La REINFEZIONE da SARS-CoV-2:
I DATI ATTUALI

CLAUDIA BALOTTA

4.2.2021

OPEN QUESTIONS

- Matter of definition:
  persistance/pronged viral shedding vs **reinfection**?
- How **common** are SARS-CoV-2 reinfections?
- How **can a SARS-CoV-2 reinfection be identified**?
- What do these observations mean for **acquired immunity**?
- What is known about the **role of reinfection in onward transmission**?

ISSUES of REINFECTION

• Some patients with laboratory-confirmed SARS-CoV-2 infection have been identified to be **PCR-positive over prolonged periods of time after infection and clinical recovery.**

• **The duration of viral RNA detection** (identification of viral RNA through PCR testing in a patient) **has been shown to be variable**, with the detection of RNA in upper respiratory specimens shown **up to 104 days after the onset of symptoms**.

• Of note, some patients have **intermittent negative PCR tests**, especially when the virus concentration in the sampled material becomes low or is **around the detection limit of a test**.

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ISSUES of REINFECTION

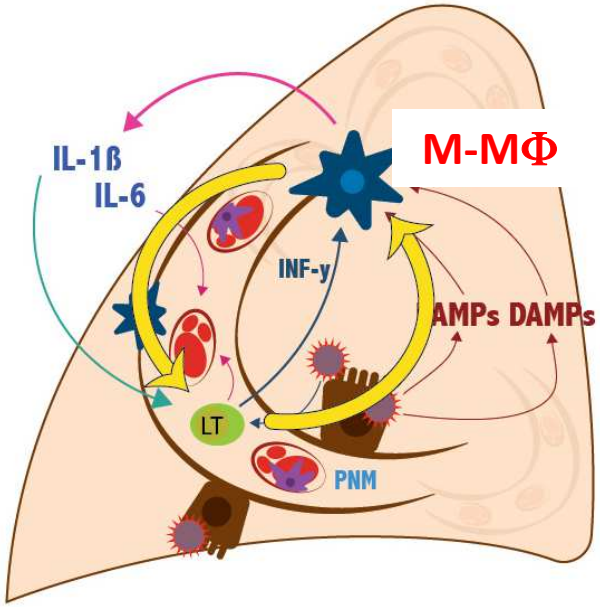
- It is important to note that **viral RNA shedding does not equate to the presence of viable, infectious virus within a patient.**

- **Classification and confirmation of suspected cases as 'confirmed' reinfections have been done in the absence of testing results and the lack of genetic sequencing.**

- **Test results must be interpreted in combination with additional epidemiological and clinical characteristics**.

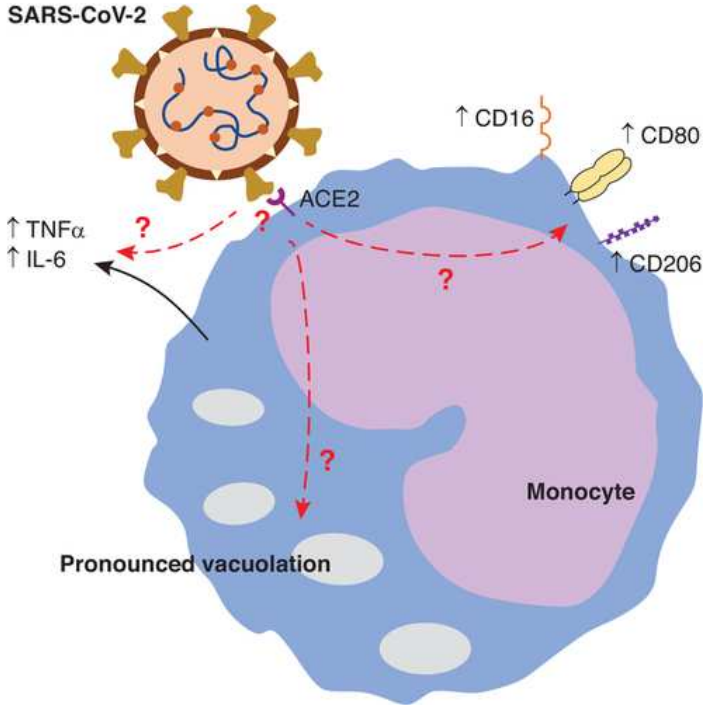
➢**To confirm a suspected reinfection it is mandatory to study the genetic sequences from nasopharyngeal swab of previuos and present infection that evaluate the diversity of viral variants.**

