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Coinfection of tuberculosis and COVID-19 limits the ability to in vitro respond to SARS-CoV-2

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ABSTRACT

Objectives: The interaction of COVID-19 and tuberculosis (TB) are still poor characterized. Here we evaluated the immune response specific for *Micobacterium tuberculosis* (Mtb) and SARS-CoV-2 using a whole-blood-based assay-platform in COVID-19 patients either with TB or latent TB infection (LTBI).
Methods: We evaluated IFN-γ level in plasma from whole-blood stimulated with Mtb antigens in the Quantiferon-Plus format or with peptides derived from SARS-CoV-2 spike protein, Wuhan-Hu-1 isolate (CD4-S).
Results: We consecutively enrolled 63 COVID-19, 10 TB-COVID-19 and 11 LTBI-COVID-19 patients. IFN-γ response to Mtb-antigens was significantly associated to TB status and therefore it was higher in TB-COVID-19 and LTBI-COVID-19 patients compared to COVID-19 patients ($p \leq 0.0007$).
Positive responses against CD4-S were found in 35/63 COVID-19 patients, 7/11 LTBI-COVID-19 and only 2/10 TB-COVID-19 patients. Interestingly, the responders in the TB-COVID-19 group were less compared to COVID-19 and LTBI-COVID-19 groups ($p = 0.037$ and 0.044, respectively). Moreover, TB-COVID-19 patients showed the lowest quantitative IFN-γ response to CD4-S compared to COVID-19-patients ($p = 0.0336$) and LTBI-COVID-19 patients ($p = 0.0178$).
Conclusions: Our data demonstrate that COVID-19 patients either TB or LTBI have a low ability to build an immune response to SARS-CoV-2 while retaining the ability to respond to Mtb-specific antigens.
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Introduction

The World Health Organization (WHO) estimated in 2019, 10 million tuberculosis (TB) cases with 1.6 million deaths worldwide (WHO, 2020a). Lung is the most frequent TB localization, but any organ can be affected (Goletti et al., 2018). Since 2020, COronaVIrus Disease-2019 (COVID-19) caused by Severe Acute Respiratory Syndrome–CoronaVirus-2 (SARS-CoV-2) spread globally with around 111 million cases reported (WHO, 2020b). There is evidence, that COVID-19 pandemic worsened TB epidemic globally due to TB-services fragmentation and additional pressures on health systems by COVID-19 resulting in weakening of the National TB programs (Migliori et al., 2020).

COVID-19 is characterized by several clinical features ranging from an asymptomatic state to severe forms with immune dysregulation that may lead to immune pathology (Falasca et al., 2020). As for TB, lung is the most frequent disease localization, and in a minority of patients, it may lead to a rapid respiratory failure and death (Gandhi et al., 2020; Motta et al., 2020).

Current evidences on TB-COVID-19 coinfection suggest that COVID-19 may occur independently of TB either before, during or after TB disease (Stochino et al., 2020; Tadolini et al., 2020a,b). However, whether COVID-19 may reactivate or worsen TB disease

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still needs to be elucidated. The impact of sequelae and the need for further rehabilitation requires further evaluation (Zampogna et al., 2021; TB/COVID-19 Global Study Group, 2021). The main determinants of mortality for COVID-19 are age and co-morbidities, HIV co-infection and poverty, and all of these have an impact on TB mortality as well (WHO, 2020a). Recently, TB has been associated with higher mortality in COVID-19-patients (Boulle et al., 2020).

Broad and coordinated SARS-CoV-2 antigen-specific adaptive immune responses is crucial to control COVID-19 (Rydyznski Moderbacher et al., 2020). *M. tuberculosis* (Mtb)-specific response is driven by Th1-responses. Interferon (IFN)-γ is mainly produced by the CD4 T-cells whereas IL-12 and TNF-α by the antigen presenting cells. IFN-γ secretion enhances the macrophage microbicidal mechanisms, regulate Th17-cells and the tissue damage whereas TNF-α has been associated with granuloma integrity (Cantini et al., 2017).

Concomitant Mtb-specific and SARS-CoV-2-specific response is not yet fully understood. Recently, it has been reported a similarity of the immune signatures associated with COVID-19 clinical severity and the spectrum of asymptomatic- and symptomatic-TB (Sheerin et al., 2020).

Still undescribed are the biological effects of the interaction of the two infections that may recall the concept of 'cursed duet' that in the past was used to describe TB and HIV coinfection (Goletti et al., 1996).

