Isolation of nearly 4000 SARS-CoV-2 shows increase of contagiousness associated with alpha then delta variant

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Abstract

Culture inoculation of 6722 nasopharyngeal samples since February 2020 allowed isolation of 3637 SARS-CoV-2 and confirmed that isolation rate is correlated to viral load, regardless symptomatology or vaccination status. Moreover, the delta variant is associated with higher viral loads and therefore higher rates of viral isolation, explaining its greater contagiousness.

Introduction

Since February 2020 and diagnosis of first cases of Covid-19 in south of France, our BSL3 laboratory was involved in massive SARS-CoV-2 isolation attempt with up to 3790 samples inoculated and 1941 strains isolated [1,2]. As a consequence, we early established a clear correlation between viral load, assessed by cycle threshold values (Ct) obtained by real time reverse transcriptase-PCR (qPCR) and isolation of the virus in cell culture, which is an indirect marker of patient contagiousness. In relation with our studies, it was suggested that asymptomatic SARS-CoV-2-infected patients would have a lower viral load and/or would be less contagious [3]. Furthermore, since the introduction of Covid-19 vaccination, it has been also suggested that patients infected post-vaccine immunization would be less contagious [4]. In order to evaluate these two aspects, we analyzed different groups of patients for whom these data were available in our collection. The present study covers different periods of time corresponding each to the predominance in our country of a SARS-CoV-2 genotype: February-September 2020 during which close Wuhan-Hu-1 strain derivatives predominated; October-December 2020 during which the Marseille-4 (a.k.a 20A.EU2 (Nextstrain clade (https://clades.nextstrain.org/)) or B.1.160 (Pangolin classification (https://cov-lineages.org/resources/pangolin.html))) variant predominated; January-June 2021 during which the alpha variant (Pangolin lineage B.1.1.7) predominated; and most recently, since July 2021, the delta variant (Pangolin lineage B.1.617) predominated.

Materials and methods

Between February 2020 and the 27th of July 2021, a total of 8930 samples have been inoculated in our BSL3 laboratory, including 6731 nasopharyngeal samples. qPCR and culture were performed as previously described [1,2]. In the present work, only nasopharyngeal samples were analyzed, and samples with a Ct>35 at time of diagnosis were excluded because they were associated with <3% of positive culture and were at greater risk

of false positive results [2,5]. The information on the symptomatic or asymptomatic status of patients, when available, was collected from the anonymized database of patients tested in our laboratory. For vaccinated people, the information was collected since January 2021, and as part of the present study we defined vaccinated patients as those diagnosed ≥15 days after their first injection of any of the four vaccines available in Europe (https://www.ecdc.europa.eu).

Statistical analysis was performed on GraphPad prism 5.03 using One-way Anova or Mann-Whitney tests. SARS-CoV-2 culture was done as previously described, except for samples inoculated the last study's week during which cultures were only observed for 2 weeks (we considered the third week could be omitted as it accounted for only 1% of isolates) [2].

Results

We could isolate 3637 (54%) SARS-CoV-2 from the 6722 patients' samples inoculated. Culture positivity was inversely proportional to Ct at diagnosis as Ct was significantly lower for patients with a positive than negative culture (mean±standard deviation: 23.2±4.83 versus 28.3±4.9, respectively; $p<0.0001$) (Figure 1a). Symptomatic or asymptomatic status was known for 3761 and 543 patients respectively. Mean Ct was significantly lower in asymptomatic than symptomatic patients (23.1±5.9 versus 26.1±5.4, respectively; $p<0.0001$) (Figure 1b). Regarding culture isolation, it was positive for 50% (1882/3761) of symptomatic patients compared to 69% (372/543) of asymptomatic patients ($p<0.0001$) (Figure 1c). Since January 2021, we inoculated 309 samples from patients having received ≥1 dose of Covid-19 vaccine. Vaccine administered was Pfizer-BioNTech, AstraZeneca, Moderna, and Janssen in 162, 33, 8 and one patient, respectively; vaccine administered was unknown for 105 patients. In the same period of time, we also inoculated 433 samples for unvaccinated patients. Ct at diagnosis were significantly lower for vaccinated