MONOCITES/MACROPHAGES and SARS-CoV-2 INFLAMMOSOME & PYROPTOSIS in COVID-19



Pyroptosis of macrophages that have phagocytosed viruses rapidly releases a myriad of alarmins, including **viral particles, cytokines, chemokines, LDH, ATP, and ROS,** prompting an immediate **reaction from surrounding immune cells and thus inducing a pyroptotic chain reaction.**

ATYPICAL MONOCITES



Brandt D. Pence, Journal of Leukocyte Biology, 19 November 2020

Early reported cases of reinfection and key information

ADDITIONAL CASES: The Netherlands (n=50), Brazil (95), Sweden (150), Mexico (285), and Qatar (at least 243).

interval of about 2 to 4 months

Location	Age of patient	First episode	Interval	Second episode	Publication
Hong Kong	33 years	Symptomatic	142 days	Asymptomatic	Peer-reviewed
Nevada, USA	25 years	Symptomatic	48 days	Symptomatic with hospitalisation	Pre-print
Belqium	52 years	Symptomatic	93 days	Symptomatic	Peer-reviewed
Ecuador	46 years	Symptomatic	63 days	Symptomatic	Pre-print
India	25 years	Asymptomatic	108 days	Asymptomatic	Pre-print
India	28 years	Asymptomatic	111 days	Asymptomatic	Pre-print

WGS

Lack of antibody testing information after the first infection.

Two cases were healthcare workers.

RECURRENCE or REINFECTION?

Recurrent SARS-CoV-2 RNA positivity after COVID-19: a systematic review and meta-analysis

Mahalul Azam[1]✉, Rina Sulistiana[1], Martha Ratnawati[2], Arulita Ika Fibriana[1], Udin Bahrudin[3], Dian Widyaningrum[4] & Syed Mohamed Aljunid[5]

Present study aimed to estimate the incidence of recurrent SARS-CoV-2 RNA positivity after recovery from COVID-19 and to determine the factors associated with recurrent positivity. We searched the PubMed, MedRxiv, BioRxiv, the Cochrane Library, ClinicalTrials.gov, and the World Health Organization International Clinical Trials Registry for studies published to June 12, 2020. Studies were reviewed to determine the risk of bias. A random-effects model was used to pool results. Heterogeneity was assessed using I^2. Fourteen studies of 2568 individuals were included. The incidence of recurrent SARS-CoV-2 positivity was 14.8% (95% confidence interval [CI] 11.44–18.19%). The pooled estimate of the interval from disease onset to recurrence was 35.4 days (95% CI 32.65–38.24 days), and from the last negative to the recurrent positive result was 9.8 days (95% CI 7.31–12.22 days). Patients with younger age and a longer initial illness were more likely to experience recurrent SARS-CoV-2 positivity, while patients with diabetes, severe disease, and a low lymphocyte count were less likely to experience. Present study concluded that the incidence of recurrent SARS-CoV-2 positivity was 14.8% suggesting further studies must be conducted to elucidate the possibility of infectious individuals with prolonged or recurrent RNA positivity.

COVID-19 REINFECTION

Understanding protection from SARS-CoV-2 by studying reinfection

Nature Medicine, Vol 26, November 2020:1678–1685

Understanding the risk of reinfection with SARS-CoV-2 in exposed cohorts provides an avenue to understanding the path to protection against SARS-CoV-2 for vaccine development.

Julie Overbaugh

The critical question: whether and how well a first infection protects against reinfection?

Several studies suggested that while reinfection could occur any time after a first infection, **the risk was highest soon after the first infection**, potentially **suggesting that the main risk was before the antibody response to the virus fully matured**,.

The window of protection is soon after a first infection, with limited protection over time.

Specific responses decay over time, as reported for endemic coronaviruses.

Current data suggest that **antibodies to SARS-CoV-2 are induced to peak levels within weeks of infection, but there are variable data on how quickly they wane over time.**

Reinfection occurs in people with notably weak immune responses?

Need of follow-up of well-characterized longitudinal cohorts including **people who are at continued risk of exposure,** due to occupation or other factors.