Therefore, in this study we evaluated the impact of SARS-CoV-2 and Mtb concomitant infections on the immune response specific for each pathogen, using a whole-blood-based assay platform.

Material and method

Study population

Ethical Committee of National Institute of Infectious Diseases (INMI) Lazzaro Spallanzani—IRCCS approved the study (approval number 59/2020). HIV-uninfected subjects were consecutively and prospectively enrolled at INMI from April to December 2020. Informed-written consent was required to participate in the study.

A positive nasopharyngeal swab for SARS-CoV-2 was used to define COVID-19 patients. The disease (at the height of disease severity) was classified as mild, moderate, severe and critical (WHO, 2020c).

In pulmonary-TB, diagnosis was based on a positive Mtb culture from respiratory samples, in extrapulmonary-TB was based on positive Mtb-specific molecular testing (TRCReady M.TB, Tosoh, Japan; Home-made PCR (IS6110) GeneXpert, Cepheid; Genotype MTBDRPlus Hain Lifescience) or culture from biological specimens or identification of acid fast bacilli or TB-specific histo-pathological findings in tissue samples. Clinical and radiologic criteria and decision of physician to give the patient a full course of TB treatment defined the clinical TB diagnosis. LTBI diagnosis was based on a positive score to QuantiFERON Plus test (QFT) or by the presence of radiological apical scars indicative of previous TB exposure, after excluding TB disease.

Patients were classified as NO-COVID-19 if they were healthy donors (HD) or had bacterial pneumonia, or echinococcosis and scored negative to SARS-COV-2 IgG serology. Demographic and clinical information were collected at enrollment. Part of the patients samples were used in published studies (Petrone et al., 2020; Petrone et al., 2021)

Stimuli

SARS-CoV-2 CD4 pool of peptides (CD4-S) have been described (Grifoni et al., 2020a; Grifoni et al., 2020b; Weiskopf et al., 2020). Briefly, the peptide megapool design was carried out on the Wuhan-Hu-1 reference isolated (GenBank ID: MN908947) and consists of 253 overlapping 15-mers by 10 spanning the entire spike protein (CD4-S; n = 253).

IFN-γ whole-blood assay

Six hundred microliter of whole-blood were stimulated or not with CD4-S at 0.1 μg/mL and Staphylococcal enterotoxin B as positive control at 200 ng/mL. CD4-S concentration was selected based on dose-titration experiments previously reported (Petrone et al., 2020). Plasma was harvested after over-night stimulation at 37 °C (5% CO_2) and stored at −80° until use.

QFT-Plus assay

QFT-Plus assay was performed and results analysed according to the reported criteria (http://www.quantiferon.com/irm/content/PI/QFT/PLUS/2PK-Elisa/UK.pdf).

ELISA

Plasma IFN-γ level was evaluated by ELISA (www.quantiFERON.com) and values were subtracted from the unstimulated control.

Statistical analysis

Data were analyzed using SPSS software (Version 19 for Windows, Italy SRL, Bologna, Italy), and Graph Pad (GraphPad Prism 8 XML ProjecT). For continuous measures, medians and interquartile ranges (IQR) were calculated. Kruskal–Wallis test (for comparisons among groups) or the Mann–Whitney U test with Bonferroni correction (for pairwise comparisons) were used. Chi-square test was used for categorical variables.

Results

Clinical and demographical characteristics of the enrolled subjects

We enrolled 92 subjects: 84 were classified as COVID-19 and 8 as NO-COVID-19. COVID-19-patients were further defined as COVID-19, TB-COVID-19 and LTBI-COVID-19. Table 1 shows the clinical and demographical information.

Within the COVID-19-patients, 4 showed comorbidities: 2 had diabetes, 1 had multiple sclerosis (MS) and 1 chronic renal failure. TB-COVID-19-patients had pulmonary TB (n = 5, 50%), or extrapulmonary TB (n = 1, 10%) either culture- or molecular-confirmed; 4 (40%) patients had pulmonary and extrapulmonary TB and among them 2 had clinical pulmonary TB (Table 1). Time of diagnosis of TB vs COVID-19 is detailed in Table 1. Lymphocyte counts were the highest in COVID-19, followed by LTBI-COVID-19 and TB-COVID-19 (medians: 1.5, 1.35, 0.97, respectively); however, the difference among these groups was not significant (p = 0.39, Table 1). Finally, severity stage is shown in Table 1 for all the three groups.

