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COMMENTARY

Elevated Rates of Indeterminate Results on QuantiFERON-TB Gold Plus in COVID-19 Patients

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ABSTRACT Interferon gamma release assays are used to screen various patient populations for latent tuberculosis infection. In this issue of the *Journal of Clinical Microbiology*, J. D. Ward, C. Cornaby, and J. L. Schmitz (J Clin Microbiol 59:e00811-21, 2021, https://doi.org/10.1128/JCM.00811-21) investigated an increased indeterminate rate in the QuantiFERON-TB Gold Plus assay among COVID-19 patients that was independent of immunosuppressive agents and lymphopenia. In their study, COVID-19 patients with indeterminate QuantiFERON-TB Gold Plus results trended toward decreased survival as well as increased serum interleukin-6 (IL-6) and IL-10 levels, although the differences were not statistically significant. They suggest that this pattern of cytokine expression supports an impairment of Th1, specifically interferon gamma production, in critically ill COVID-19 patients, as indicated by indeterminate QuantiFERON-TB Gold Plus results. Clinicians should be aware of the increased rate of indeterminate QuantiFERON-TB Gold Plus results in critically ill COVID-19 patients.

Interferon gamma (IFN-γ) release assays (IGRAs) are recommended by both the CDC and the Infectious Disease Society of America as a method for screening patients for latent tuberculosis (1, 2). Patients are screened for latent tuberculosis prior to initiation of immunosuppressive therapy or during presentation of respiratory illness in which tuberculosis is in the differential diagnosis, among other reasons (1). IGRAs have multiple advantages over the traditional tuberculin skin test, including the need for only one patient visit, the use of objective measurements, and no cross-reactivity with Bacillus Calmette-Guerin (3). Another advantage is that IGRAs contain a control for T-cell function, which is important in patients in which the functionality of T cells cannot be assumed, i.e., patients with immunosuppressive conditions or on immunosuppressive therapy. This prevents a false-negative interpretation, which can occur in the tuberculin skin test due to the lack of a control placement. IGRAs are being increasingly used for latent tuberculosis screens in the United States, although statistics on the use of IGRAs versus tuberculin skin tests are not readily available.

Two IGRAs are commercially available. Qiagen manufactures the QuantiFERON-TB Gold Plus (QFT) assay, and Oxford Immunotec, which was recently acquired by PerkinElmer, manufactures the T-SPOT.*TB* assay. Both IGRA methodologies utilize the patient's T cells and antigen-presenting cells to measure IFN-γ as an indicator of a memory T-cell response to tuberculosis peptides (4, 5). Both assays include a phytohemagglutinin positive control, called the mitogen, which assesses for functional T cells that are required for IGRAs. The mitogen additionally accounts for proper shaking and incubation of the tubes for the QFT assay (5). Failure to achieve sufficient IFN-γ production in response to the mitogen results in an indeterminate (QFT) or invalid (T-SPOT.*TB*) result interpretation, indicating that a positive or negative result cannot be determined. Both assays also include a negative control, called the nil, which contains no peptides. The nil measures the amount of IFN-γ in the patient's specimen without antigen stimulation. The nil is subtracted from the mitogen and tuberculosis peptide tubes or wells when interpreting the results.

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In this issue of the *Journal of Clinical Microbiology*, Ward et al. report a doubling in the QFT indeterminate rate at the University of North Carolina (UNC), from a baseline of 8.7% to 15.5% during the months of March to April 2020, as the COVID-19 pandemic began in the United States (6). In Spain, Solanich et al. reported an indeterminate QFT rate of 35.4% in 96 patients who were screened for latent tuberculosis infection because they were receiving or preparing to receive immunosuppressive therapy for COVID-19 (7). In Italy, Torre et al. reported an indeterminate QFT rate of 36.4% in COVID-19 patients at multiple institutions (8). Quest Diagnostics, one of the largest reference laboratories in the United States, observed QFT indeterminate rates soar at certain regional locations in April 2020, with the northeast region (notably including New York City) reaching indeterminate rates as high as 21% compared to a baseline of 10% (data not published). In comparison, Qiagen suggests an indeterminate rate of $\leq$3% is within normal expectations (personal communication); the QFT-Plus package insert reports an indeterminate rate of 2.5% in the sensitivity summary (5). Patient population significantly impacts an institution's baseline indeterminate rate.

To define the mechanisms responsible for the increased indeterminate rates observed in QFT tests performed on severely ill COVID-19 patients, Ward et al. performed a retrospective observational cohort study that compared patients with confirmed COVID-19 and an age-matched control group tested March through September 2020 (6). The study included both inpatient and outpatient adults treated at the UNC Health Care system during the study period. The COVID-19 cohort consisted of patients with a positive SARS-CoV-2 PCR within 10 days of the collection of the QFT, resulting in a study size of 48 patients. The control group included 75 patients.

When comparing the COVID-19 patients to the control cohort, the difference in the rate of indeterminate QFT results was highly statistically significant, with a staggering 64.58% indeterminate rate in the COVID-19 patient cohort versus 2.97% in the control arm (6). Additionally, control patients produced 6-fold more IFN-γ in the mitogen tube than COVID-19 patients, at 7.87 IU/ml versus 1.29 IU/ml (6) for the mitogen minus nil.

