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Short communication

Preliminary observations on IGRA testing for TB infection in patients with severe COVID-19 eligible for immunosuppressive therapy

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ABSTRACT

COVID-19, the novel coronavirus pandemic, has already spread around the globe affecting more than 18 million people. As previously observed with other coronaviruses, SARS-CoV-2 deeply dysregulate the immune system eliciting respiratory failure and a state of systemic hyperinflammation in severely ill individuals. Immunotherapy is often used to downgrade the detrimental effects of the disease sustained by high-level of cytokines. Those treatments, however, are known to undermine patients' ability to contain tuberculosis (TB) infection. This study aims to describe interferon-γ release assay (IGRA) results in severe COVID-19 patients eligible for immunosuppressive treatment. Aggregate data were gathered from five hospitals in Milan, Italy, from March 1 to May 15, 2020 and retrospectively analyses. Results were summarized using absolute frequencies and percentages and compared using a two-sided Chi-squared test. Overall, 462 COVID-19 patients were eligible for immunosuppressive therapy, among which 335 were tested using IGRA testing. More than one-third of them (122/335; 36.4%) had an indeterminate IGRA result because of insufficient immune response to mitogen control, 19 (5.7%) tested positive and 194 (57.9) negative. The majority of patients with lymphocytopenia (*i.e.*, total lymphocyte count [TLC] below 1000 cells/mm^3) had indeterminate IGRAs (81/155; 52.3%). The proportion becomes even higher in patients with severe lymphocytopenia (*i.e.*, TLC<500 cells/mm^3) (36/57; 63%). Our results suggest a possible negative impact of COVID-19 related immune dysregulation on TB infection assessment and management. Close monitoring of individuals with or without retesting of individuals with indeterminate IGRAs and further basic science investigations should to be sought to better comprehend their implication on TB epidemiology.

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1. Introduction

COVID-19, an infectious disease caused by SARS-CoV-2, has widely spread across the globe after a first onset in Wuhan, China, in December 2019. Italy, and notably the Lombardy Region, has been one of the most affected countries in terms of registered cases, severe complications, and deaths [1].

As previously observed for other coronaviruses (SARS-CoV and MERS-CoV), SARS-CoV-2 is responsible for a state of severe immune dysregulation. The overresponse of the innate immunity and the concomitant reduction of the lymphocyte subsets ($CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and natural killer cells) results in a diffuse damage of pulmonary parenchyma as well as a systemic hyperinflammatory and pro-coagulative state [2,3]. In order to counter-balance the host immune and reduce its harmful effects, different anti-inflammatory therapies have been proposed, including the monoclonal antibody against interleukin-6 (IL-6) receptor (tocilizumab) and the IL-1 receptor antagonist (anakinra), two cytokine-blocking agents usually employed in the treatment of cytokine storm syndromes and other rheumatologic conditions. Such treatments do not usually require to carefully rule out tuberculosis (TB) infection (TBI)—a state in which TB bacilli are contained by a persistent host immune response—through tuberculin skin test (TST) or interferon-γ release assay (IGRA) [4]. However, a severe state of SARS-CoV-2 infection and the concurrent administration of immunosuppressive agents might play a role in making TBI progress into disease [5,6] and ultimately worsen its outcome, because of the lung damage produced by COVID-19 [7–9].

In general, IGRA testing may results indeterminate in 2–11% of cases, particularly in subjects with chronic conditions such as rheumatologic or immunosuppressive states [10]. Furthermore, a low lymphocyte count is a well-known potential cause of false-negative TST and/or indeterminate IGRA results, the latter due to an insufficient immune response to the mitogen control [11,12].

Many Italian COVID-19 Units included IGRA testing in the panel of preliminary exams to prescribe anti-inflammatory treatments. Given the necessity for the quickest start of therapy to contrast COVID-19-related massive cytokines release, the usual laboratory turn-around time for response (on average, 3–7 days) was considered not compatible with clinical urgency. Considering the short treatment course of cytokine-blocking agents in COVID-19 (1–2 doses for tocilizumab; 2 weeks or less for high-dose anakinra) and the relatively brief duration of the iatrogenic immunosuppression (4 weeks after drug administration for tocilizumab; until drug discontinuation for anakinra), some COVID-19 Units seldom wait for IGRA results before starting these therapies, while others simply do not include TBI screening as a pre-requisite.

Aim of this retrospective study was to describe the IGRA testing results among severe COVID-19 patients eligible for immunosuppressive treatment.