patients than unvaccinated patients (21.5±4.5 versus 23.4±5.4, respectively) (Figure 1d) ($p<0.0001$). We isolated 80% (249/309) of SARS-CoV-2 among vaccinated patients versus 66% (287/433) among unvaccinated patients ($p<0.0001$) (Figure 1e). For the 150 vaccinated patients for whom this information was available, 134 (89%) were symptomatic and 16 (11%) were asymptomatic (Figure 1f). For the 209 unvaccinated patients for whom this information was available, 167 (80%) were symptomatic and 42 (20%) were asymptomatic. The proportion of symptomatic patients was statistically different from that among vaccinated patients ($p=0.008$) (Figure 1f). Finally, we tested if the predominant SARS-CoV-2 variant at time of diagnosis was associated with a different Ct and culture positivity rate. We studied four periods: (i) February-September 2020 (Wuhan-Hu-1 close virus derivatives (French original viruses)), (ii) October-December 2020 (predominance of 20A.EU2 variant, in 1684/2135 (79%) of the samples with viral genotype available); (iii) January-June 2020 (predominance of alpha variant; 9060/14495 (63%) samples with viral genotype available); and (iv) July 2021 (delta variant, 1932/2031 (95%) samples with viral genotype available). There was no significant difference between mean Ct of the periods with the French original SARS-CoV-2 and during which the 20A.EU2 variant predominated (25.6±5.5 versus 25.7±5.5 respectively). In contrast, mean Ct was significantly lower for periods during which the alpha (22.6±5.2) then delta variants (19.7±3.4) predominated than for the two former periods, and between January-June than in July ($p<0.0001$) (Figure 1g). In addition, the culture isolation rate was inversely correlated with the Ct (Figure 1h) ($p<0.0001$).

Discussion

This work allowed us to still confirm a relationship between qPCR Ct at diagnosis and SARS-CoV-2 isolation [1,2]. The same correlation between culture positivity rate and Ct (as a proxy of viral load) was observed by others authors despite culture viral isolation sensitivity could vary dramatically according to the procedure [6,7]. In our work, such correlation was

independent of the period of study, of the presence or absence of clinical symptoms, and of the vaccination status of the patients. Unexpectedly, we observed that patients symptomatic at SARS-CoV-2 diagnosis had lower viral loads and culture isolation rate than asymptomatic patients, regardless they were vaccinated or not (Figures 1b-c, 1f). We believe that this could be due to the fact that asymptomatic patients may test earlier than symptomatic patients in the course of infection, when viral loads are highest. Still more surprising are greater viral loads and culture isolation rates in vaccinated patients compared to those unvaccinated (Figures 1d-e). A bias in selection of patients coming to our institute to be tested for SARS-CoV-2 infection is possible, but such correlation has already been observed among healthcare workers immediately after vaccination for whom the absolute risk of testing SARS-CoV-2-positive was increased without obvious explanation [8]. Rate of culture isolation was correlated with the Ct in nasopharyngeal samples and the predominantly circulating SARS-CoV-2 genotype. Indeed, Ct during periods during which for the alpha variant largely predominated was lower than those during which French original SARS-CoV-2 and 20A.EU2 variants predominated. This is in agreement with a study of 341 patients infected between November and December 2020 in London that reported that those infected with the alpha variant (n=198 (58%) had a lower Ct than those infected with a non-alpha variant virus (29 versus 32; $p<0.0011$) [9]. Despite a short period of time (only July 2021) and a small effective of patients (n=94) were analyzed, the delta variant was notwithstanding associated with a lower mean Ct than the alpha variant, an observation in agreement with other recent reports [10]. Indeed, two recent US studies reported similar conclusion, as patients infected with the delta variant had higher viral loads than those infected with former variants, regardless they were vaccinated or not [11]. The lack of information about the proportion of fully-vaccinated patients (2-dose course of vaccine $\geq$14 days before SARS-CoV-2 infection) could be considered a weakness in our work. However, Ct were also similar among samples from

patients fully-vaccinated or not in the study by Brown et al. [12] who enrolled 74% of patients fully-vaccinated and 90% infected with the delta variant. Overall, the present work confirms that higher viral loads observed with the delta variant are correlated to higher positivity rate of culture virus isolation, and therefore to higher contagiousness of patients, regardless of the vaccination status of the patients and/or the presence or absence of clinical symptoms.

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Conflict of Interest: The others authors declare no conflict of interest.

Ethical approval : The protocol was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (No.: 2020-029 and 2021-023).

Informed consent: All subjects provided informed consent in accordance with the Declaration of Helsinki.

