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Policy for Screening SARS-CoV-2 Variants in the Emergency Room Revealed 2 Cases of Moderate Infection Due to Alpha Variant in Patients Vaccinated with ChAdOx1-S

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Research Article

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ABSTRACT

With the evolution of the COVID-19 pandemic and the emergence of variants, it is of importance to monitor the outbreak on the ground. Only genome sequencing can accurately type variants, but this method is expensive, time-consuming and unsuitable for routine use. The detection of major mutations of concern with a RT-qPCR although more limited is a rapid, robust and affordable alternative.

INTRODUCTION

The Emergency Department is one of the primary gateways for patients with COVID-19. In our institution, more than 10 percent of the tests carried out in the laboratory concern patients admitted *via* the emergency rooms. Over the months since the beginning of the pandemic, Variants of Concern (VOC) and Variants of Interest (VOI) have appeared and are spreading in the community. Some authors report the link between transmissibility or immune escape and mutations in the spike protein of SARS-CoV-2 [1-4]. However, precisely determining the type of variant

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requires sequencing of the viral genome. In addition to the fact that Next-Generation Sequencing (NGS) can only be done in specialized laboratories, the lead time often exceeds one week and is not compatible with clinical practice. Moreover the method is expensive and laborious and is not suitable for a mass routine. In recent months, other alternatives have been developed and consist in the detection by RT-qPCR of the mutations most frequently encountered in VOCs with clinical implications. Even if these analyses provide less genomic information compared to NGS, they have the advantage of being about 8 times cheaper than NGS and can be included routinely on a PCR platform for conventional screening. As a result, the results can be obtained 24 hours after obtaining a first-line positive test. The main objective of our study is to assess the impact of the presence of SARS-CoV-2 mutations on the outcome of patients with COVID-19.

MATERIALS AND METHODS

During 37 days, we reviewed the records of patients consulting the emergency room and diagnosed with SARS-CoV-2. The N501Y and E484K mutations and the H69/V70 deletion were assessed by RT-qPCR.

Settings

The evaluation was carried out in a 550-bed public hospital in the Brussels region between 5th March and 11th April 2021. All consecutive patients admitted to one of our 3 emergency rooms with any indication for a COVID-19 testing and with a positive SARS-CoV-2 result were included in the study. For each patient, a clinical follow-up was carried out over a 2-month period.

Data collection

The medical records were reviewed retrospectively by a single emergency medicine supervisor. The metrics collected were demographics, history of COVID-19, notion of previous COVID-19 vaccination (including vaccine type, number of doses received and date of last injection) and clinical data. Measured parameters were also recorded (PO_2 at admission and worst, CRP, D-Dimers) as well as the outcome of each patient (hospitalization, transfer to another hospital, admission to the ICU, length of stay and mortality).

Diagnostic of COVID-19 infection

Patients were considered positive according to the results of the RT-qPCR. The analyses were performed as follows: all the samples tested were fresh nasopharyngeal swabs taken from UTM-RT swabs (Copan SpA, Brescia, IT) or from Vacuette Virus Stabilization Tube (Greiner Bio-One International GmbH, Kremsmünster, Austria). First-line molecular analyses were performed either by Transcription Mediated Amplification using the Aptima® SARS-CoV-2 assay kit (Hologic, San Diego, CA, USA) on a Panther platform, or by PCR using the Allplex® kit (Seegene Technologies, Seoul, South Korea) after extraction with the STARMag Viral DNA/RNA 200 C Kit (Seegene Technologies, Seoul, South Korea) on a STARLet platform (Hamilton Company, Reno, NV, USA). The positive results were retested with RT-qPCR with extraction using the Novaplex™ SARS-CoV-2-variants I kit (Seegene Technologies, Seoul, South Korea), targeting the RdRP gene and the following mutations: deletion H69/V70, N501Y and E484K. The search for mutations is considered positive when the cycle threshold (Ct) is less than or equal to 33 cycles. The amplification step was performed using a CFX96 C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA).

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Statistical analysis

Statistical analysis was carried out using MedCalc version 10.4.0.0 (MedCalc Software, Ostend, Belgium). Descriptive statistics were used to analyze the data. A P-value<0.05 is considered significant.