SARS-CoV-2 infection and antibody development

Antibody identification/antibody titres are recognised as a correlate of antiviral immunity, and anti-receptor-domain antibody levels are known to correspond to plasma viral neutralisation activity **developed in most individuals between day 10 and day 21 after infection.** Most patients (>91%) develop IgG seropositivity and neutralising antibodies (>90%) following primary infection.

Antibody levels to other coronaviruses wane over time, (**range: 12–52 weeks**). SARS-CoV-2 antibody levels remain up to 94 days after infection, and **the antibody titres peak between 3-4 weeks, remaining relatively stable up to 4 months**. Nevertheless, the neutralising activity significantly **decreases over time**.

It has been discussed that the magnitude of the antibody response appears to be associated with disease severity. Wang et al observed that the antibody response in mild illness patients was significantly lower, with lower levels of IgM response and lower levels of neutralising antibodies, when compared with severe COVID-19 patients.

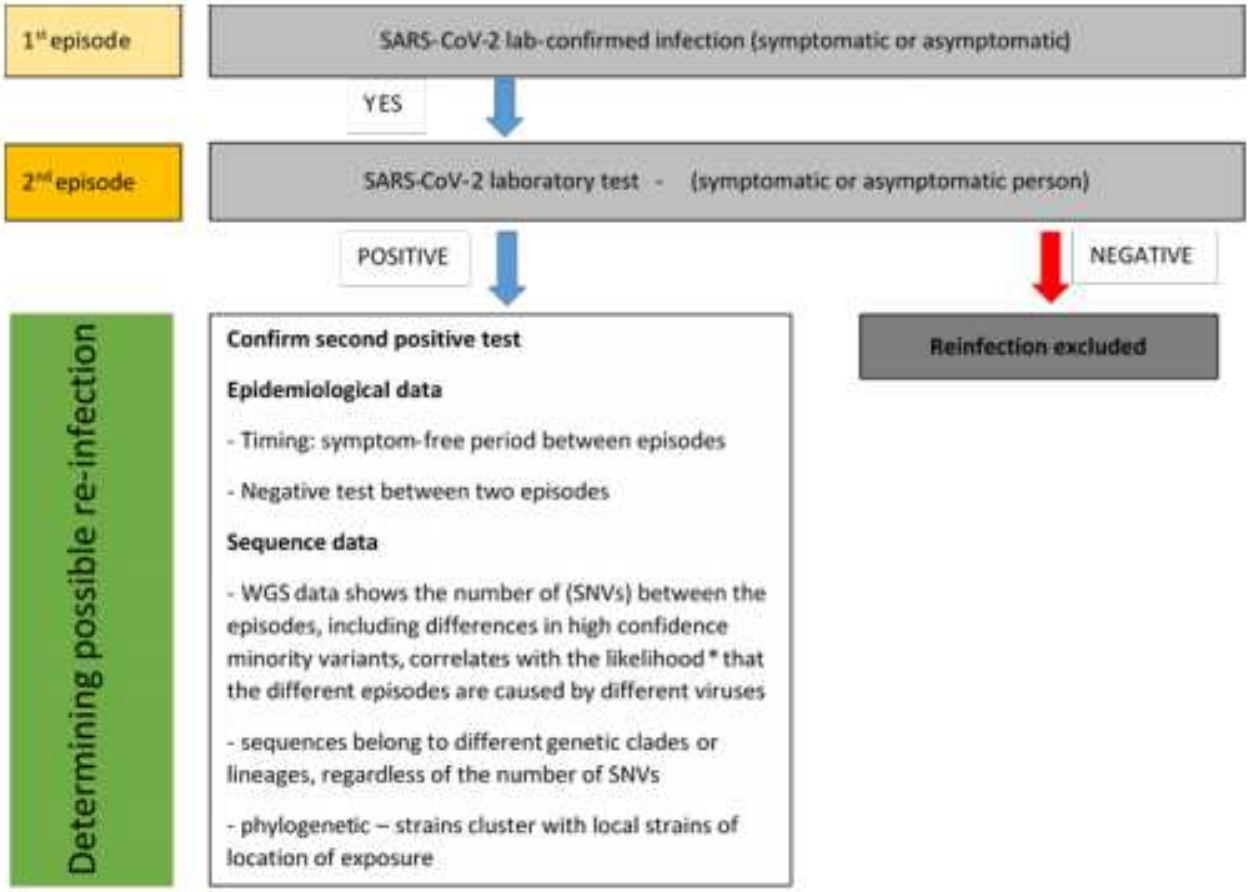
The magnitude and time period of decreasing antibody levels as well **as the impact of cellular immunity has not been sufficiently studied on larger population groups over extended periods**.

