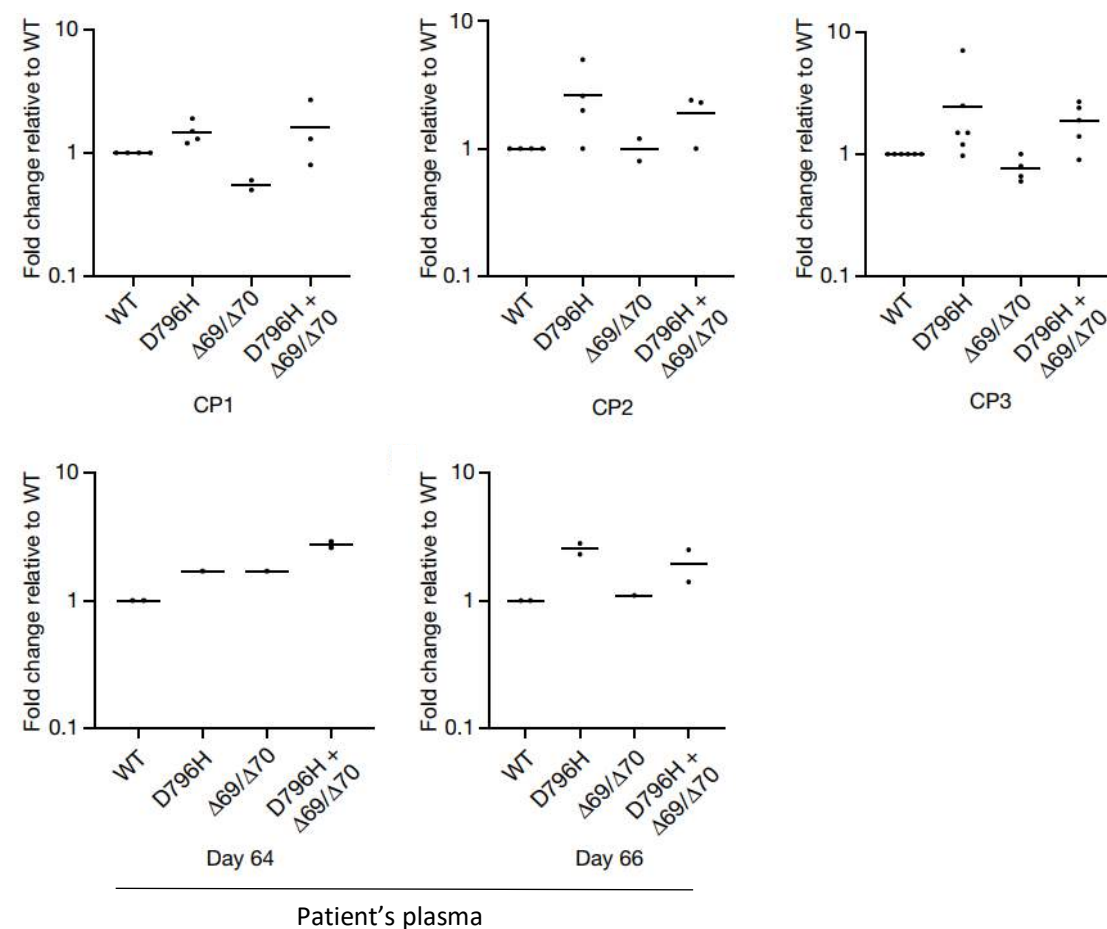


- ❖ Highly dynamic population in immunocompromised patient
- ❖ Combination of deletion and spike mutation conferred selective advantage

SARS-CoV-2 evolution during treatment of chronic infection

Spike mutations impair neutralizing antibody potency

- spike(Δ H69/ Δ V70) had twofold higher infectivity, spike(D796H) lower infectivity, and spike (D796H, Δ H69/ Δ V70) had similar infectivity compared to wild-type spike
- Spike(D796H) and spike(D796H, Δ H69/ Δ V70) were less sensitive to neutralization by convalescent plasma, but not spike(Δ H69/ Δ V70)
- Neutralization potency of 8 tested RBD-specific monoclonal antibodies was not affected. Non-RBD-specific antibody COVA1-21 showed 3-5 fold reduction in potency against spike(D796H, Δ H69/ Δ V70) and spike(Δ H69/ Δ V70)



