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Interferon Gamma Release Assay Mitogen Responses in COVID-19

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Background: Elevated cytokine release and T cell exhaustion have been associated with COVID-19 disease severity. T cell activity may be indirectly measured through interferon gamma release assays (IGRAs), which use mitogen stimulation of T lymphocytes as a positive control. In our institution, an unexpectedly high rate of indeterminate IGRAs was noted in COVID-19–positive patients. We aimed to evaluate the clinical characteristics associated with indeterminate IGRA results and the difference in mitogen responses between COVID-19–positive and COVID-19–negative patients.

Methods: We reviewed all patients, regardless of COVID status, who were admitted between March 1, 2020, and May 31, 2020, and for whom an IGRA was collected. For COVID-19–positive individuals, we evaluated the statistical association of their IGRA results and clinical characteristics, including demographics, laboratory values, comorbidities, and hospitalization-related outcomes. For both COVID-19–positive and COVID-19–negative individuals, we evaluated IGRA mitogen control values relative to COVID-19 status as stratified by absolute lymphocyte count.

Results: In COVID-19–positive patients, indeterminate IGRA results were statistically associated with white blood cell count and other markers of inflammation. When comparing COVID-19–positive and COVID-19–negative patients, we noted a statistically significant difference in IGRA mitogen control values when stratified by absolute lymphocyte count.

Conclusions: Indeterminate IGRA results were associated with higher levels of inflammatory markers. Furthermore, when comparing mitogen responses between COVID-19–positive and COVID-19–negative patients, there was an observed difference in T cell reactivity between the groups. The mitogen response in IGRAs may serve as a surrogate for T cell function and a marker of disease severity in COVID-19.

Key Words: COVID-19, IGRA, lymphopenia, SARS-CoV-2

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In January 2020, the World Health Organization received reports of a cluster of atypical pneumonia cases in Wuhan, China.[1] The disease was later named COVID-19 and the causative virus identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[1] The presence of certain clinical comorbidities, such as diabetes and cardiovascular disease, has repeatedly been shown to be a significant predictor of mortality.[2–4] One proposed mechanism underlying this mortality is the elevated release of cytokines followed by the uncontrolled activation and subsequent exhaustion of T cells.[5] Although lymphopenia is a well-known clinical hallmark of COVID-19,[6,7] functional T cell exhaustion, as manifested by the low expression of interferon gamma (IFN-γ), is also predictive of disease severity.[8]

The Qiagen QuantiFERON-TB Gold Plus (QTB) is a commonly used IFN-γ release assay (IGRA) that detects the release of IFN-γ by a patient's T lymphocytes when exposed to *Mycobacterium tuberculosis* (MTB) ESAT-6 and CFP-10 antigens.[9] For its positive control, the QTB test requires that a patient's lymphocytes also be exposed to a proprietary mitogen.[10] In patients with functional lymphocytes, the mitogen stimulates T cells to produce IFN-γ, regardless of whether the patient is infected with MTB. Per manufacturer guidelines, patients with a low mitogen response (compared with a negative control) in the setting of a negative MTB antigen response are deemed to have indeterminate test results, whereas those with a normal mitogen response in the setting of a negative antigen response are deemed to have negative test results.[11]

Early in the COVID-19 pandemic, patients admitted to the Thomas Jefferson University Hospital with COVID-19 were screened using the QTB test as a prerequisite to receiving tocilizumab. The Thomas Jefferson University Hospital Clinical Microbiology Laboratory noticed an increase in the percentage of QTB tests that were indeterminate relative to prior rates. A quality control investigation by the laboratory found that the indeterminate rate was elevated (28.9%) during April and May 2020, compared with indeterminate rates from the preceding 5 months (16.8%), and that most of the indeterminate results were in COVID-19 patients. Here, we explore the hypothesis that COVID-19–positive patients with indeterminate QTB test results have different clinical characteristics compared with those who have negative test results. Furthermore, we evaluate the hypothesis that there is a significant difference in mitogen response between COVID-19–positive patients and COVID-19–negative patients as stratified by absolute lymphocyte count (ALC).

MATERIALS AND METHODS

As a part of a cross-sectional design, we reviewed the IGRA results for all inpatients at our 717-bed hospital in central Philadelphia from March 1 through May 31, 2020. Interferon gamma release assays were performed using the QTB test following the manufacturer's recommendations for specimen collection and processing. Interferon gamma enzyme-linked immunosorbent assays (Qiagen, Germantown, Maryland) were performed on a Dynex Agility automated enzyme-linked immunosorbent assay processor according to manufacturer's instructions. All QTB tests were collected as a part of routine laboratory work for COVID-19–positive patients before possible administration of tocilizumab.

