The SARS-CoV-2 mRNA vaccine breakthrough infection phenotype includes significant symptoms, live virus shedding, and viral genetic diversity

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Abstract

Little is known about SARS-CoV-2 'vaccine-breakthrough' infections (VBI). Here we characterize 24 VBI in predominantly young healthy persons. While none required hospitalization, a proportion endorsed severe symptoms and shed live virus as high as 4.13×10^3 PFU/mL. Infecting genotypes included both variant-of-concern (VOC) and non-VOC strains.

Key words: SARS-CoV-2, vaccine breakthrough, symptoms, patient reported outcomes, live virus

Introduction

SARS-CoV-2 vaccines have been administered in the United States and elsewhere in the world since late 2020. Several of these vaccines demonstrated high efficacy in Phase III clinical trials (1, 2). A number of vaccine effectiveness studies have recapitulated protection against virologically proven SARS-CoV-2 infection (3-6). In the USA, over 100 million persons have received a SARS-CoV-2 vaccine dose, including many US Military Health System (MHS) beneficiaries (7, 8).

Our understanding of the clinical and virological phenotype, and functional impact of SARS-CoV-2 vaccine breakthrough infections (VBI) remains very limited. The mRNA-1273 and BNT162b2 mRNA Phase III clinical trials demonstrated no severe COVID-19 cases after the 2nd dose of vaccine (1, 2). These trials measured the severity of COVID-19 in the context of clinical outcomes such as hospitalization, critical illness, and death (1, 2). These studies did not focus on patient-reported outcomes such as symptom severity. Similarly, post-Phase III observational studies have focused on endpoints such as infection frequency and hospitalization requirements, rather than subjective outcomes (6, 9). Such patient-reported outcomes represent an extensive additional burden of the SARS-CoV-2 pandemic, yet it remains unclear whether SARS-CoV-2 VBI is associated with symptoms severe enough to interfere with daily activities or employment.

The virological phenotype of SARS-CoV-2 VBI is also unclear. While data show a reduction in qPCR-estimated viral load in VBI (10), it is unclear if live virus shedding occurs in VBI, thereby representing an ongoing transmission risk. Further, it is unclear whether VBI occurs with non-variant-of-concern (VOC) genotypes (11). We therefore present an extensive clinical, serological, and virological characterization of SARS-CoV-2 VBI among subjects enrolled in a cohort of U.S. MHS beneficiaries. We particularly focus on the functional impact and detection of infectious virus in SARS-CoV-2 infections among the vaccinated.

Methods

MHS beneficiaries presenting with a positive SARS-CoV-2 test, a COVID-19 like illness, or a high-risk SARS-CoV-2 exposure were eligible for enrollment into the ongoing EPICC Study, a SARS-CoV-2 natural history study enrolling at nine U.S. Military Treatment Facilities since March 2020 (see Supplementary Material).

We evaluated EPICC-enrolled subjects with a history of PCR confirmed SARS-CoV-2 infection a minimum of 14 days post final dose of SARS-CoV-2 vaccination. Structured interview and medical record review were used to determine demographics, comorbidities, medications, SARS-CoV-2 vaccine type, and vaccine dose timing. Clinical outcomes, including hospitalization, were abstracted from clinical records. Symptom severity and functional outcomes in VBI were assessed by questionnaires which included subjective symptom severity, ability to perform daily activities, duration of illness, and days-to-recovery. We also measured personal and household infection risk factors. Nasal, nasopharyngeal and/or oropharyngeal swabs were collected and sent for qPCR, viral culture, and SARS-CoV-2 whole genome sequencing (see Supplementary Materials). Venous sera were collected and sent for anti-spike (S) IgG and anti-NP IgG binding antibodies (see Supplementary Materials).

Results

From March 2020 through May 03 2021, the EPICC study enrolled 1547 subjects (1229 outpatients, 318 inpatients) with confirmed SARS-CoV-2 infection. We observed a total of 24 infections that occurred ≥ 14 days after the final dose of a SARS-CoV-2 vaccine with a median illness onset of 50.5 days (IQR = 31.5 - 73.5, range = 15-95) from final vaccination dose (Table 1). Infections that occurred 7 - 14 days after final dose of vaccination are characterized in Table S1. The other EPICC subjects were not vaccinated before infection. The mean age was 37.8 years (SD = 13.4, range 20.9 - 77.7), and 71% were male. Most infections (67%) were observed in those without comorbidities.