How can a SARS-CoV-2 reinfection be identified?

Other respiratory viruses, such as seasonal influenza cause COVID-19 similar symptoms and should be considered as differential diagnoses.

- **Epidemiological information**
  - Time elapsed between the first episode and the suspected second episode of infection
- **Clinical information**
  - **Existence of underlying disease/immunosuppressive therapy/immune modulators (diminished immune response)**
- **Information on testing / by test result and specimen**
  - Specimen type (e.g. respiratory, saliva)
  - **For RT-PCR results – Ct values.**
- **Immune assessment tests**
  - Duration/persistence, type and titres of antibodies [range]
  - Detection of neutralising antibodies
  - **T cell immunity and biomarkers** such as CD40L
- **Virus culture**
- **Comparative genomic analyses**
  - WGS [– the number of single nucleotide variations (Single Nucleotide Variations) between the episodes. The virus is expected to mutate by approximately 2 SNVs per month.
  - **Stronger evidence of reinfection** if the sequences recovered from the two episodes belong to **different genetic clades or lineages, regardless of the number of SNVs**.

Flow chart for assessing a reinfection in a previously confirmed COVID-19 case



* The virus is expected to mutate by approximately 2 SNVs per month

SNV: single nucleotide variations

WGS: Whole genome sequencing

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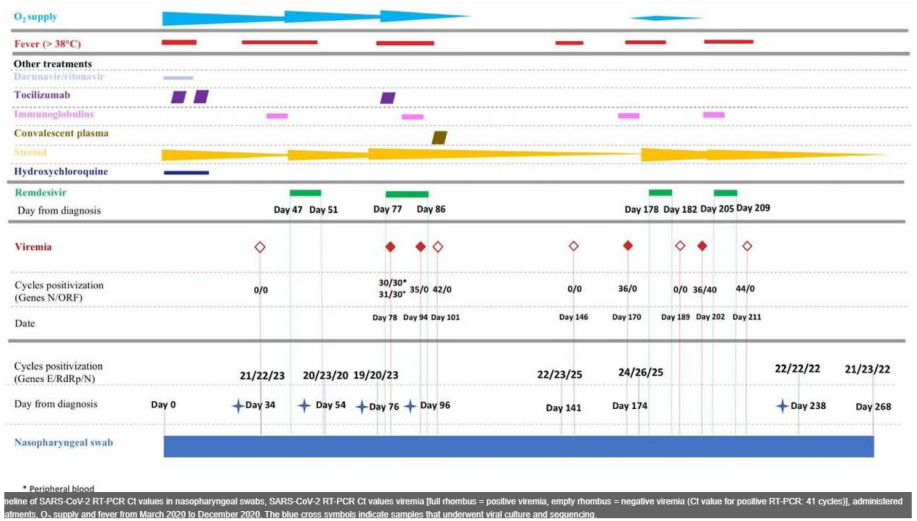
PERSISTENZA: DATI ITALIANI (1)

medRxiv
THE PREPRINT SERVER FOR HEALTH SCIENCES

The longest persistence of viable SARS-CoV-2 with recurrence of viremia and relapsing symptomatic COVID-19 in an immunocompromised patient – a case study

Chiara Sepulcri, Chiara Dentone, Malgorzata Mikulska, Bianca Bruzzone, Alessia Lai, Daniela Fenoglio, Federica Bozzano, Annalisa Bergna, Alessia Parodi, Tiziana Altosole, Emanuele Delfino, Giulia Bartalucci, Andrea Orsi, Antonio Di Biagio, Gianguglielmo Zehender, Filippo Ballerini, Stefano Bonora, Raffaele De Palma, Guido Silvestri, Andrea De Maria, Matteo Bassetti

doi: https://doi.org/10.1101/2021.01.23.21249554



meline of SARS-CoV-2 RT-PCR Ct values in nasopharyngeal swabs, SARS-CoV-2 RT-PCR Ct values viremia [full rhombus = positive viremia, empty rhombus = negative viremia (Ct value for positive RT-PCR: 41 cycles)], administered atments, O_2 supply and fever from March 2020 to December 2020. The blue cross symbols indicate samples that underwent viral culture and sequencing

8-month persistence (271 days) of SARS-CoV-2 RT-PCR positive nasopharyngeal swab in an **immunosuppressed patient with non-Hodgkin lymphoma**, with four symptomatic relapses of COVID-19.