Among patients for whom a QTB had been performed, we identified those who tested positive for SARS-CoV-2. For these patients, we confirmed that the patients were admitted for a new diagnosis of COVID-19 by individual chart review. Furthermore, we collected the following elements from real-time electronic health record data, also confirmed by individual chart review: basic demographics, date of hospitalization, date of symptom onset,

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TABLE 1. Clinical Characteristics of COVID-19–Positive Patients and Bivariate Analyses Based on QuantiFERON Test Results

	QuantiFERON Test Results				
	Indeterminate		Negative		*P* for Fisher Exact Test or Student *t* Test
Clinical Variables	**n = 48**	**% of Total or Mean Value**	**n = 27**	**% of Total or Mean Value**	
Demographics					
Age group, y					
<25	2	2.7%	1	1.3%	0.54
25–44	5	6.7%	5	6.7%	
45–64	21	28%	12	16.0%	
65–74	10	13.3%	7	9.3%	
≥75	10	13.3%	2	2.7%	
Sex					
Female	14	18.7%	10	13.3%	0.61
Male	34	45.3%	17	22.7%	
Race/ethnicity					
White	11	14.6%	8	10.7%	0.65
African American	19	25.3%	10	13.3%	
Asian	11	25.3%	4	5.3%	
Hispanic	5	6.7%	5	6.7%	
Unknown	2	2.7%	0	0.0%	
Laboratory values					
ALC	48	0.81×10^3 cells/uL	26	1.1×10^3 cells/uL	0.0656
LDH	46	617.30 IU/L	26	419.31 IU/L	0.0001
CRP	47	19.48 mg/dL	26	13.28 mg/dL	0.0374
D-dimer	46	7022.74 ng/mL	26	1470.39 ng/mL	0.0024
Ferritin	48	1920.88 ng/mL	27	1288.89 ng/mL	0.1685
Fibrinogen	41	650.46 mg/dL	22	685.31 mg/dL	0.5983
WBC count	48	10.7×10^9 cells/L	27	7.4×10^9 cells/L	0.0138
ANC	48	9180 cells/uL	26	5460 cells/uL	0.0035
Comorbidities					
Diabetes mellitus					
No	25	33.3%	17	22.7%	0.47
Yes	23	30.7%	10	13.3%	
Hypertension					
No	15	20.7%	14	18.7%	0.090
Yes	33	44.0%	13	17.3%	
Obesity					
No	32	42.7%	17	22.7%	0.803
Yes	16	21.3%	10	13.3%	
Coronary artery disease					
No	42	56.0%	24	32.0%	0.586
Yes	6	8.0%	3	4.0%	
Smoker					
No	44	58.7%	26	34.7%	0.65
Yes	4	5.3%	1	1.3%	
Use of immunosuppressive medications					
No	47	62.7%	27	36.0%	1.00
Yes	1	1.3%	0	0.0%	
Clinical outcomes					
Died					
No	34	45.3%	22	29.3%	0.41
Yes	14	18.7%	5	6.7%	
Use of invasive mechanical ventilation					
No	25	33.3%	16	21.3%	0.632
Yes	23	30.7%	11	14.7%	

Continued next page

TABLE 1. *(Continued)*

	QuantiFERON Test Results				
	Indeterminate		Negative		*P* for Fisher Exact Test or Student *t* Test
Clinical Variables	**n = 48**	**% of Total or Mean Value**	**n = 27**	**% of Total or Mean Value**	
Use of noninvasive mechanical ventilation					
No	29	39.7%	24	32.9%	0.028
Yes	17	23.3%	3	41.1%	
ECMO					
No	43	57.3%	27	36.0%	0.153
Yes	5	6.7%	0	0.0%	
ICU admission					
No	19	25.3%	15	20.0%	0.230
Yes	29	38.7%	12	16.0%	
Use of HFNC					
No	21	28.0%	12	16.0%	1.00
Yes	25	33.3%	15	20.0%	
Medication use					
Anticoagulation use					
No	1	1.3%	2	2.7%	0.551
Yes	45	60.0%	25	33.3%	
Corticosteroid use					
No	14	18.7%	13	17.3%	0.142
Yes	32	42.7%	14	18.7%	
Remdesivir use					
No	42	56.0%	27	36.0%	0.290
Yes	4	5.3%	0	0.0%	
Hydroxychloroquine use					
No	26	34.7%	15	20.0%	1.00
Yes	20	26.7%	12	16.0%	
Tocilizumab use					
No	21	21.0%	15	20.0%	0.472
Yes	25	33.3%	12	16.0%	