2. Methods

We retrospectively assessed the results of IGRA testing performed in patients with severe COVID-19 admitted in five hospitals of the Milan area by collecting and analysing aggregate data with no access to patients' medical records; therefore, no ethical clearance was required. All the centres employed QuantiFERON-TB Gold Plus® (QTP) (DiaSorin; Salluggia, Italy) as IGRA testing before starting immunosuppressive treatment, according to local protocols. Results of IGRA testing were assessed according to manufacturer's criteria. Screening policies differed widely according to local decision processes, ranging from testing all possible candidates to not testing any of them.

Data were summarized using absolute frequencies and percentages and comparison between groups, defined by IGRA results, and compared using a two-sided Pearson's Chi-squared test using R software (version 3.6.3).

3. Results

Overall, between 1st March and 15th May 2020, of 462 patients who eventually received cytokine-blocking agents, 335 (72.5%) performed IGRA testing (Table 1). Among those tested with IGRA, 122/335 subjects (36.4%) had an indeterminate result (*i.e.*, lack of response to the mitogen control phytohemagglutinin) with no difference between the five hospitals. Positive and negative results were instead observed in 5.7% and 57.9% patients, respectively. All subjects were tested for HIV infection, with all negative results and none of them started TB preventive treatment (TPT) before or during immunosuppressive therapy independently of the QTP result.

Lymphocytopenia (total lymphocyte count [TLC] <1000 cells/mm^3) characterized 155/335 patients (42.2%), of which 57 with severe lymphocytopenia (TLC<500 cells/mm^3). Interestingly, indeterminate IGRA results mostly concentrated in patients with lymphocytopenia (81/155; 52.3%), especially if severe (36/57; 63%), while were present only in 41/180 (22.8%) of patients with TLC >1000 cells/mm [3]. As expected, positive IGRA results were overrepresented among patients originating from high TB incidence countries (defined with a TB incidence $>$ 50/100,000 population based on the World Health Organization estimates) compared to low burden countries with 8/31 (25.8%) compared to 11/304 (3.6%) subjects, respectively.

The majority of the patients tested with IGRA survived (289; 86.3%) and a higher death rate was documented among those with indeterminate IGRA result (23/122; 18.9%) compared to those either IGRA positive or negative (23/190; 12.1%) (p-value = 0.047).

TPT was administered only in two patients with a positive IGRA result.

4. Discussion

This data support the role of severe damage of the immune response secondary to COVID-19 underlying the possible threatening consequences about TB epidemiology after the pandemic [13]. Indeed, it is unclear how an indeterminate IGRA result should be interpreted when registered in patients with acute viral infections [14]. Yet, it is disputed how to manage these patients especially as the need of a TPT after only few doses of immune suppressive treatment is not well documented [15]. Additionally, age and possible co-morbidities may discourage treatment start and hinder its completion because the development of undesirable adverse events. An indeterminate IGRA result in these anergic patients, with no data on the influence of COVID-19 on the TBI progression, may delay eventually TB diagnosis and care leading to poor outcome [16,17]. For all these reasons, we propose a follow-up period of at least two years with periodic (*i.e.*, at month 2, 6, 12, and 24 after the first test) clinical, immunological (with IGRA testing), and radiological reassessment to those resulted indeterminate as displayed in Fig. 1. This reassessment may be supported by previous study suggesting a change in IGRA result after months from the indeterminate result [10].

Being a retrospective analysis of aggregated data, many confounders may have been overlooked, including the presence of chronic rheumatologic or systemic diseases or acute conditions known to affect IGRA results [10]. Nevertheless, we believe that COVID-19-driven immune disturbance (both quantitative and qualitative) plays a major role due the high number of indeterminate IGRA testing.

Table 1
Baseline characteristics of COVID-19 patients tested with IGRA at hospital admission.