During each relapse, the patient received a cycle of remdesivir; **however, viable virus remained detectable on nasopharyngeal swabs.**

Viral infectivity assessed by **viral culture on five nasopharyngeal swab samples:** cultures were all positive for viral replication with cytopathic effect observed at 48 hours.

Comparing the isolated and sequenced viral strains by genomic analysis we observed an increasing number of mutations from 7 on April to 20 in November.

Sequence Name	Clade	Pangolin	Total Aminoacid Changes	ORF1a	ORF1b	S	ORF3a	N	ORF14
BC1_Genova_Liguria_2020-04-27	20B	B.1.1	7	P1640L	P314L	D614G		R203K,G204R	G50N
BC2_Genova_Liguria_2020-05-17	20B	B.1.1	13	K291E, K291N, T1322R, T1638I, R3561K, A4396V	P314L	D614G	S74F	R203K,G204R	G50N
BC3_Genova_Liguria_2020-06-08	20B	B.1.1	15	T1322R,T1638I,R3561K,A3705V,S4398L	P314L	D614G,W1214C,I1221K,G1223C	G44A	R203K,G204R	G50N
BC4_Genova_Liguria_2020-06-18	20B	B.1.1	13	T283P,K291T,K292T,V306I,T1322R,T1638I,S4398L	P314L	D614G		R203K,G204R	G50N
BC5_Genova_Liguria_2020_11_17	20B	B.1.1	20	R24C,V86F,T1322R,V1570L,T1638I,T4087I,S4398L	P314L,C455Y	H69Y,H69P,V70G,D614G,S982A	T151I	R203K,G204R	G50N

PERSISTENZA e REINFEZIONE : DATI ITALIANI (2)

52-year-old male patient **with bladder carcinoma**, who attended the oncology department at Maggiore Hospital-Novara

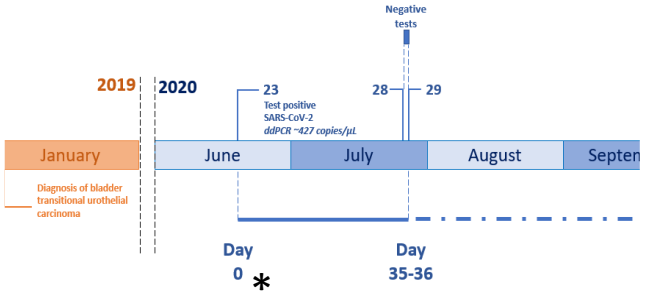
23 June (day 0) → cough and fever, RT-PCR assay of a nasopharyngeal swab specimen **positive for COVID-19** (ct 25-26)

day 35 and 36 → **two consecutive nasopharyngeal swabs negative for SARS-CoV-2** infection

day 170 → he had fever and **RT-PCR assay of a nasopharyngeal swab** was positive indicating a recurrence of Covid-19 (ct 34-36).

On day 176, he died from shock and respiratory failure.

Whole genome sequencing was performed from the nasopharyngeal swab harvested **on day 0, day 170 and post- mortem**.



seqName	Clade	Lineage	ORF1b
Day 0	20B	B.1.1	P314L
Day 170	20A	B.1	P314L
Day 175	20B	B.1.1	P314L

B.1

B.1.1

De Andrea M, Lai A, Bergna A, Zehender G, Borgogna C, Gariglio M

REINFEZIONE: DATI ITALIANI

RESEARCH LETTER

Assessment of SARS-CoV-2 RNA Test Results Among Patients Who Recovered From COVID-19 With Prior Negative Results