RESULTS

One hundred and twenty-five COVID-19-positive patients were included over the 37 days of the study: 52 women, 73 men, median age (95% Confidence Interval (CI); extremes) 50.7 years (50.4-56.8; 14.0-95.1). No patient reported a history of COVID-19 in the past. Five patients were vaccinated, all with ChAdOx1-S vaccine (Vaxzevria®, AstraZeneca, Oxford, UK), 2 of whom received 2 doses. Admission took place 4, 7 and 14 days after the first dose for patients who received one dose and 2 and 9 days after the second dose for patients who received both doses. Table 1 shows the characteristics of patients being vaccinated. At admission, 74 patients (59.2%) complained of breathing difficulties, 27 (21.6%) had digestive symptoms, 54 (43.2%) had fever and 70 (56%) had degradation of the general state. 71 patients (56.8%) were hospitalized, among them 21 were in intensive care unit (ICU) and 6 patients were redirected to other hospitals. The duration of hospitalization (2-month review) was 12 days (95% CI: 8.8-14.2) and 7 patients were still hospitalized prior to data analysis. Eight patients (6.4%) died within 1 month of observation (time between admission and death [95% CI]: 13.5 days [6.1-23.2]). The causes of mortality were all in relation to COVID-19: viral pneumonia leading to hypoxemia (N=2) or cardiopulmonary arrest (N=1), COVID-19-related acute respiratory distress syndrome (N=4) and secondary pulmonary embolism (N=1).

Table 1. Characteristics of patients being vaccinated with ChAdOx1-S.

	Gender	Age	Risk factors	N doses of vaccine	Delay last dose (day)	PaO_2 (mm Hg)	CRP (ml/dL)	D-dimers (ng/mL)	Admission to ICU	Length of stay in hospital (days)	Death (delay)	Ct
Patient 1	f	60.5	None	2	2	101	70,6	2104	No	23	No	21.35
Patient 2	m	82.3	None	2	9	66	34.0	1129	No	15	No	23.45
Patient 3	m	85.3	None	1	7	51	157.0	1720	No	2	Yes (2 Days)	19.08
Patient 4	f	77.8	None	1	4	67	41.0	1726	No	5	No	19.24
Patient 5	m	69.4	Hypertension	1	14	73	134.0	>10000	Yes	14	No	23.10

Table 2 shows the measured PO_2, CRP and D-dimers in hospitalized, ICU admitted and deceased patients. Values were compared using a Mann-Whitney U-test. No differences were observed regarding D-dimers levels in the different categories of patients. CRP levels are significantly higher in patient requiring hospitalization, in patients admitted in ICU and in patients who deceased. The PaO_2 is globally lower in ICU patients. The following treatments were administered in the emergency room: low-molecular-weight heparin 44.8%; corticosteroids 40.0%; antibiotics 13.6%. Treatment was symptomatic (paracetamol, cough medicine) in 19.2% of patients. Mutations E484K, N501Y and deletion H69/V70 were found in 6.4%, 84.8% and 82.4% of patients, respectively. Table 3 shows the relationship between combinations of mutations and patient outcomes. A Kruskal-Wallis ANOVA did not permit to highlight a significant difference between patients having different variants regarding hospitalization, admission to

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ICU and mortality. All 8 patients who died (medium age [95% CI]: 72.6 years [60.1-84.8]) had a positive test for H69/V70 deletion and N501Y mutation together. One of these patients had received a dose of ChAdOx1-S seven days earlier. The median Ct (95% CI) of the control RdRP gene was 20.9 (20.5-22.4) cycles.

Table 2. Parameters in categories of patients.

	PaO_2 upon admission (mm Hg)		Worst PaO_2 (mm Hg)		CRP mg/dL		D-dimers (ng/mL)	
Hospitalized	69.4	P=0.063	66.9	N/A	90.2	P<0.0001*	1544.6	P=0.14
Not hospitalized	83.3		N/A		13		1580	
Admitted in ICU	61.9	P=0.0135*	56.8	P<0.0001*	121.4	P=0.0079*	2086.7	P=0.40
Not admitted in ICU	72.7		71.6		76.1		1308.3	
Non survivors	63.1	P=0.0559	57.9	P=0.09	125.1	P=0.0131*	1187.2	P=0.68
Survivors	72.6		70.8		68.9		1586.4	
Note: *a P-value <0.05 is considered significant.								