Sensitivity of mutants to convalescent plasma

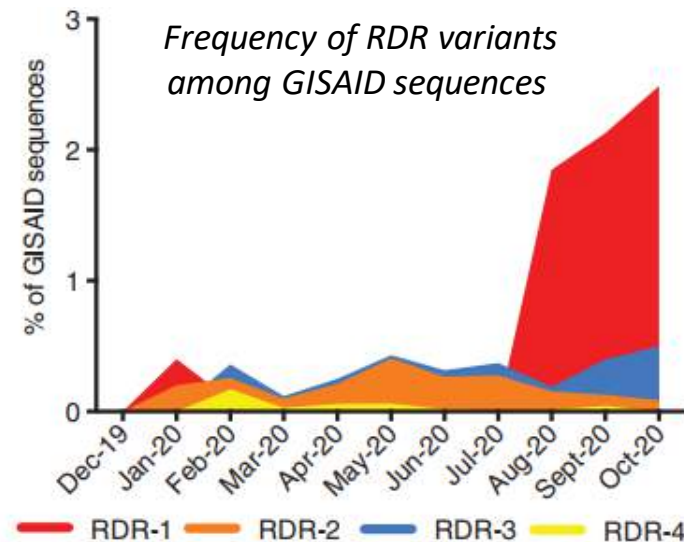
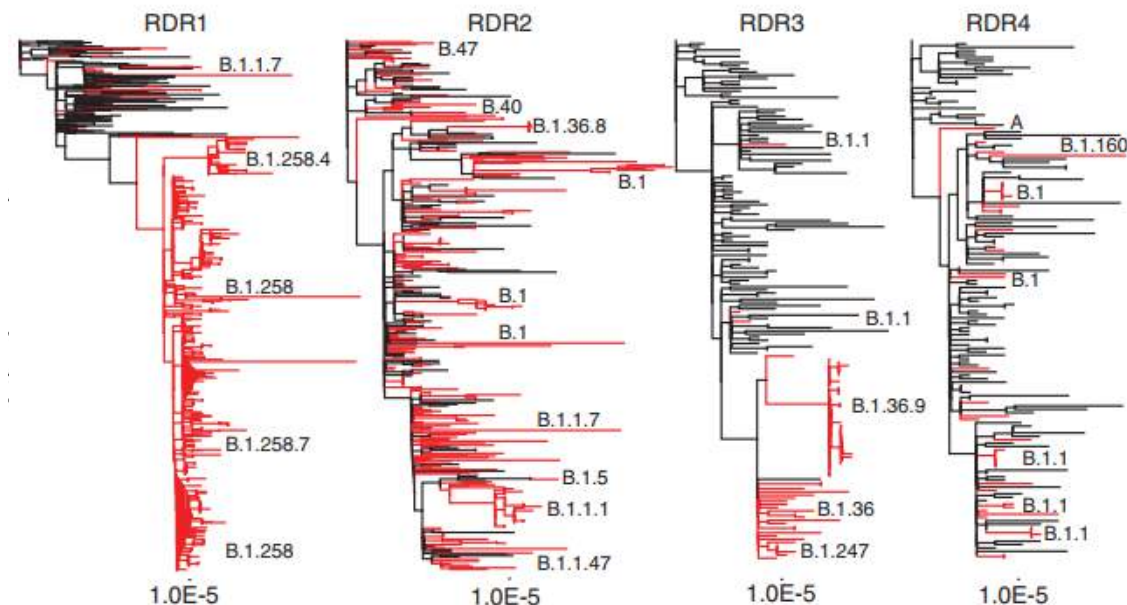
- Pseudotyped viruses bearing indicated mutations
- The serum dilution required to inhibit 50% of virus infection (ID50) is shown, expressed as a fold change relative to the wild-type virus

SARS-CoV-2 evolution – Recurrent deletion regions

Comparison of deletions acquired in an immunocompromised patient (PLT11) and patient sequences from GISAID

- 4 recurrent deletion regions identified – Convergent evolution under a common selective pressure
- RDRs 2 and 4: frequent loss of S glycoprotein residues 144/145 in RDR2 and residues 243 and 244 in RDR4. RDR1: frequent loss of residues 69 and 70 → B.1.1.7
- Temporal and geographic distribution: these mutations have been present throughout the pandemics
- *In vitro* testing: these viral mutants are resistant to neutralisation by the monoclonal antibody 4A8 but not by patient sera → Naturally arising variants of SARS-CoV-2 can have altered antigenicity

Phylogenetic analysis of deletion variants (red branches) and genetically diverse nondeletion variants (black branches)