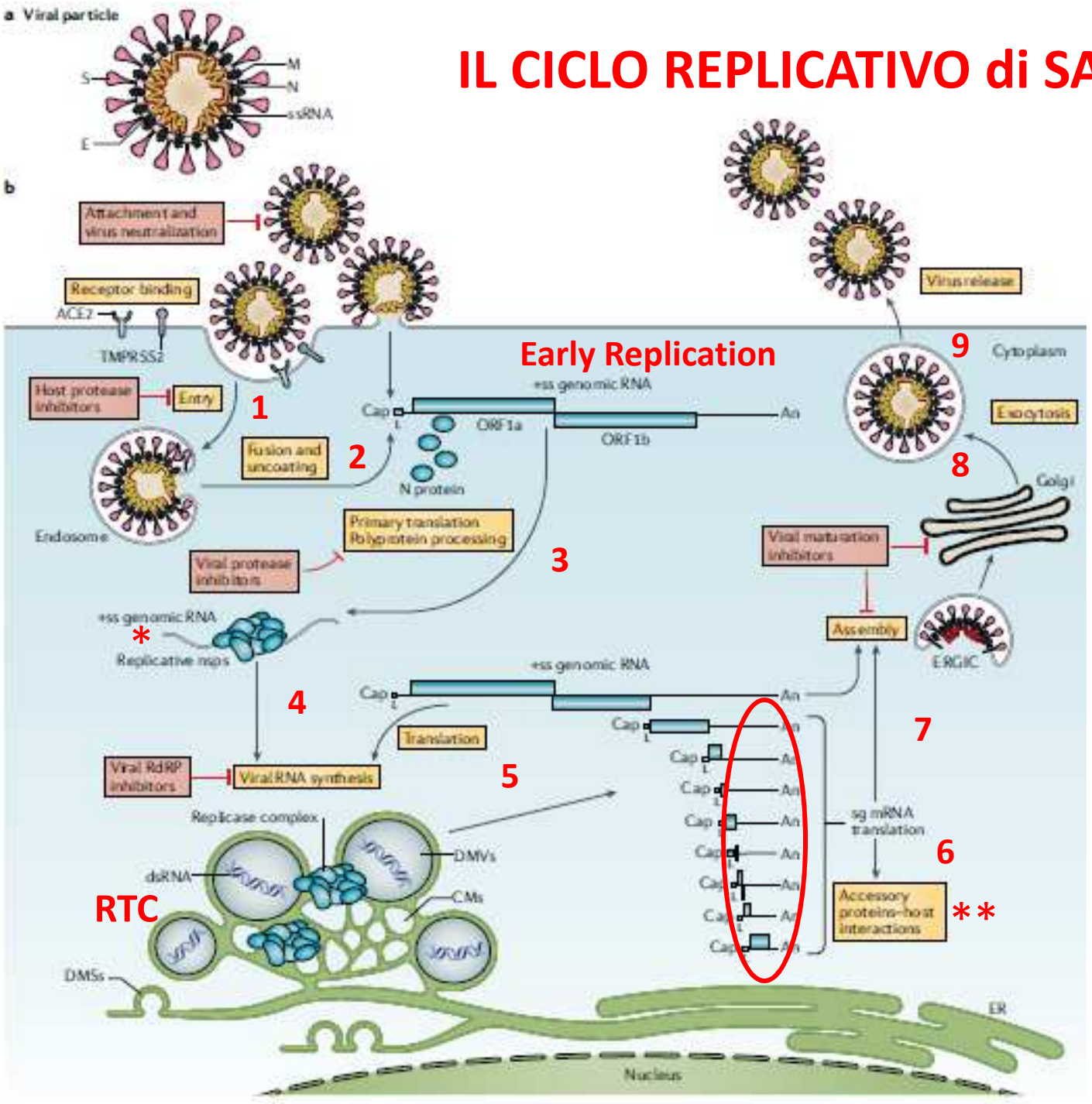
Liotti, et al JAMA Internal Medicine ***November 12****, 2020*

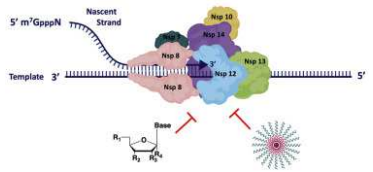
32/176 (18%) pazienti clinicamente guariti e studiati a una media di **48.6 giorni** ritornavano ad essere positivi ma solo **1 paziente** (3.1%) ha una **virus potenzialmente capace di replicare a 39 giorni** sulla base della presenza di **RNA sub genomici (ssRNA).**

Table. Testing Results for NOS Samples Obtained at COVID-19 Diagnosis or After COVID-19 Recovery in 32 Study Patients[a]