NO-COVID-19-subjects were HD (n = 5) or had echinococcosis (n = 1) or bacterial pneumonia (n = 2). All NO-COVID-19 scored QFT-Plus negative and all of them tested negative for SARS-COV-2 specific IgG. In subjects with a serology result available, the testing was performed concomitantly (or within a week) with the whole-blood test.

Tuberculosis comorbidity impairs the SARS-CoV-2-specific immune response

All COVID-19-patients were tested for QFT-Plus. Within the COVID-19-patients, 59 (93.7%) had a negative QFT-Plus result and 4

Table 1
Demographical and clinical characteristics of the enrolled subjects.

	COVID-19	TB-COVID-19	LTBI-COVID-19	NO COVID-19	p Value
N (%)	63	10	11	8	
Age median N (IQR)	55 (44–63)	45 (34–49)	63 (50–67)	61 (51–69)	**0.05**
Male N (%)	42 (66.7)	6 (60)	4 (36.4)	4 (50.0)	0.25
Origin N (%)				**0.0005**	
Western Europe	42 (66.7)	1 (10.0)	7 (63.6)	7 (87.5)	
Eastern Europe	0 (0)	1 (10.0)	0(0)	1 (12.5)	
Asia	16 (25.4)	5 (50.0)	0(0)	0 (0)	
Africa	3 (4.7)	2 (20.0)	1 (9.1)	0 (0)	
North America	0 (0)	0 (0)	0 (0)	0 (0)	
South America	2 (3.2)	1 (10.0)	3 (27.3)	0 (0)	
Swab positive results N (%)[a]	63 (100.0)	10 (100.0)	11 (100.0)	0 (0)	**NA**
TB diagnosis N (%)					**NA**
Microbiological	–	9 (90)	–	–	
Clinical	–	1 (10)	–	–	
Site of TB disease N (%)					
Pulmonary	–	5 (50.0)	–	–	
Extrapulmonary	–	1 (10.0)	–	–	
Pulmonary and extrapulmonary	–	4 (40.0)	–	–	
Serology positive results N/N available	12/18	2/4	3/3	0/8	**0.005**
Time of diagnosis of TB vs COVID					**NA**
Previous TB	–	3 (30)	–	–	
Concomitant TB-COVID-19	–	4 (40)	–	–	
Previuos COVID-19	–	3 (30)	–	–	
COVID-19 Severity N (%)[b]					0.47
Mild	16 (26.2)	1 (10.0)	1 (9.1)	–	
Moderate	25 (41.0)	7 (70.0)	7 (63.6)	–	
Severe	8 (13.1)	0 (0)	1 (9.1)	–	
Critical	12 (19.7)	2 (20.0)	2 (3.3)	–	
Lymphocytes count N (IQR)	1.5 (0.95−2.03)	0.97 (0.72−1.79)	1.35 (1.19−2.19)	–	0.39
QFT-Plus results N (%)					**NA**
Positive	0 (0)	5 (50.0)	9 (81.8)	0 (0)	
Negative	59 (93.7)	4 (40.0)	1 (9.1)	8 (100.0)	
Indeterminate	4 (6.3)	1 (10.0)	1 (9.1)	0 (0)	
TB1	0 (0)	5 (50.0)	8 (72.7)	0 (0)	
TB2	0 (0)	5 (50.0)	8 (72.7)	0 (0)	
Concordant TB1 and TB2	63 (100.0)	10 (100.0)	9 (81.8)	8 (100.0)	
CD4-S results N (%)[c]					**0.005**
Positive	**35 (55.6)**	**2 (20.0)**	**7 (63.6)**	**0 (0)**	[e]
Mild	10 (62.5)[d]	0 (0)[d]	1 (100)[d]	–	
Moderate	18 (72.0)[d]	2 (28.6)[d]	5 (71.4)[d]	–	
Severe	3 (37.5)[d]	0 (0)[d]	0 (0)[d]	–	
Critical	4 (33)[d]	0 (0)[d]	1 (50.0)[d]	–	
Negative	**28 (44.4)**	**8(80.0)**	**4 (36.4)**	**8 (100.0)**	[e]
Mild	6 (37.5)[d]	1 (100)[d]	0 (0)[d]	–	
Moderate	7 (28.0)[d]	5 (71.4)[d]	2 (28.6)[d]	–	
Severe	5 (62.5)[d]	0 (0)[d]	1 (100.0)[d]	–	
Critical	8 (66.7)[d]	2 (100)[d]	1 (50.0)[d]	–	
Immune suppressive therapy N (%)					
Under cortisone therapy	22 (35.5)	6 (60.0)	3 (37.3)	–	0.25
CD4-S positive response[f]	10 (45.5)	0 (0)	2 (66.7)	–	0.07
QFT-Plus positive results[g]	0	2 (33.3)	2 (66.7)	–	0.34
QFT-Plus Indeterminate results	3 (13.6)	1 (16.7)	0 (0)	–	0.77