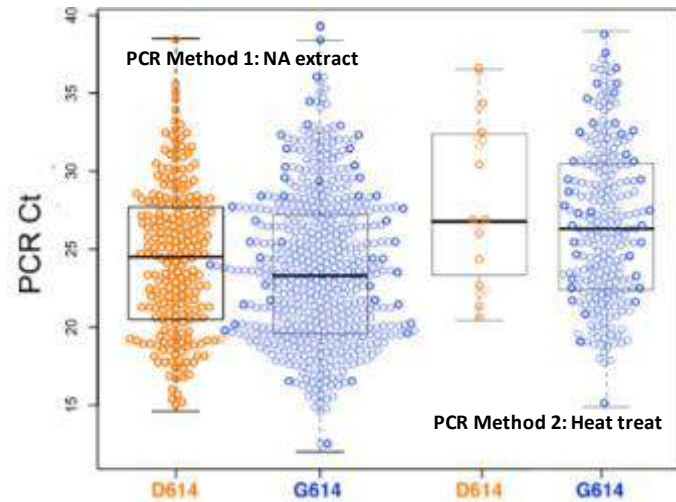


Changes in SARS-CoV-2 Spike

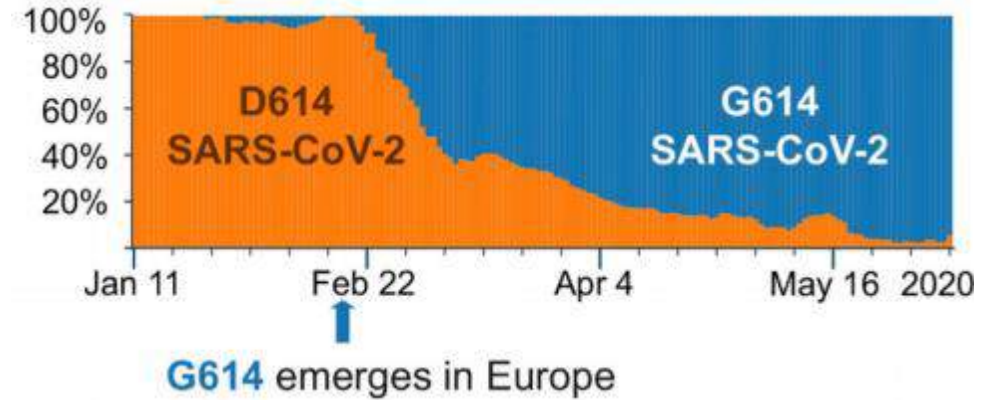
SARS-CoV-2 variant with Spike G614 has replaced D614 as the dominant pandemic form:

- Spike D614G amino acid change is caused by an A-to-G nucleotide mutation at position 23,403 in the Wuhan reference strain

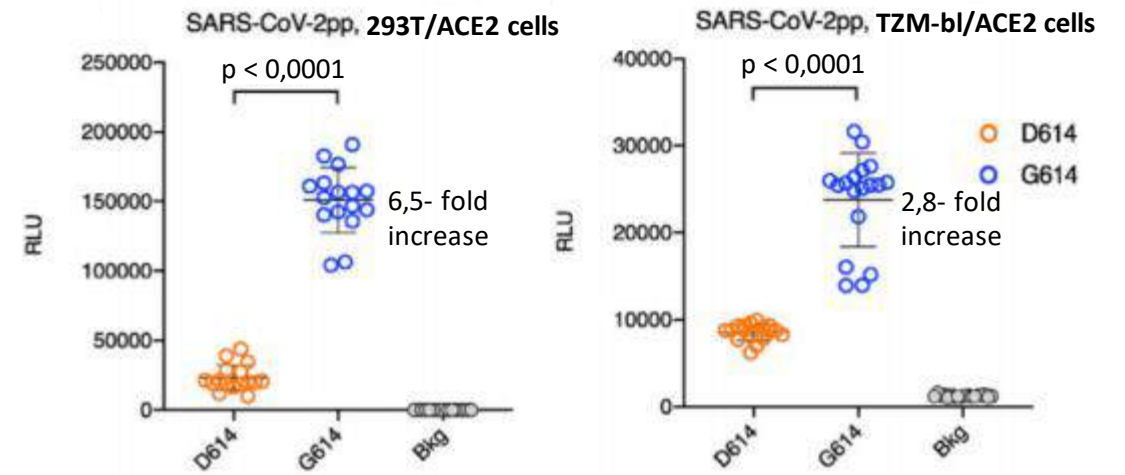
G614 Is Associated with Potentially Higher Viral Loads in COVID-19 Patients but not with disease severity:



- G614 is associated with a lower cycle threshold (Ct) required for detection (higher viral loads)



Recombinant lentiviruses pseudo typed with the G614 Spike more infectious than corresponding D614 S-pseudo typed viruses



Limits: this mutation is not single (e.g. associated to P314L in ORF1b) and represents the vast majority of cases in France among non-travelers since the very beginning of the outbreak

Spike mutation D614G & SARS-CoV-2 fitness

What is the impact on viral spread and vaccine efficacy of the spike protein mutation D614G ?

D614G amino acid substitution reached over 74% of all published sequences by June 2020.

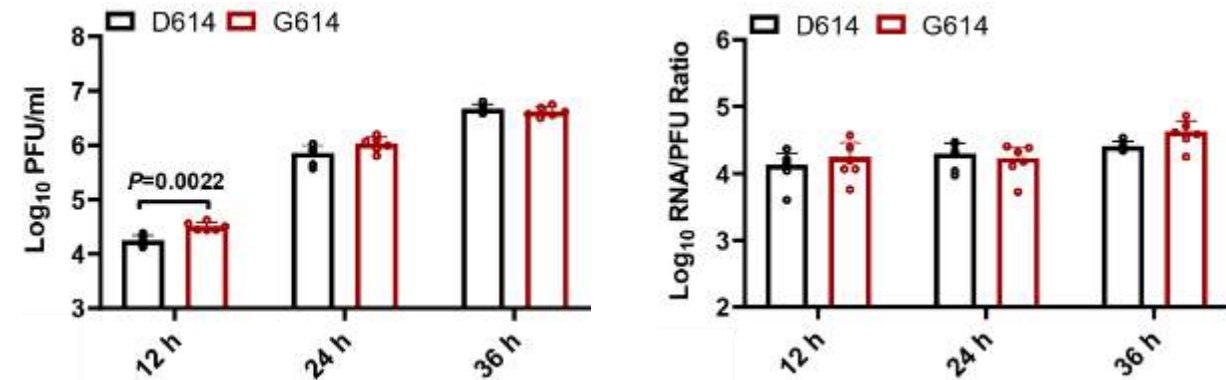
Effect on viral replication in cell culture:

- Use of Vero E6 cells to test a pair of recombinant isogenic viruses presenting a D614 or G614
 - Two viruses replicated to comparable levels
 - No difference was found on calculated the genomic RNA/PFU ratios.
- **D614G mutation does not affect viral replication or virion infectivity in Vero E6 cells**

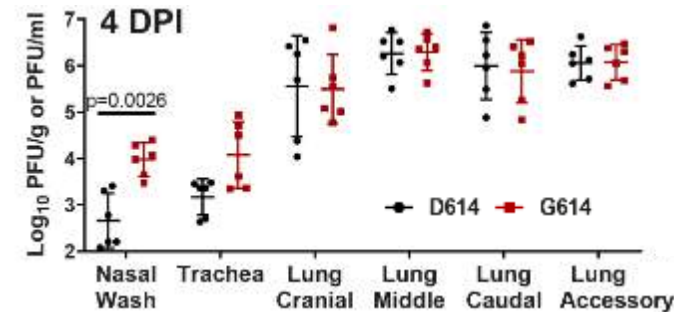
In vivo relevance of D614G mutation:

- Hamster model: intranasally infecting with D614 or G614
- Hamster infected with G614 produced higher infectious viral titers in the upper airway but not on lungs
- The RNA/PFU ratios of G614 virus were lower than D614 in upper airway but differences are negligible in lungs.

Viral replication and genomic RNA/PFU ratios of D614 and G614 viruses produced from Vero E6 cells



D614G substitution increases SARS-CoV-2 replication in the upper airway, but not the lungs, of hamsters



Spike mutation D614G & SARS-CoV-2 fitness

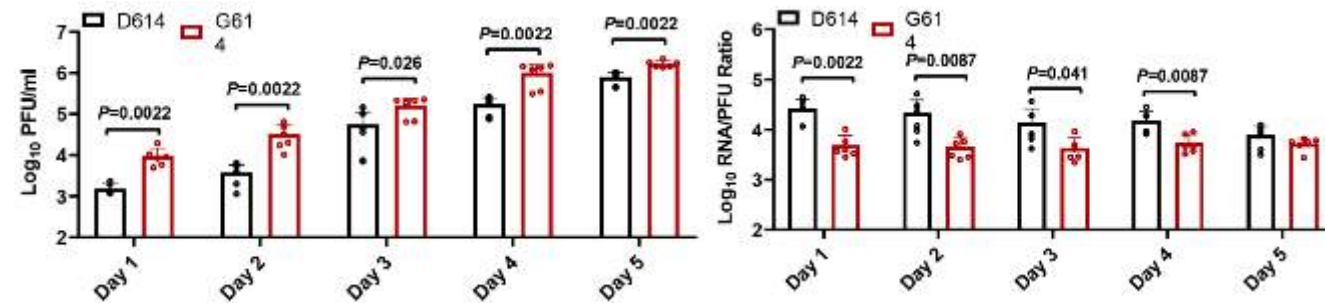
In primary human airways tissue model:

- Infectious viral titers of G614 were higher than those of D614
- RNA/PFU ratios of D614 virus were 1.4- to 5.3-fold higher than those of G614 virus
- G614 enhances viral replication through increased virion infectivity in primary human upper airway tissues
- **Suggest the role of D614G mutation in viral transmissibility**

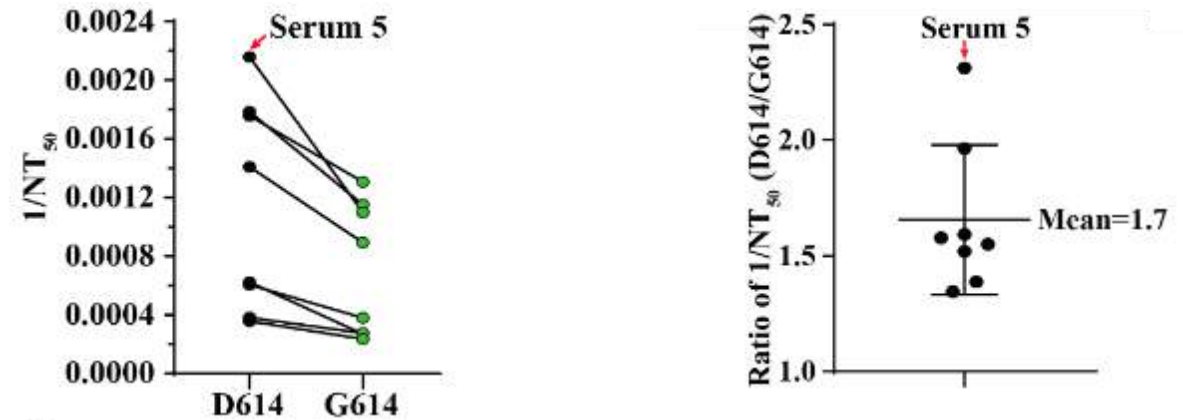
Effect on neutralization susceptibility:

- D614G may confer higher susceptibility to serum neutralization
- D614G may modulate spike protein conformation to affect mAb neutralization
- **Mutation may not reduce the ability of vaccine to protect against COVID-19**
- **Importance to test therapeutic mAbs against G614**
- **Importance to monitor the impact of future mutations emergence with the introduction and use of vaccines**

D614G substitution increases SARS-CoV-2 replication in primary human airway tissues



Neutralizing activities of hamster sera against D614 and G614



Variants Of Concern - Spike mutations

The nonsynonymous spike mutation N501Y encodes for an amino acid substitution on the RBD domain.

Observed in two important circulating variants:

- B.1.1.7 (UK) strain – N501 mutation in RBD
- B.1.351 (South Africa) – N501Y, E484K and K417N mutations in RBD

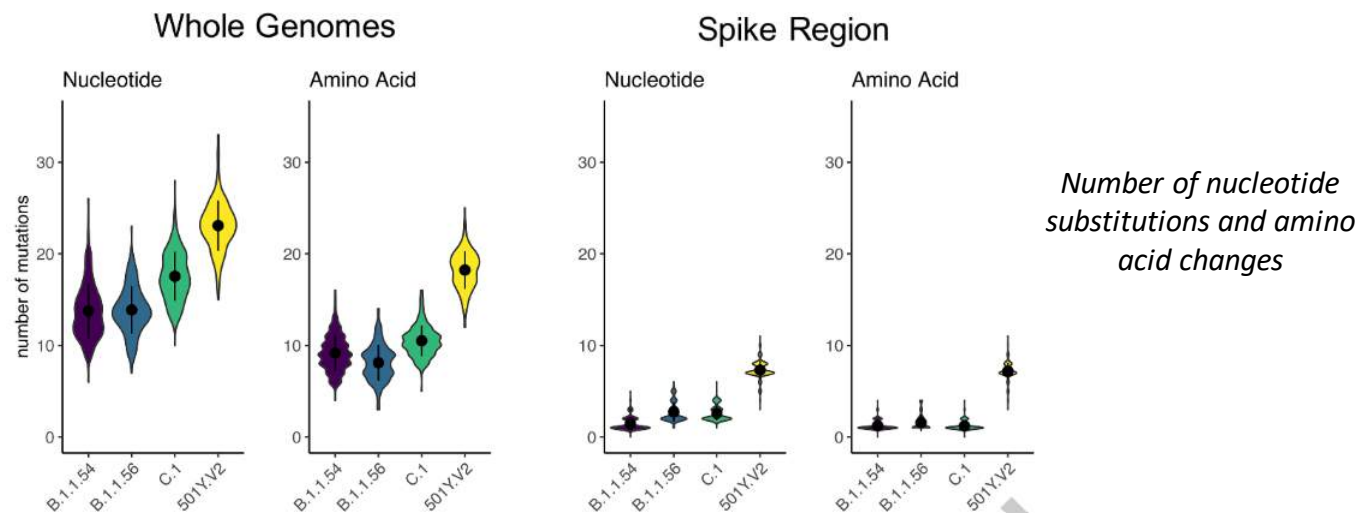
In silico and structural analysis showed that:

- N501Y does not induce large conformational changes in the RBD → **possibly not affecting antibody recognition**
- N501Y increases ACE2 affinity due to a lower binding energy and favorable nonbonded interactions of the Y501 residue

→ **B.1.1.7 might be more transmissible.** In B.1.351, N501Y increased affinity might be counterbalanced by E484K and K417N, which are not favorable for interaction with ACE2.

B.1.351 lineage (VOC 501Y.V2)

- Marked **hypermutation**: 6 non-synonymous mutations in the spike protein by 15/10/20, then 3 more by 30/11/20, plus deletion of 3 amino acids
- Mutations N501Y, E484K and K417N are at **key residues of the RBD** – the two latter are key for neutralizing antibody binding
- E484 and N501 pattern of nucleotide variation suggests evolution under **positive selection**
- B.1.351 most likely evolved by mutation on circulating **intermediate mutants**