Sample	COVID-19 samples tested											Days of recovery sampling since diagnosis
	Diagnosis				Recovery							
	Genomic RNA (C_T value)			Subgenomic RNA (C_T value)	Genomic RNA (C_T value)			Subgenomic (C_T value)	RNA load, copies/mL	Serology (positive or negative result)		
Sample No.	*E* gene	*RdRP* gene	*N* gene	*E* gene	*E* gene	*RdRP* gene	*N* gene	*E* gene	*N* gene	IgG	IgA	
1	31.6	31.3	31.2	34.5	29.3	30.7	31.2	39.1	1.2×10^4	Positive	Positive	39
2	27.0	26.9	30.0	36.0	30.0	30.5	31.2		8.9×10^3	Positive	Positive	31
3	19.3	20.8	22.1	35.2	31.5	34.7	32.8		3.3×10^3	Positive	Negative	44
4	21.6	22.0	22.9	36.4	31.8	31.4	32.3		5.5×10^3	Positive	Positive	34
5	30.0	32.8	38.1	30.2	31.8	34.3	34.5		3.2×10^3	Positive	Positive	62
6	20.8	20.9	22.3	37.3	32.2	32.8	34.1		5.3×10^3	Positive	Positive	37
7	27.3	29.9	31.3	36.9	32.3	30.9	32.7		6.4×10^3	Positive	Positive	39
8	26.9	27.0	31.2	38.1	35.0	34.4	36.1		4.0×10^2	Positive	Positive	71
9	22.5	23.7	24.9	31.0	38.8	33.6	33.9		2.6×10^3	Negative	Negative	42
10	21.3	21.4	28.9	38.9		32.2	33.4		1.2×10^4	Positive	Positive	56
11	26.6	26.9	28.1	33.0		32.8	33.2		1.3×10^4	Positive	Positive	54
12	22.8	24.2	25.3	31.0		34.2	33.7		6.9×10^3	Positive	Positive	55
13	25.8	25.8	26.1	39.8	NA	34.8	39.1		3.0×10^2	Positive	Positive	36
14	20.8	20.4	21.1	32.0		35.0	35.1		1.9×10^3	Positive	Positive	56
15	29.4	30.1	32.2	37.0		36.5	39.2		3.2×10^3	Positive	Positive	36
16	27.9	29.1	31.1	32.0		38.1	39.3		1.6×10^1	Positive	Positive	77

IL CICLO REPLICATIVO di SARS-CoV-2





* **Proofreading Activity Complex**

RTC: Replication Transcription Complex

** **nps/immune system interaction**

POTENTIAL INHIBITORS

RISK of REINFECTION

Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers

S.F. Lumley, D. O'Donnell, N.E. Stoesser, P.C. Matthews, A. Howarth, S.B. Hatch, B.D. Marsden, S. Cox, T. James, F. Warren, L.J. Peck, T.G. Ritter, Z. de Toledo, L. Warren, D. Axten, R.J. Cornall, E.Y. Jones, D.I. Stuart, G. Screaton, D. Ebner, S. Hoosdally, M. Chand, D.W. Crook, A.-M. O'Donnell, C.P. Conlon, K.B. Pouwels, A.S. Walker, T.E.A. Peto, S. Hopkins, T.M. Walker, K. Jeffery, and D.W. Eyre, for the Oxford University Hospitals Staff Testing Group*

BACKGROUND

The relationship between the presence of antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the risk of subsequent reinfection remains unclear.