Hypertension, obstructive airway disease, diabetes, and chronic kidney disease were the most common comorbidities noted (Table 1). One subject reported receiving immunosuppressant medication (mycophenolate and prednisone) for a renal transplant.

Most cases were active duty military service members (19/24, 79%). 15/24 (63%) were healthcare workers, and 13/23 (57%) reported close contact with a COVID-19 case in the last month. In the prior month, 19/23 (83%) reported staying 6-feet away from people in public more than half the time. The majority lived with children and/or another adult (Table 1).

No VBI resulted in hospitalization. 3/21 (14%) reported severe symptoms (based on the question "Overall, how would you rate your symptoms at their worst up until this point in time?"). Illness duration was up to two weeks in those study participants who reported feeling back to a usual state of health ("back to normal") at the time of assessment (Table 1). The assessment occurred a median 6 (IQR 4-12) days after illness onset.

Quantitative PCR was performed on upper respiratory tract specimens from 22 cases collected a median of 6 days post symptom onset (IQR = 4-10, range 0-18 days). 13 were positive by qPCR with a median RNA abundance of 1.08×10^4 GE/reaction (IQR $21.52 - 10.59 \times 10^4$ GE/reaction, range $2.60 - 1.42 \times 10^6$ GE/reaction). 10 of these 13 qPCR-positive specimens were successfully genotyped and included the variants-of-concern (VOC) B.1.1.7 (n = 2), P.1 (n =1) and B.1.429 (n=2) in addition to non-VOC strains B.1.1 (n = 1), B.1.1.519 (n = 1), B.1.2 (n = 2), and B.1.243 (n = 1) strains. qPCR-positive specimens in which no genotype was determined were associated with low sequencing coverage and high CT values (N1 CT > 33). Respiratory tract specimens from 6 qPCR-positive cases were analyzed by viral culture, three of which had viral loads of 113, 200, 4130 PFU/mL on specimens collected between day 6-7 post-symptom-onset.

Anti-S IgG serology results were available in 19 of 24 of subjects, with the first sera collected a median of 12 days (IQR = 7 – 16, range 4 – 25 days) after illness onset. All participants were anti-S

IgG positive by their first sera collection, with the exception of a 65 year old immunosuppressed renal transplant who tested negative to anti-S IgG on day 6 post symptom onset (36 days after a second dose of BNT162b2), and seroconverted to anti-S IgG by 28 days post symptom onset. Anti-NP IgG serology results were available in 6 of 24 subjects, four of who were anti-NP IgG positive by day 15 - day 22 after symptom onset. The remaining two subjects were anti-NP seronegative at day 6 and day 29 after illness onset (latest available time-points), respectively.

Conclusions:

We have observed SARS-CoV-2 post-vaccine infections across a range of ages in this cohort, predominantly in those with no comorbidities and no immunosuppression. The number of VBI in our study population remain low to date.

We note a proportion of VBI were associated with functional impact and symptoms self-reported as severe. No SARS-CoV-2 VBI led to hospitalization, correlating with results from mRNA-1273 and BNT162b2 clinical trials. However, the typical duration of illness was significant, with symptoms documented for as long as two weeks in those who had recovered (n = 6). Many infections occurred in subjects at higher risk for SARS-CoV-2 – with 55% of cases in healthcare workers – as well as risks for secondary household transmission. In this case series, the frequency of VBI by occupation, and vaccine product received, needs to be interpreted carefully in the context of vaccine prioritization and implementation strategy in the U.S. MHS (8). The high frequency of Pfizer vaccine receipt in this case series reflects the product being most used at EPICC sites.

While our study did not compare viral loads or genotypes between vaccinated and unvaccinated subjects, we note VBI in genotypes not previously associated with significant vaccine immune escape *in-vitro*, including B.1.1, B.1.1.519, B.1.2, and B.1.243 genotypes. Our results also underscore the emerging vaccine escape risk of the P.1 and B.1.429 variants. Sieve analyses from larger sample sizes are required to definitively confirm specific genotypes with a higher risk of vaccine

breakthrough. We observed live virus shedding in VBI as high as 4130 PFU/mL at day 7 post-symptom-onset; though relatively low magnitude, the presence of infectious virus which may indicate a transmission risk of VBI (12).