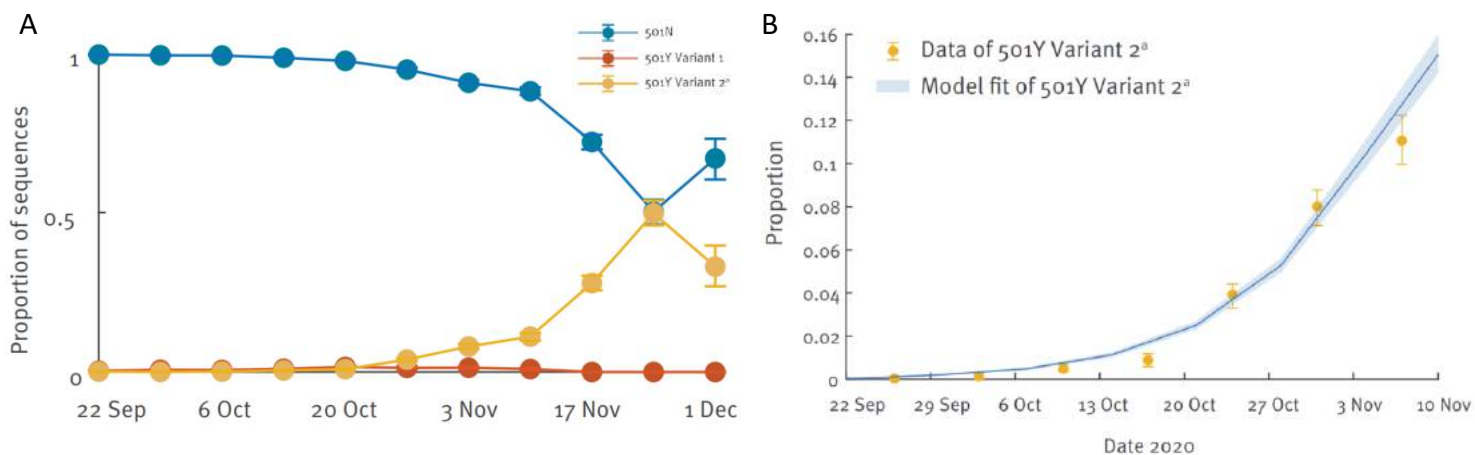
- B.1.351 likely emerged in Nelson Mandela Bay in early August
- It has a selective advantage, from increased transmissibility and/or immune escape

Variants Of Concern - Lineage B.1.1.7

N501Y in the UK:

- 501Y Variant 1 without deletion $\Delta 69/70$ – Sept to mid-November, mainly in Wales
- 501Y Variant 2 with deletion $\Delta 69/70$ (B.1.1.7) – from late Sept, mainly in England

→ **501Y Variant 2 became the dominant lineage in England**, increasing from **0.1%** in early October to **49.7%** in late November



Observed and fitted weekly proportion of 3 circulating variants (A) and of Variant 2 (B) (22 Sept - 01 Dec 2020)

501Y Variant 2 (B1.1.7) is highly transmissible due to high R_0 but not shorter generation time

- 501Y Variant 1 R_0 : 10% (95% CrI: 6-13%) higher than 501N
- 501Y Variant 2 R_0 : **75%** (95% 70-80%) **higher than 501N**

Increased B.1.1.7 growth rate

- Several models identified a substantially increased growth rate. One model estimated a growth rate of 0.104 days^{-1} → 77% increase in R_0
- Similar increases in R estimated in other countries: Denmark 55%, Switzerland 74%, USA 59%

Mechanistic hypothesis for the rapid spread

- Increased transmissibility is the most fitting model, followed by longer infectious period

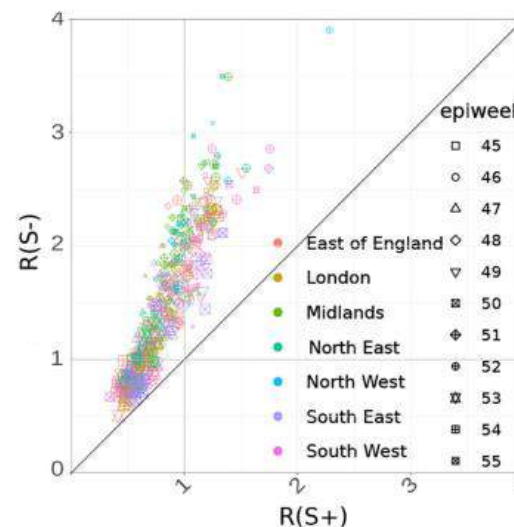
Projection of Covid-19 dynamics in England

- Regardless of control measures, all regions might experience a wave of cases and death in early 2021
- No substantial vaccine roll-out → cases, hospital and ICU admissions, deaths in 2021 could exceed those of 2020
- The primary benefit of accelerated vaccine roll-out is help to avert case resurgences following NPI relaxation

Variants Of Concern - Lineage B.1.1.7

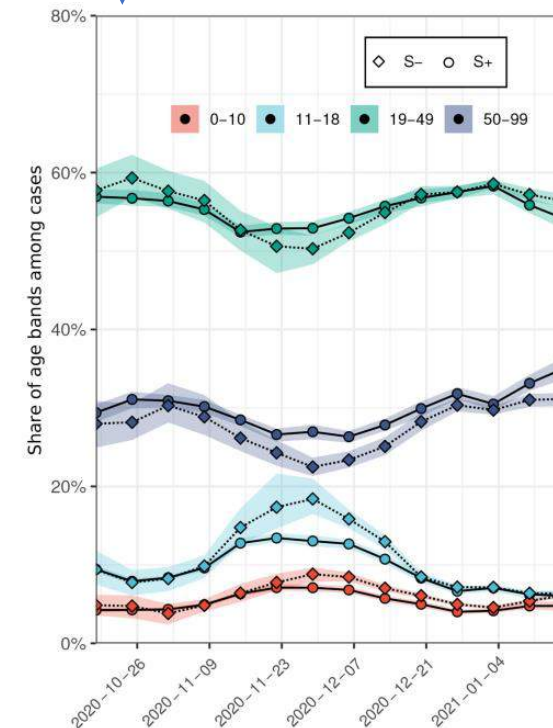
31,390 VOC (B.1.1.7 lineage) and 52,795 non-VOC sequences – 01 Oct 2020 to 16 Jan 2021, England