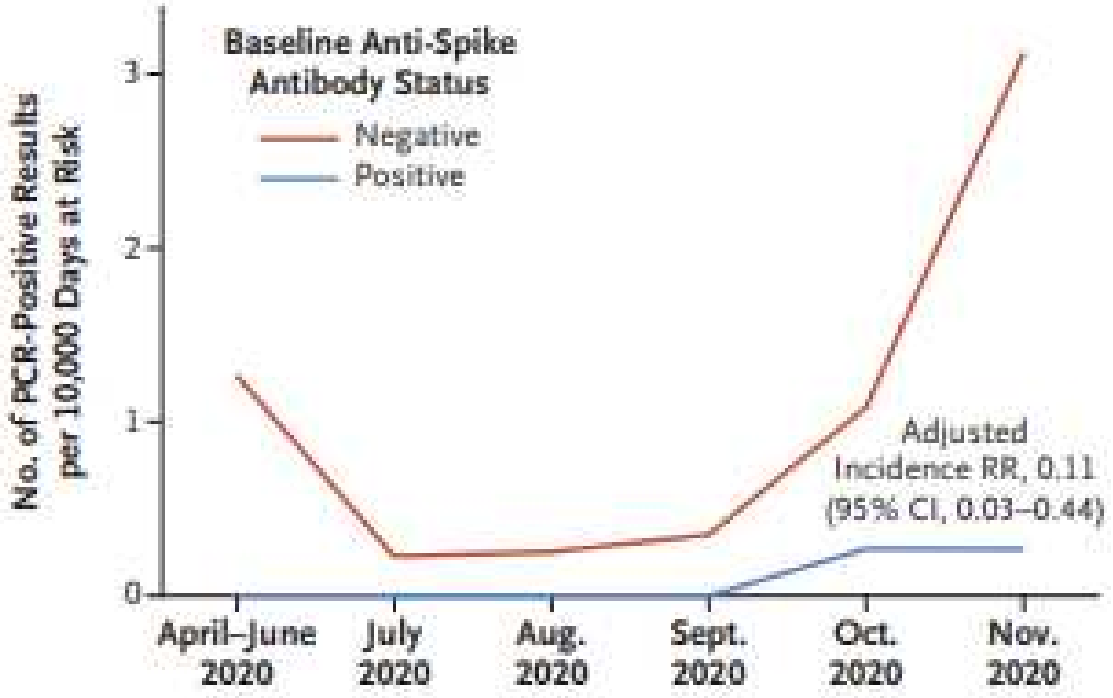
METHODS

We investigated the incidence of chain reaction (PCR) in seroposit ing testing of asymptomatic and in the United Kingdom. Baselin (primary analysis) and anti-nucle lowed for up to 31 weeks. We est results and new symptomatic inf age, participant-reported gender,

RESULTS

A total of 12,541 health care workers participated and had anti-spike IgG measured; 11,364 were followed up after negative antibody results and 1265 after positive results, including 88 in whom seroconversion occurred during follow-up. A total of 223 anti-spike–seronegative health care workers had a positive PCR test (1.09 per 10,000 days at risk), 100 during screening while they were asymptomatic and 123 while symptomatic, whereas 2 anti-spike–seropositive health care workers had a positive PCR test (0.13 per 10,000 days at risk), and both workers were asymptomatic when tested (adjusted incidence rate ratio, 0.11; 95% confidence interval, 0.03 to 0.44; P=0.002). There were no symptomatic infections in workers with anti-spike antibodies. Rate ratios were similar when the anti-nucleocapsid IgG assay was used alone or in combination with the anti-spike IgG assay to determine baseline status.

RISK of REINFECTION



Days at Risk						
Seronegative	456,963	307,508	316,141	312,027	332,704	329,469
Seropositive	316	19,474	31,601	34,011	36,824	37,098

Figure 1. Observed Incidence of SARS-CoV-2–Positive PCR Results According to Baseline Anti-Spike IgG Antibody Status.

CONCLUSIONS

The presence of anti-spike or anti-nucleocapsid IgG antibodies was associated with a substantially reduced risk of SARS-CoV-2 reinfection in the ensuing 6 months. (Funded by the U.K. Government Department of Health and Social Care and others.)

https://bnonews.com/index.php/2020/08/covid-19-reinfection-tracker/

COVID-19 reinfection tracker

Published 5 months ago on August 28, 2020
By **BNO News**

This article is updated daily.

The table below shows confirmed cases of COVID-19 reinfection. The first confirmed case of reinfection was reported in Hong Kong in late August, and 46 others were reported over the next few months, along with thousands of suspected cases.

SUSPECTED CASES	DEATHS
10,721	**30**

CONFIRMED CASES OF REINFECTION

CASES	DEATHS	RECOVERED	AVERAGE INTERVAL
47	2	40	84 days

CONCLUSIONS

Reinfection seems to be a limited event at this stage of pandemic.

However, the proportion of **immunocompromised hosts,** who may be more susceptible to recurrent infection, and the **circulation of viral variants** **pose serious concerns about the risk of reinfection.**

The **role of cellular immunity** in the prevention of COVID-19 reinfection was not studied in the reported cases and needs to be investigated.

The **number** of mutations as well as the **positions** of the mutations in the genome might help to understand the possibility of reinfections and **possible immune response escape.**

Present concerns are also related to reinfection as the consequences of vaccine escape mutants as well as the result of resistance to developing therapeutic agents (monoclonal antibodies).

GRAZIE per l'ATTENZIONE!