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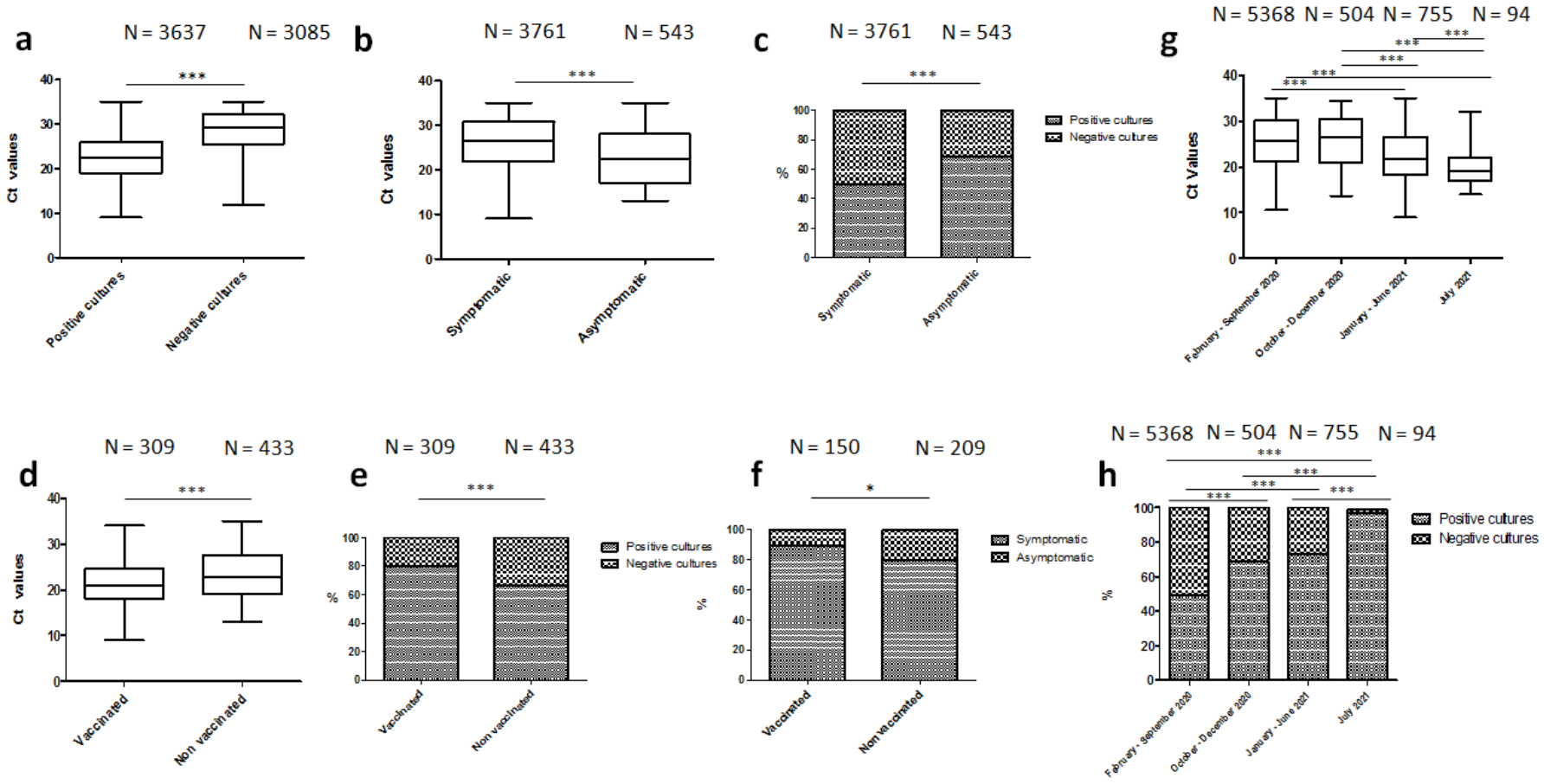
Figure 1. Correlation between viral load evaluated by RT-PCR Ct (a, b, d, g), symptomatology (b, c, f), vaccine status (d, e, f), time of sampling (g, h) and isolation yield (c, e, h).


a
N = 3637
N = 3085
***
Ct values
Positive cultures
Negative cultures
b
N = 3761
N = 543
***
Ct values
Symptomatic
Asymptomatic
c
N = 3761
N = 543
***
%
Positive cultures
Negative cultures
Symptomatic
Asymptomatic
g
N = 5368
N = 504
N = 755
N = 94
***
Ct Values
February - September 2020
October - December 2020
January - June 2021
July 2021
d
N = 309
N = 433
***
Ct values
Vaccinated
Non vaccinated
e
N = 309
N = 433
***
%
Positive cultures
Negative cultures
Vaccinated
Non vaccinated
f
N = 150
N = 209
*
%
Symptomatic
Asymptomatic
Vaccinated
Non vaccinated
h
N = 5368
N = 504
N = 755
N = 94
***
%
Positive cultures
Negative cultures
February - September 2020
October - December 2020
January - June 2021
July 2021