date of first positive COVID-19 test, discharge date and disposition, length of stay, mortality, need for intensive care unit (ICU) stay, use of invasive or noninvasive mechanical ventilation, use of high flow nasal cannula (HFNC), use of extracorporeal membrane oxygenation (ECMO), medical comorbidities (hypertension, hyperlipidemia, coronary artery disease obesity, obesity, use of immunosuppressive medications, diabetes, and active smoking), laboratory studies within 7 days of COVID-19 diagnosis [white blood cell (WBC) count, absolute neutrophil count (ANC), ALC, C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, ferritin, and fibrinogen], and medication use. For patients with more than one IGRA test, the IGRA result closest to the date of COVID-19 test positivity was used in the analyses.

For both COVID-19–negative and COVID-19–positive patients for whom an IGRA had been performed, we evaluated QTB mitogen control values relative to COVID-19 status and ALC value (low $<0.8 \times 10^9$/L). Values were grouped by COVID-19 status (positive vs negative) and ALC status (low vs normal).

In patients with a positive COVID-19 test, simple counts and proportions were used to describe categorical variables of interest, whereas simple counts and means were used to describe continuous variables of interest. The association between categorical variables and QTB test results was evaluated using Fisher exact test ($\alpha = .05$). For continuous variables, the means were compared between patients with negative results and those with indeterminate results using Student *t* tests ($\alpha = .05$). Missing values were excluded from the analysis. These analyses were performed using Stata software (version 16.1, StataCorp, College Station, Texas).

Scatterplots were created to display mitogen control values for COVID-19–positive and COVID-19–negative patients as stratified by low and normal ALC values. Within both ALC strata, mitogen control values for COVID-19–positive and COVID-19–negative patients were compared using Mann-Whitney *U* tests ($\alpha = .05$). The scatterplots and statistical analyses were generated using GraphPad Prism 8.3.1 (GraphPad Software Inc, San Diego, California).

ETHICS

This investigation (protocol #20E.709) was approved by the Thomas Jefferson University institutional review board. Given the minimal risk to privacy, the institutional review board granted a waiver of consent.

RESULTS

For the study period, 188 QTB tests were identified among 180 patients. Of those 180 patients, 75 were COVID-19–positive. Through chart review, the COVID-19–positive patients were confirmed as patients admitted with new infections. Of the 75 patients, 48 had indeterminate QTB test results and 27 had negative results. The time from symptom onset to a positive COVID-

19 test was similar between those patients with an indeterminate QTB test and those with a negative QTB test (11 and 10 days, respectively). Table 1 shows the baseline characteristics of all patients. The mean age was similar, and most patients (68%) were male with no statistical differences in test result by sex or race/ethnicity.

Indeterminate QTB test results were statistically associated with higher levels of COVID-19–related inflammatory markers, including LDH, CRP, and D-dimer. Indeterminate results were also associated with higher WBC and ANCs. There were no statistically significant differences between the 2 groups with respect to comorbidities. With regard to clinical outcomes, there were no statistically significant differences between the groups except for the use of noninvasive mechanical ventilation, which was higher in the QTB-negative group. However, although the differences did not reach statistical significance, there were more patients who died, more who were admitted to the ICU, and more who required mechanical ventilation, ECMO, or HFNC among those with an indeterminate QTB test result. Finally, there were no differences between the QTB-indeterminate and QTB-negative cohorts regarding the use of specific medications, including anticoagulation, corticosteroids, remdesivir, hydroxychloroquine, or tocilizumab.

Figure 1 displays the results of QTB mitogen control values for COVID-19–positive and COVID-19–negative patients as stratified by ALC value. For patients in both ALC strata, the mitogen control values for COVID-19–negative patients were significantly higher than those for COVID-19–positive patients.