COVID-19: COronaVIrus Disease 19; N: number; TB: tuberculosis; QFT: quantiferon; CD: cluster differentiation; NA: not available.
[a] Info available at diagnosis for 63 COVID-19 (100%), 10 COVID-19/TB (100%), 11 COVID-19/LTBI (100%) individuals.
[b] At the highest of the disease. 2 COVID-19 patients were excluded as asymptomatic.
[c] Results are scored positive or negative based on the published cut off of 0.16 IU/mL (Petrone et al., 2020).
[d] The proportion is evaluated having as denominator the patient within the same COVID-19 severity stage.
[e] COVID-19 responders vs COVID-19/TB responders p value: 0.037; COVID-19/TB responders vs COVID-19/LTBI responders p value: 0.044.
[f] COVID-19 responders vs COVID-19/TB responders p value: 0.04; COVID-19/TB vs COVID-19/LTBI responders p value: 0.02.
[g] Only among those capable to respond to *M. tuberculosis* antigens as COVID-19/TB vs COVID-19/LTBI responders.

(6.3%) scored indeterminate (Table 1). Interestingly, only 5 (50%) TB-COVID-19-patients scored positive to QFT-plus and the remaining patients scored either negative (n = 4, 40%) or indeterminate (n = 1, 10%). Patients with a positive result had concordant TB1 and TB2 results (Table 1). By contrast, the majority of LTBI-COVID-19-patients scored QFT-Plus positive (n = 9, 81.8%). In these patients, concordant TB1 and TB2 results were found in 7/9, 1 patient had a positive TB1 score and 1 patient had a positive TB2 result (Table 1).

IFN-γ response to QFT-Plus antigens TB1 and TB2 was evaluated in all the subjects enrolled. As expected the IFN-γ level in COVID-19-patients was significantly lower compared to TB-COVID-19 and LTBI-COVID-19-patients in response to TB1 ($p = 0.0007$ and $p < 0.0001$, respectively; Figure 1A) and TB2 ($p = 0.0002$ and $p < 0.0001$, respectively; Figure 1B). IFN-γ level in response to TB1 or TB2 did not significantly differ between COVID-19 and NO-COVID-19-subjects.

COVID-19-patients either TB or LTBI were evaluated for the ability to respond to CD4-S SARS-CoV-2-specific antigen. Considering the already published cut-off for CD4-S of 0.16 IU/mL (Petrone et al., 2020), 35/63 (55.6%) COVID-19-patients scored positive, 7/11 (63.6%) LTBI-COVID-19-patients had a positive CD4-