	Total *N*	No. of positive IGRA results *n* (%)	No. of negative IGRA results *n* (%)	No. of indeterminate IGRA results *n* (%)	P-value[b]
Total	335	19 (5.7)	194 (57.9)	122 (36.4)	
Sex					.028
Male	248	17 (6.9)	150 (60.4)	81 (32.7)	
Female	87	2 (2.3)	44 (50.6)	41 (47.1)	
Nationality					<.001
Italians	252	10 (4.0)	138 (54.7)	104 (41.3)	
Foreign-born	83	9 (10.8)	56 (67.5)	18 (21.7)	
Age					.073
0–65 years	209	11 (5.3)	131 (62.7)	67 (32.0)	
>65 years	126	8 (6.3)	63 (50)	55 (43.7)	
TB epidemiology[a]					<.001
Low incidence country	304	11 (3.6)	179 (58.9)	114 (37.5)	
High incidence country	31	8 (25.8)	15 (48.4)	8 (25.8)	
TB history					.305
No history of previous TB	326	18 (5.5)	191 (58.6)	117 (35.9)	
History of previous TB	9	1 (11.1)	3 (33.3)	5 (55.6)	
TLC (cells · mm^{-3})					<.001
>1000	180	14 (7.8)	125 (69.4)	41 (22.8)	
500–1000	98	5 (5.1)	48 (49)	45 (45.9)	
<500	57	0 (0.0)	21 (36.8)	36 (63.2)	
TB preventive therapy					.003
TPT done	2	2 (100.0)	0 (0.0)	0 (0.0)	
TPT not administered	333	17 (5.1)	194 (58.3)	122 (36.6)	
Treatment outcome					.038
Survivors	289	19 (6.6)	171 (59.2)	99 (34.3)	
Deaths	46	0 (0.0)	23 (50.0)	23 (50.0)	

Abbreviations: IGRA, interferon-γ release assay; TB, tuberculosis; TPT, tuberculosis preventive treatment; TLC, total lymphocyte count; WHO, World Health Organization.

[a] A high TB incidence country has been defined as a TB incidence >50 per 100,000 based on WHO estimates [4].

[b] P-value is computed using Pearson's Chi-squared test in R version 3.6.3 applying a significance level of 95% in a two-sided distribution.

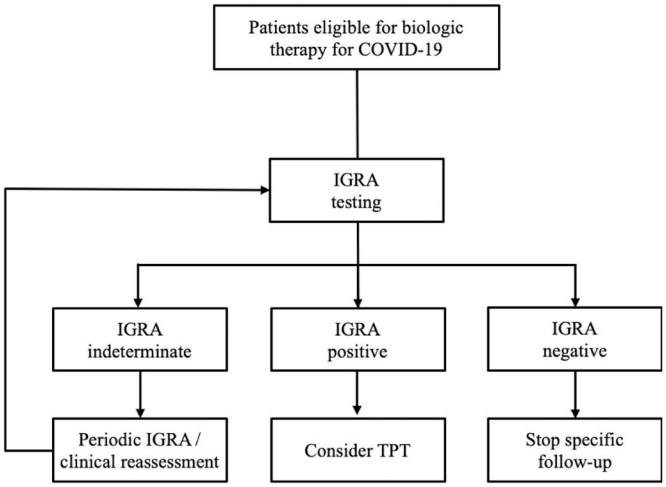

Fig. 1. Algorithm for IGRA testing and subsequent proposed follow-up for TB infection in COVID-19 patients.
The algorithm displays the proposed follow-up for COVID-19 laboratory-confirmed patients underwent to IGRA testing. TPT could be considered based on national TB guidelines and individual clinical features for patients resulted IGRA-positive at baseline or anytime during follow-up if IGRA result positive. Periodic IGRA testing and/or clinical Follow-up should last at least for the first two years after COVID-19 diagnosis. Those IGRA-negative patients will undergo eventually to COVID-19 follow-up according to specific local or national guidelines.
Abbreviations: COVID-19, coronavirus disease 2019; IGRA, interferon-γ release assays; TB, tuberculosis; TPT, tuberculosis preventive treatment.

As countries with high rate of TB are the ones most at risk to struggle for COVID-19, prospective controlled studies are urgently needed to confirm our results and their clinical implication [15]. Also, an international consensus should be agreed on how to manage individuals with positive or indeterminate IGRA affected by COVID-19 to avoid surge in TB cases.

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CRediT authorship contribution statement

Alessandro Torre: Data curation, Investigation, Writing - original draft, Writing - review & editing. **Stefano Aliberti:** Conceptualization, Data curation, Investigation, Methodology, Writing - review & editing. **Paola Francesca Castellotti:** Data curation, Investigation, Writing - original draft, Writing - review & editing. **Daniela Maria Cirillo:** Conceptualization, Investigation, Methodology, Supervision, Writing - review & editing. **Antonella Grisolia:** Data curation, Investigation, Writing - review & editing. **Davide Mangioni:** Conceptualization, Data curation, Investigation, Writing - review & editing. **Giulia Marchetti:** Data curation, Writing - review & editing. **Roberto Rossotti:** Data curation, Investigation, Writing - review & editing. **Pierachille Santus:** Data curation, Methodology, Investigation, Writing - review & editing. **Giorgio Besozzi:** Data curation, Investigation, Supervision, Validation, Writing - review & editing. **Simone Villa:** Formal analysis, Visualization, Writing - review & editing. **Luigi Ruffo Codecasa:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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