- Population sizes of VOC and non-VOC: estimated growth rate difference 0.33/week (95%CI: 0.09-0.062) → VOC reached 50% frequency within 2.5-3 month after its emergence
- Ratio of reproductive numbers:
 - 25 Oct – 16 Jan : 1.89 (95% CrI: 1.43-2.65)
 - By mid-January: 1.54 (95%CI: 1.34-1.82), coinciding with increasing VOC frequency
- R_t :
 - VOC R_t was greater than non-VOC for all week pairs
 - Mean ratio of R_t was 1.70 (95%CI: 1.22-2.49) for Nov-Jan and declined to 1.5 by mid-Jan
 - **VOC has a transmission advantage**
- Age distribution:
 - Age 19-49: consistently over-represented relative to their share population, little difference between VOC and non-VOC cases
 - Age 11-18: over-represented relative to their share population, differences between VOC and non-VOC cases statistically significant for 3 weeks in November (2nd lockdown, schools remained open).



Reproduction no. Of S- (VOC) et S+ (non-VOC) over time and between regions

Age distribution of S+ (VOC) and S- (non-VOC) cases over time



Variants Of Concern - Lineage B.1.1.7

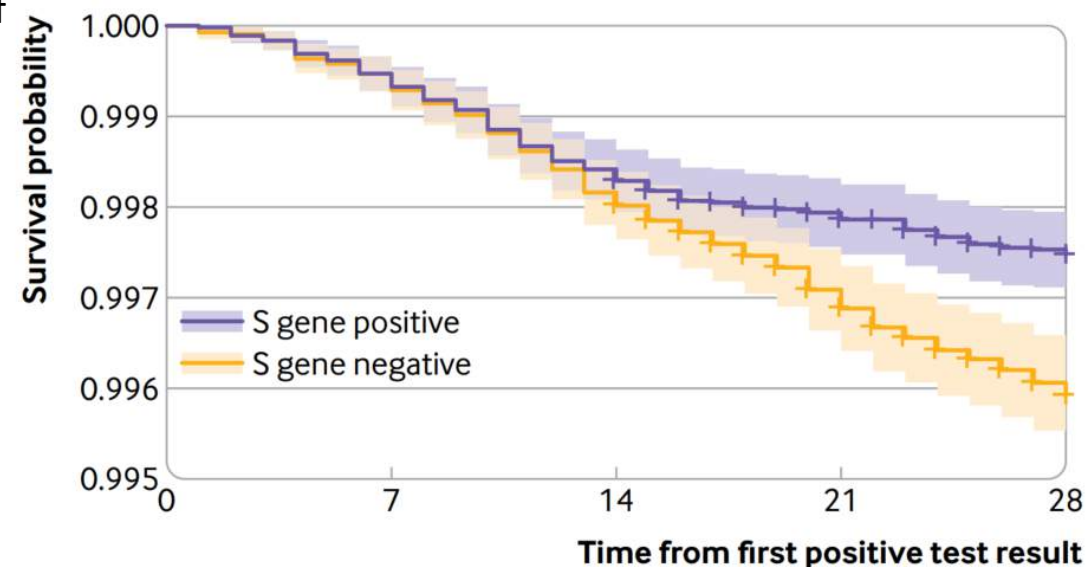
Sample

- >30 year-old SARS-CoV-2 positive community individuals (UK, 1 Oct 2020 – 28 Jan 2021), identified as S positive (previous variants) or S negative (B.1.1.7)
- 54 906 pairs of participants (S-pos and S-neg), matched on age, sex, ethnicity, index of multiple deprivation, lower tier local authority region, sample date of positive specimen → minimum bias

Main outcome: death within 28 days of first positive test

- 227 deaths in S-neg arm, 114 in S-pos arm → **Hazard ratio (HR) 1.64** (95% CI, 1.32-2.04; P<0.001)
- Rate of death in S-pos and S-neg diverged after day 14
 - Day 0-14 – HR was not increased
 - Day 15-28 – HR 2.40 (1.66-3.47)
- No evidence of asymmetrical delays in time from hospital admission
- Higher viral load at timing of sampling in S-neg arm
 - Either due to intrinsic property of the variant → higher mortality associated with high viral load
 - Or to timing in testing: S-neg patients presenting at peak of infectiousness

Kaplan-Maier survival curve for S-pos (previously circulating variants) and S-neg (B.1.1.7)



Infection with B.1.1.7 is associated to higher mortality
Most probable HR 1.64, or 64% increased risk of death

Variants Of Concern - Lineage B.1.1.7

2,245,263 individuals who had a positive community test (1 Nov 2020 – 14 Feb 2021).