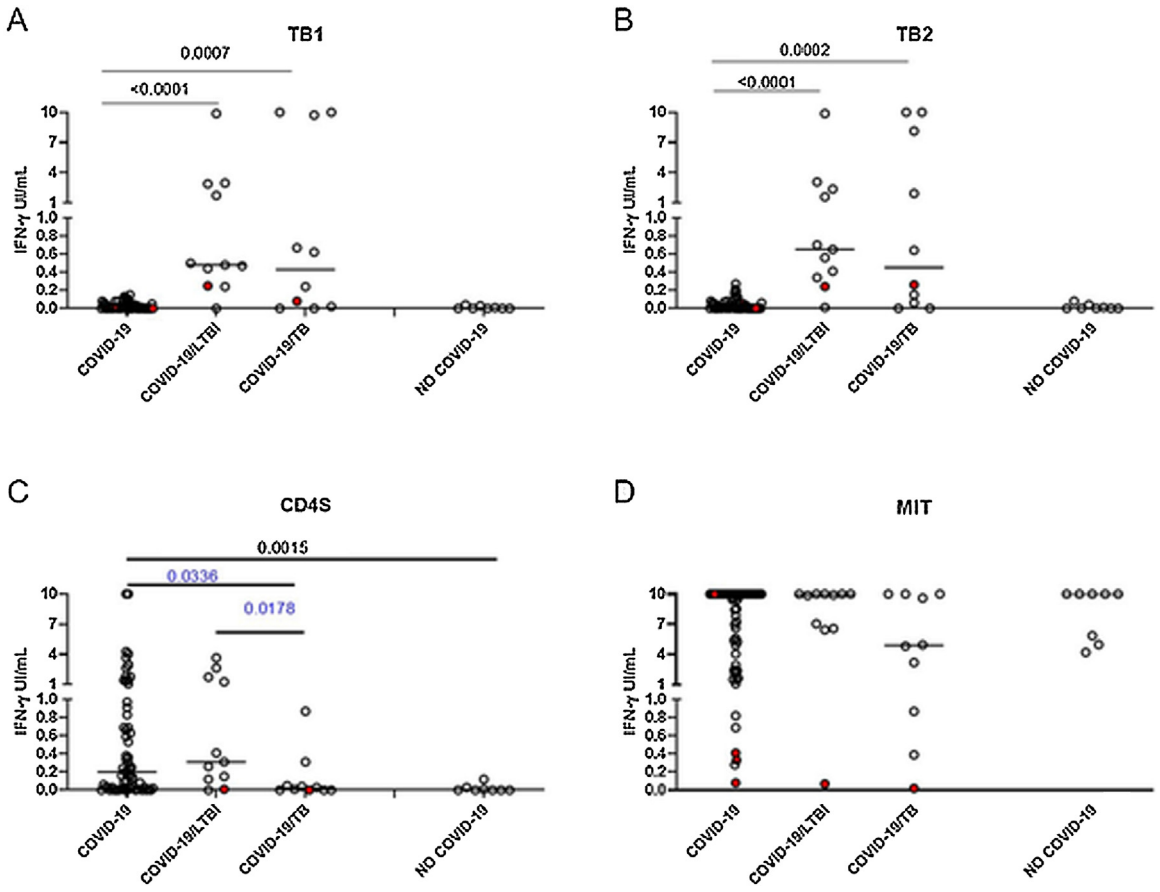

Figure 1. Tuberculosis comorbidity impairs the SARS-CoV-2-specific immune response. Evaluation of the IFN-γ response to TB1 (A), TB2 (B), CD4S (C) and mitogen in COVID-19, TB-COVID-19, LTBI-COVID-19 and NO COVID-19 subjects. A. The IFN-γ level in COVID-19-patients was significantly lower compared to TB-COVID-19 and LTBI-COVID-19-patients in response to TB1. B. The IFN-γ level in COVID-19-patients was significantly lower compared to TB-COVID-19 and LTBI-COVID-19-patients in response to TB2. C. TB-COVID-19-patients showed the lowest IFN-γ levels in response to CD4-S compared to "COVID-19" patients and to LTBI-COVID-19-patients. D. No significant differences were found in response to the mitogen among the groups evaluated. Footnotes: Horizontal bars represent medians. IFN-γ was measured by ELISA in harvested stimulated plasma. Responses were compared using the Mann–Whitney test with Bonferroni correction; differences were considered significant at p-values of ≤0.05 or 0.016. Red dots indicate patients scored indeterminate to QFT-Plus. Blue p values indicate differences almost significant. COVID-19: CoronaVIrus Disease-2019; TB: tuberculosis; LTBI: latent TB infection; CD: cluster differentiation; MIT: mitogen.

S response and only 2/10 (20%) TB-COVID-19-patients scored CD4-S-positive (Table 1). Interestingly, the number of responders in the TB-COVID-19 group was lower compared to COVID-19 only and LTBI-COVID-19 groups (Table 1). Then, we stratified the no-responders to CD4-S based on the COVID-19 disease. Interestingly, within the COVID-19-patients, we observed a trend of absence of a viral-specific response along with the severity grade of the disease. Differently, among the TB-COVID, no trend was observed (80% were no responders) (Table 1). Moreover, as shown in Figure 1C, although not significant, TB-COVID-19-patients showed the lowest IFN-γ levels in response to CD4-S compared to COVID-19-patients and to LTBI-COVID-19-patients. CD4-S-specific response was significantly higher in COVID-19-patients compared to NO-COVID-19-subjects (p = 0.0015, Figure 1C). No significant differences were found in response to the mitogen among the groups evaluated (Figure 1D). We also evaluated cortisone therapy impact on the ability to respond to CD4-S in COVID-19-patients, TB-COVID-19-patients and LTBI-COVID-19-patients. No significant differences were found between patients that received cortisone and those not treated with steroids (p = 0.19; p = 0.053; p = 0.89 respectively). Interestingly, among patients receiving cortisone, none of the TB-COVID-19 responded to CD4-S, whereas 45.5% of the COVID-19-patients and 66.7% of the LTBI-COVID-19 responded, although no significant difference was found (Table 1).