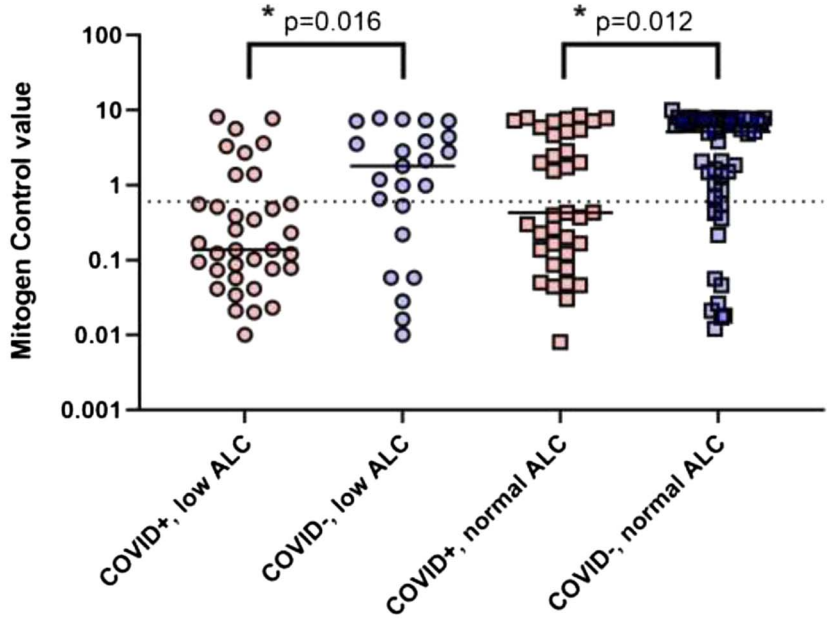
DISCUSSION

The immunopathogenesis of SARS-CoV-2 infection has been difficult to define given the complex pathways that lead from the hyperactivation of T cells to their ultimate depletion and impairment.[12] Lymphopenia is a common finding in patients with COVID-19 with disease states of varying degrees of severity.[13] The degree of lymphopenia has been correlated with disease severity and mortality.[14] However, there are also qualitative T cell defects in patients with COVID-19. In symptomatic patients, there is an increase in T cell expression of exhaustion markers, such as programmed cell death protein 1 and NKG2A, as well as the diminished capacity to produce IFN-γ.[14,15] This diminished capacity may be the mechanism underlying our current findings.

When comparing mitogen responses between COVID-19–positive and COVID-19–negative patients, there is an observed difference in T cell reactivity between the groups. In our study, COVID-19–positive patients expressed less INF-γ than COVID-19–negative patients, which may suggest some degree of exhaustion. Consequently, if IGRA results are used as a surrogate for T cell function, indeterminate results may suggest T cell exhaustion given the low mitogen response. By contrast, a negative test may suggest less- or nonimpaired T cell function, given the normal mitogenic response.

We have observed that, among COVID-19 patients, there were statistically significant differences in a number of inflammatory markers when comparing those with IGRA-negative results and those with IGRA-indeterminate results. Furthermore, in terms of clinical outcomes that suggest disease severity, there were more patients who died, more who were admitted to the ICU, and more who required mechanical ventilation, ECMO, or HFNC among those with an indeterminate IGRA result, although these differences were not statistically significant. If IGRA results are, in fact, reasonable surrogates for T cell function, there may be a role for their use in predicting COVID-19 disease severity.

There are limitations to our study. This study was cross-sectional with a small sample size. Deriving causal relationships in this setting is a challenge. The timing of QTB test collection and the presence of unmeasured confounders may have impacted our results. One concern is the effect of immunomodulators. Per institutional protocol, QTB tests were collected before tocilizumab administration. However, corticosteroid use may have occurred before test collection. This may have, in turn, affected lymphocytic response to mitogen stimulation. Furthermore, categorization of



ALC, Absolute Lymphocyte Count

FIGURE 1. Comparison of QuantiFERON mitogen control values in patients with low and normal ALCs, grouped by COVID-19–positive and COVID-19–negative status.

patients as COVID-19–positive or COVID-19–negative in our study was based on the results of laboratory testing with molecular assays, but it is possible that some patients in our COVID-19–negative groups were infected with SARS-CoV-2 at some point in their clinical course but were not diagnosed because of imperfect clinical sensitivity or timing of testing. In future studies, we can draw upon larger sample sizes with the consideration of the timing of our predictor variable—QTB test results—and potential clinical outcomes. This will allow us to generate the necessary models to evaluate QTB tests as an indicator of COVID-19 disease severity. If borne out in larger observational studies, QTB tests and other IGRAs could become a rapid tool in identifying patients that may have a deficient immune response to SARS-CoV-2 and are, therefore, destined for poor clinical outcomes.

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