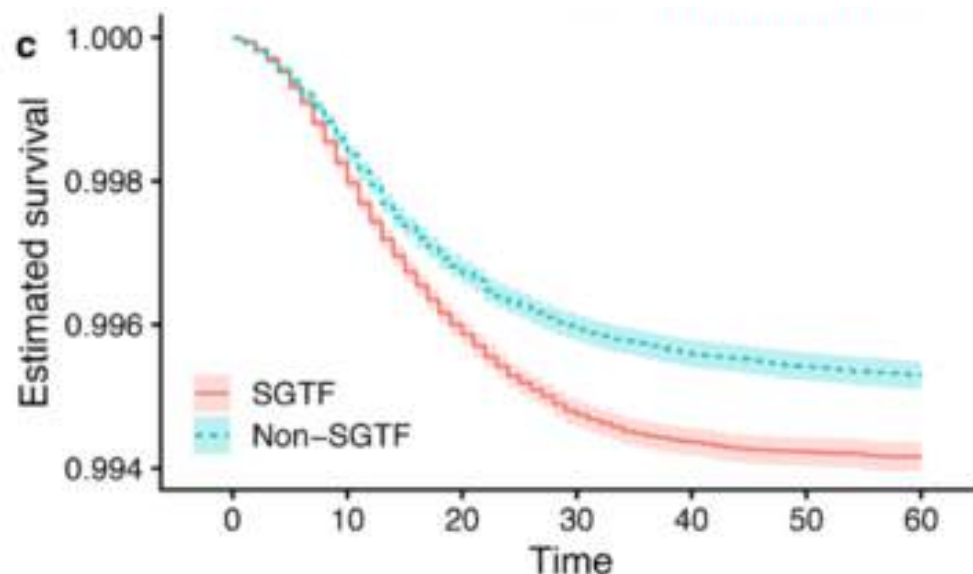
Prevalence

- 1,146,534 (51.1%) had a conclusive SGTF (S-Gene Target Failure) reading, of these, 58.8% had SGTF (→ **B.1.1.7 variant**)
- SGTF prevalence was lower in older age groups: 59.0% in 1-34 yo, 55.4% in ≥85 yo
- SGTF status was strongly associated with age and place of residence
- SGTF prevalence **increased over time**: from 5.8% (Nov 2020) to 94.3% (Feb 2021)

Mortality

- 19,615 people died in the study group (0.87%). 17,452 of observed deaths (89.0%) met criteria to be defined as Covid-19 death
- Crude Covid-19 death rate was **1.84 deaths per 10,000 person-days in the non-SGTF group vs. 1.42 deaths per 10,000 person-days in the non-SGTF group**
- **Absolute mortality risk** within 28 days of a positive SARS-CoV-2 test:
 - Females aged 70-84: 2.9% without SGTF, 4.4% with SGTF (95% CI 4.0–4.9%)
 - Females aged ≥85: 13% without SGTF, 19% with SGTF (17-21%)
 - Males aged 70-84: 4.7% without SGTF, 7.2% with SGTF (6.4-7.9%)
 - Males aged ≥85: 17% without SGTF, 25% with SGTF (23-27%)

Survival among individuals tested in the community in England with and without SGTF (Kaplan-Meier plot, 95% Cis)



	SGTF						
At risk	674539	625587	549622	448192	303131	155715	0
Censored	0	47638	122338	223187	368094	515475	671181
Events	0	1314	2579	3160	3314	3349	3358
	Non-SGTF						
At risk	471995	469441	463358	450813	420343	374946	0
Censored	0	1783	7095	19279	49595	94921	469822
Events	0	771	1542	1903	2057	2128	2173

B.1.1.7 shows a substantial increase in absolute risk amongst older age groups, but the risk of COVID-19 death following a positive test in the community remains below 1% ≤70 years old

1. Which type of virus is SARS-CoV-2?

- RNA viruses that belong to the *betacoronavirus* genus

2. What is the stability and viability of SARS-CoV-2?

- Stability is similar to that of SARS-CoV-1 under experimental circumstances tested
- Aerosol and fomite transmission of SARS-CoV-2 is plausible

3. What do we know about viral load and shedding according to different samples?

- Highest positive rates of SARS-CoV-2 in bronchoalveolar fluid among severe patients
- No influence of sex, age and disease severity on viral loads, has been observed
- Viral shedding may begin 2 to 3 days before first symptoms
- Detection of viral RNA does not necessarily mean that infectious virus is present, especially for low viral loads and >8 days from symptoms onset
- Viral load may be an independent risk factor associated to mortality

4. Alternative to the nasopharyngeal swab for SARS-CoV-2 detection?

- Saliva sample might be a good alternative to the NPS with several advantages, but asymptomatic populations are poorly characterized

5. What is the impact of RBD mutations for SARS-CoV-2? What are the characteristics of the current Variants of Concern?

- D614G may increase transmission by increasing viral load in the upper airways without clinical impact
- D614G may have higher susceptibility to serum neutralization --> may not reduce the ability of vaccine to protect against COVID-19
- N501Y and other RBD mutations appear to increase transmissibility of B.1.1.7 and B.1.351 lineages
- An increased risk of mortality seems associated to B.1.17 lineage

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