While we did not have sera collected before infection in these subjects, our finding of a lack of anti-S IgG seroconversion 36 days after final vaccine dose in an immunosuppressed renal transplant participant suggests a failure to develop an appropriate humoral response to the vaccine, as has been noted in a small study of BNT162b2 in renal transplant recipients. We further observed that anti-NP seroconversions did not occur in all PCR-positive VBI cases who were tested, with one case not seroconverting to anti-NP-IgG by 29 days after symptom onset.

Our findings are descriptive, preliminary, and can inform further study, including comparison of risk factors, viral load, and subjective outcomes with unvaccinated SARS-CoV-2 infections. Such comparisons require larger sample sizes of VBI, particularly to adjust for confounding. However, these findings offer several early insights into the clinical and viral phenotype of VBI, including data not typically collected in clinical trials or vaccine effectiveness studies (1-4, 6, 9).

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The investigators have adhered to the policies for protection of human subjects as prescribed in 45 CFR 46. The study protocol was approved by the Uniformed Services University Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

Potential conflicts:

SDP, MPS, THB, and DT report that The Uniformed Services University (USU) Infectious Diseases Clinical Research Program (IDCRP), a U.S. Department of Defense institution, and the Henry M. Jackson Foundation (HJF) were funded under a Cooperative Research and Development Agreement to conduct an unrelated Phase III COVID-19 monoclonal antibody immunoprophylaxis trial sponsored by AstraZeneca. The HJF, in support of the USU IDCRP, was funded by the Department of Defense Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense to augment the conduct of an unrelated Phase III vaccine trial sponsored by AstraZeneca. Both these trials were part of the US Government COVID-19 response. Neither is related to the work presented here. THB, MPS, and DT report that they are U.S. military service members. This research was funded by U.S. Department of Defense, Defense Health Program. AG reports support from NIAID, DMRDP, outside the submitted work.

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Tables:

Table 1. Characteristics and outcomes of n = 24 vaccine breakthrough infections[a]	**n (%)**[d]
Demographic characteristics	
Age (mean, SD, range years)	37.8 (13.4), 20.9 - 77.7
Male	17 (71%)
Race/Ethnicity	
Asian	2 (8%)
Black	1 (4%)
Hispanic	3 (13%)
Native Hawaiian	1 (4%)
White	17 (71%)
Occupational characteristics and military status	
Active Duty	19 (79%)
Dependent	4 (17%)
Retired	1 (4%)
Healthcare worker	15 (63%)
Risk behaviors	
Lives with another adult	15

	(71%)
Lives with children	7 (50%)
	13
Close contact with person with COVID-19 in past month[f]	(57%)
	19
Stayed 6 ft away from people in public > half the time in past month	(83%)
	15
Increased frequency of handwashing in the past month	(65%)
	19
Wore mask all the time in the past month	(83%)
Comorbidities	
	8
Any comorbidity	(33%)
Multiple comorbidities	3 (13%)
Hypertension	4 (17%)
Asthma or chronic obstructive pulmonary disease	3 (13%)
Obesity	2 (8%)
Diabetes	2 (8%)
Chronic kidney disease	2 (8%)
Renal transplant	1 (4%)
History of venous thromboembolism	1 (5%)
Immunosuppressant medication	1 (4%)
	16
None	(67%)
Vaccine product received	

	22
BNT162b2 (Pfizer-BioNTech)	(92%)
mRNA-1273 (Moderna)	2 (8%)
	50.5
	(31.5-
	73.5,
Illness onset from time of final dose - median days (IQR, range)[e]	15-95)
Symptom severity	
Never had symptoms	5 (24%)
Mild	7 (33%)
Moderate	6 (29%)
Severe	3 (14%)
Critical	0 (0%)
Illness outcomes, and other characteristics	
Prior SARS-CoV-2 infection	0 (0%)
Hospitalized	0 (0%)
Feeling back to normal[b]	6 (35%)
Days to recovery – median days (range)[c]	5 (0-14)

[a]Restricted to those with illness onset ≥ 14 days after final dose of vaccination

[b]At time of interview

[c]In those recovered by time of interview (n = 6)

[d]Denominator varies based on response rate

[e]Derived from time to earliest SARS-CoV-2 test positivity in those without symptoms

[f]Based on the question: *"In the month before you were ill, tested for, or exposed to COVID-19, did you have close contact (e.g., caring for or living with) a person who tested positive for COVID-19 or had symptoms of COVID-19 such as fever and/or acute respiratory illness?".*