Table 3. Distribution of variants in emergency room and overall.

	No mutation detected	HV69/70	N501Y + E484K	HV69/70 + N501Y	HV69/70 + N501Y + E484K	E484K	Other combinations
Emergency room							
Hospitalization	5	3	5	58	-	0	-
	35.7%	75.0%	71.4%	58.6%	-	0.0%	-
ICU	1	2	0	18	-	0	-
	20.0%	66.7%	0.0%	30.5%	-	0.0%	-
Death	0	0	0	8	-	0	-
	0.0%	0.0%	0.0%	8.1%	-	0.0%	-
Over all data							
N overall	209	60	44	693	5	7	11
% over all	20.3%	5.8%	4.3%	67.3	0.5%	0.7%	1.1%
N Emergency	14	4	7	99	0	1	0
% Emergency	11.2%	3.2%	5.6%	79.2%	0.0%	0,8%	0.0%

DISCUSSION

Positioning of RT-qPCR for VOC detections in clinical practice

Monitoring SARS-CoV-2 variants is essential because some VOCs have clinical implications. This monitoring is already assessed at the level of national monitoring programs, but sequencing capacities are limited and field actors can also play a role in the detection of VOC and VOI. Emergency departments are involved in screening for COVID-19, both for at-risk contacts and for patients with symptoms compatible with COVID-19. In our institution, the

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results of molecular tests of SARS-CoV-2 are available within 24 hours, while the search for the most common mutations of VOC in Belgium is carried out most often within 48 hours. In addition, since the end of this study, first-line tests have sometimes been performed directly at the patient's bedside using a rapid point-of-care molecular screening method. The performance is equivalent to the classic RT-qPCR and produces a qualitative result in just 10 minutes. Then for positive COVID-19 results, a RT-qPCR for VOCs detection allowing a detection of the most frequent mutations is now systematically perform. We can detect H69 and V70 ΔH69/ΔV70) deletions and N501Y and E484K mutations that affect all spike protein. The implementation of this new procedure makes it possible to return a positive COVID-19 result by specifying the mutations detected within 24 hours. Moreover, highlighting VOCs by PCR is more robust than the NGS. Indeed, the performance of NGS is poor for Ct above 25, while PCR can detect mutations up to Ct 33, and therefore in samples with much lower viral loads. In our series, 31 samples out of 125 (24.8%) with a Ct>25 would potentially not have been interpretable by NGS.

The ΔH69/ΔV70 deletion was first described in Denmark and was associated with failure to detect the S gene coding the spike protein using certain molecular methods [5]. This variant is quite common in Belgium. VOC B.1.1.7, B.1.351 and P.1 emerged around September 2020 and include the N501Y mutation. VOC B.1.351 and P.1 (South African and Brazilian) combine the N501Y and E484K mutations. The VOC B.1.1.7 (UK variant) combines ΔH69/ΔV70 and N501Y but may also present the E484K mutation. This is the most frequent VOC in Belgium. While N501Y significantly increases their potential of contagion, E484K affects antibody neutralization [4,6,7].

VOC implications on ChAdOx1-S vaccine escape

Phenotypic impacts of VOCs are now better understood. For example, B.1.1.7 is involved in an increased transmissibility risk [8], and in a possible increased severity (with a risk of hospitalization [9] or death [10]). Impacts on vaccines are now detailed in the WHO COVID-19 weekly Epidemiological updates [11] but data remain fragmented. There is an ongoing need to strengthen surveillance and reporting cases of vaccines escape.

Lopez Bernal et al., recently showed that a single dose of ChAdOx1-S provides significant protection against COVID-19 and further protection against severe disease lasting at least six weeks, including against the UK variant of concern (B.1.1.7). They also concluded that a single dose of this vaccine was about 80% effective at preventing admission to hospital with COVID-19[12]. In contrast, Madhi et al. showed that a two-dose regimen of the ChAdOx1 nCoV-19 vaccine did not show protection against mild-to-moderate COVID-19 due to the variant B.1.351[13].