Discussion

In this study, we investigated for the first time to our knowledge, the biological effects of the interaction of COVID-19 and TB, evaluating the immune response specific for SARS-COV-2 and Mtb, using a whole-blood-based assay platform. Our data demonstrate that TB significantly reduces the SARS-COV-2-specific response in coinfected TB-COVID-19-patients.

Several viral infections, as measles or influenza, have been described to have a detrimental impact on TB (Durrheim et al., 2014; Whittaker et al., 2019; Ong et al., 2020) with an induced transient immunosuppression for weeks/months—leading to an increased incidence of TB disease in adults and children (Durrheim et al., 2014; Whittaker et al., 2019). In contrast, to date, COVID-19 pathogenicity mechanisms remain poorly elucidated and few studies report experience with concomitant TB, describing both good and severe COVID-19 outcomes in TB-patients (Faqihi et al., 2020; Musso et al., 2021; Stochino et al., 2020; Tadolini et al., 2020a,b; Motta et al., 2020).

Immune response in TB typically involves T-cells, mainly the CD4 compartment (Ong et al., 2020). COVID-19 is characterized by lymphopenia that is considered a marker of the disease severity

(Tan et al., 2020; Lanini et al., 2020). Further, immunosuppressive drugs may be used to treat COVID-19-patients (Cantini et al., 2020). Despite these assumptions, our data demonstrate that COVID-19-patients either TB or LTBI retain the ability to respond to Mtb-specific antigens.

In contrast, coinfected TB-COVID-19-patients have a low chance to build an immune response to SARS-COV-2. Interestingly, these patients had the lowest lymphocyte counts compared to the other two groups. The reduced/absent response to SARS-COV-antigens in whole-blood from patients with coinfection of TB-COVID-19 may be the consequence of a massive compartmentalization of the specific-T-cells in infectious foci or, as seen in other infectious diseases, by the elimination of effector T-cells when confronting with high doses of antigens (Garcia et al., 1999; Moskophidis et al., 1993). It is unknown if a lack of SARS-COV-2-specific response associates with a worse clinical outcome. Interestingly, while within the COVID-19-patients, we observed a trend of absence of a viral-specific response along with the severity grade of the disease, no such trend was found among the TB-COVID characterized as a group per se as unable to respond to CD4-S. These data suggest that the comorbidity TB-COVID-19 does impact on SARS-COV2-specific response independently of the COVID-19 severity stage.

Although a limited number of patients was evaluated, cortisone therapy did not seem having an impact on the ability to respond to SARS-CoV-2 antigens, as previously shown (Petrone et al., 2020). Interestingly, among the patients receiving cortisone, none of the TB-COVID-19 responded to CD4-S, whereas 45.5% of the COVID-19-patients and 66.7% of the LTBI-COVID-19-patients responded further suggesting that TB disease reduces SARS-COV-2-specific response.

Limitation of this work should be accounted. The small size and the heterogeneity of the groups examined hampered us to fully characterize the relationship between COVID-19 and TB. Moreover, the antigen used to evaluate the SARS-CoV-2 response, shows a moderate sensitivity for COVID-19 identification.

In conclusion, we demonstrated that TB impairs the ability to mount a SARS-CoV-2-specific immune response in co-infected subjects. These evidences, if confirmed in larger studies, may be useful in evaluating the management and diagnostic algorithms of TB and COVID-19 co-infection.

Transparency declaration

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Conflict of interest

A.S. is a consultant for Gritstone, Flow Pharma, Merck, Epitogenesis, Gilead and Avalia. D.G. received fees for scientific talk from Qiagen. The other authors declare no conflicts of interest.

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Ethical approval

The Ethical Committee of National Institute of Infectious Diseases (INMI) Lazzaro Spallanzani-IRCCS approved the study (approval number 59/2020).

Authors’ contributions

Study conception and design: DG.
Acquisition of data: VV, GC, GG, PV, EN, GM, FP, DG.
Analysis and interpretation of data: LP, EP, DG.
Drafting the article: LP, DG.
Revising the article critically for important intellectual content: LP, EP, VV, GC, GG, PV, EN, GM, AG, AS, GI, GBM, FP, DG.
Final approval of the version of the article to be published: LP, EP, VV, GC, GG, PV, EN, GM, AG, AS, GI, GBM, FP, DG.
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