Corticosteroids have been shown to cause indeterminate QFT results in previous versions of the QFT assay (9, 10). While only one small study on the impact of corticosteroids on the current version of the QFT assay (QuantiFERON-TB Gold Plus) is published (11), the results from previous assay versions can likely be extrapolated to the QFT-TB Gold Plus test (12). Thus, a reasonable hypothesis to explain increased indeterminate results is the use of corticosteroid and other immunosuppressive therapies in COVID-19 patients (13). Solanich et al. found a statistically significant association between corticosteroid therapy and indeterminate results, although they noted that 14.7% of patients who did not receive immunosuppressive therapy prior to testing still produced indeterminate QFT results (7).

Ward et al. found that COVID-19 patients who were not receiving immunosuppressive therapy and did not have preexisting immunosuppressive conditions still showed a significant decrease in mitogen-induced IFN-γ production (6). Notably, even COVID-19 patients with acceptable (i.e., valid) results (mitogen minus nil greater than 0.5 IU/ml) had lower mitogen-induced IFN-γ levels than did control patients (6). While Solanich et al. found lymphopenia to be associated with indeterminate QFT results in COVID-19 patients (7), COVID-19 patients in Ward et al.'s study were lymphopenic regardless of whether their QFT results were acceptable or indeterminate. This finding suggests that lymphopenia was not the root cause of the indeterminate results (6). In contrast, there was a statistically significant increase in both the absolute neutrophil count and the neutrophil-to-lymphocyte ratio (NLR) in COVID-19 patients, and those with indeterminate QFT had higher NLRs than those with acceptable results. A previous study evaluating QFT results in multiple patient populations also reported NLR to be a useful predictor of indeterminate QFT results (14), and Solanich et al. found neutrophilia to be associated with indeterminate QFT results as well (7).

Survival rates in Ward et al.'s study did not differ significantly between COVID-19 patients with acceptable QFT results (70.6%) and those with indeterminate QFT results

(72.7%) (6). Further, no significant differences were observed between survivors and nonsurvivors in absolute lymphocyte count, absolute neutrophil count, C-reactive protein, lactate dehydrogenase, or bilirubin (6). Finally, there was no difference in the COVID-GRAM critical illness risk score between the COVID-19 patients with acceptable and indeterminate QFT results (6). Of note, the UNC COVID-19 cohort consisted primarily of critically ill patients in the intensive care unit who were receiving high-flow oxygen (6); the lack of mildly ill COVID-19 patients may have confounded the correlation analysis. One can hypothesize that mildly ill COVID-19 patients would produce higher levels of IFN-γ in response to mitogen stimulation; thus, acceptable mitogen-induced IFN-γ results may still be correlated with surviving COVID-19.

Multiple studies have been published on the role of cytokines in COVID-19 disease, with early studies focusing on IL-6 (15–17). In Ward et al.'s study, a subset of 34 of the 48 patients in the COVID-19 cohort had at least one serum IL-6 level documented. As expected, IL-6 concentrations were elevated, with nine of the patients having IL-6 levels exceeding the maximum measurable concentration in the assay (400 pg/ml) (6). The QFT-indeterminate patients had a trend toward higher IL-6 levels, but the difference was not statistically significant (6). The authors argue that the difference may still be physiologically relevant, as the results are well above the reference range of 2.0 to 5.0 pg/ml (6). Supporting this hypothesis is the finding that the survivors had significantly lower levels of IL-6 near the time of QFT collection than did nonsurvivors (6). However, a challenge to interpreting the relationship between indeterminate QFT, IL-6 levels, and survival is that the QFT and the IL-6 test were not performed at the same time points during the disease course.

In the UNC study, additional cytokine levels were evaluated in a subset of seven patients. Of this subset, six of the patients demonstrated serum IL-6 levels above the reference range of 2.0 to 5.0 pg/ml (6). Six of the seven patients also had elevated IL-10 (6), with five of the seven patients demonstrating both elevated IL-6 and elevated IL-10. Elevated IL-6 and IL-10 have been shown to correlate with disease severity in COVID-19 patients (18). This finding suggests a shift toward a Th2 immune response, although IL-4 and IL-5 did not display an increase in the seven patients tested (6). The authors suggest that impairment of the IFN-γ response, as demonstrated by an indeterminate QFT, is an indicator of an immune response shift away from a Th1 response in critically ill COVID-19 patients.

Clinicians experienced with the use of IGRAs are likely aware of the recommendation to perform IGRAs prior to the initiation of immunosuppressive therapy (9). This study provides the important finding that an IGRA may still be of limited clinical utility in COVID-19 patients, particularly those with critical illness, due to a significantly increased rate of indeterminate results. While the T-SPOT.*TB* assay was not included in this study, the finding that lymphopenia was not associated with the indeterminate results suggests that the use of a normalized number of peripheral blood mononuclear cells (2.0×10^5 to 3.0×10^5 cells/well) in the T-SPOT.*TB* assay is not sufficient to overcome an invalid result. While diagnosis of active tuberculosis still relies on performing a smear, culture, and PCR directly from the specimen (1), there is no alternative laboratory test for screening for latent tuberculosis. Thus, the utility of laboratory testing in the identification of latent tuberculosis is limited in patients who consistently produce an indeterminate or invalid IGRA result. In the absence of an acceptable IGRA result, identification of latent tuberculosis in COVID-19 patients will require analysis of clinical signs and symptoms in conjunction with a patient's risk factors.

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