The main objective of our study was to assess the impact of the presence of SARS-CoV-2 mutations on the outcome of patients with COVID-19. We took advantage of the availability of results on the most common variants in order to

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link their presence to the clinical impact on patients admitted *via* the emergency department. As expected, samples with both ΔH69/ΔV70 and N501Y, presumptive of VOC B.1.1.7 are predominant and represent almost 80% of our positive patients. At the time of the investigation the wild virus (Wuhan-Hu-1) represented only 11.2% of the positives. More than half of the patients required hospitalization and almost 30% of them at the ICU, regardless of the results of the mutations. The differences observed in Table 3 are not significant (P > 0.05). Although a Chi Square test could not demonstrate a significant relationship between mutations and mortality, all deceased patients in this series were carriers of the putative B.1.1.7 variant. Interestingly, among them, one 85-year-old was vaccinated but had received only one dose of ChAdOx1-S vaccine one week earlier. Bernal et al.[12] have demonstrated the efficacy of ChAdOx1-S vaccine in patients over 70-year-old, but this vaccine provides a real protection only 3 weeks after the first dose. In addition to this case report, further studies are expected to assess the effect of ChAdOx1-S on mortality.

In our study we also reported two cases of hospitalized patients (60.5 and 82.3-years-old) with no vaccinal protection against moderate COVID-19 caused by variant B.1.1.7 despite two doses of vaccine. The mutations found in these patients were also ΔH69/ΔV70 and N501Y. Our current methods make it possible to identify the presumed B.1.1.7, B.1.351 and P.1 variants. As the epidemiology of COVID-19 evolves, it becomes very risky to continue to conclude that the absence of mutations detected with our methods means de presence of wild virus. Indeed, in the past few weeks, Indian variants (B.1.617) have been emerging in Belgium and cannot be detected by the Novaplex™ SARS-CoV-2-variants I kit. These variants are carriers of the L452R mutation, which can be highlighted with a variant-II version that we intend to use routinely in the third line.

CONCLUSION

Routine systematic monitoring of SARS-CoV-2 variants allows a local representation of epidemiology and its evolution. Although the search for mutations by RT-qPCR is much less precise than the sequencing, it can be within the reach of all hospitals and makes it possible to target the most common VOCs. In addition, manufacturers are working to develop new products to detect emerging variants. The hospitalization rate in our series is very high and probably reflects a bias between patients with COVID-19 coming into the emergency room and those who are consulting their family doctor. Overall mortality was 6.4% and included one patient who received a vaccine dose. Of the patients included, two people received two doses of ChAdOx1-S vaccine and still contracted COVID-19 with a symptomatology severe enough to justify their hospitalization. To our knowledge, this is the first description of moderate COVID-19 infection with variant B.1.1.7 in patients having received both doses of this vaccine.

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REFERENCES

1. Wibmer CK, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med.2021;27:622-625.
2. Wu K, et al. mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. bioRxiv. 2021;1-20.
3. WHO, SARS-CoV-2 Variants – Disease Outbreak News, 2020, December 31.Reliefweb.2020
4. Leung K, et al. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020, Euro Surveill. 2021;26:1-7.
5. Bal A, et al. Two-step strategy for the identification of SARS-CoV-2 variant of concern 202012/01 and other variants with spike deletion H69-V70, France, August to December 2020. Euro Surveill. 2020;26:1-3.
6. Wang Z, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature. 2021;592: 616-622.
7. Jangra S, et al. SARS-CoV-2 spike E484K mutation reduces antibody neutralization. Lancet Microbe.2021;2:283-284.
8. Curran J, et al. Transmission characteristics of SARS-CoV-2 variants of concern. medRxiv.2021;1-33.
9. Aleem A, et al. Emerging Variants of SARS-CoV-2 And Novel Therapeutics Against Coronavirus (COVID-19). StatPearls Publishing.2021.
10. NERVTAG paper on COVID-19 variant of concern B.1.1.7. 2021;1-19.
11. COVID-19 Weekly Epidemiological Update. WHO.2021;1-32.
12. Bernal J L, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on COVID-19 related symptoms, hospital admissions, and mortality in older adults in England: Test negative case-control study. BMJ.2021; 373:1-11.
13. Madhi S A, et al. Efficacy of the ChAdOx1 nCoV-19 COVID-19 Vaccine against the B.1.351 Variant. N Engl J Med.2021; 384:1885-1898.