Longitudinal Evaluation of the QuantiFERON-TB Gold Plus Assay in Hospitalized COVID-19 Patients with a First Indeterminate Result: Resolution of Inflammation and Restoration T-lymphocyte Counts and Interferon-gamma Production

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BACKGROUND

Several studies reported an increased rate of indeterminate QuantiFERON-TB Gold Plus (QFT-P) assay results in patients with severe Coronavirus Disease (COVID)-19, due to peripheral blood T-lymphocyte depletion and dysfunction [1 2 3 4 5 6].

OBJECTIVES

Aim of the study was to longitudinally evaluate QFT-P responses in patients who survived COVID-19, with a previous indeterminate result.

METHODS

In the Infectious Disease Unit, all patients hospitalized for COVID-19 underwent QFT-P assay on admission. In a subgroup of patients, the test was reassessed after recovery.

Demographics, clinical and laboratory data were collected. All statistical analyses were performed using GraphPad.

Comparison between groups was performed with an independent unpaired t-test; the level of statistical significance was <0.05.

RESULTS

We observed 223 patients with an indeterminate QFT-P assay among 949 patients hospitalized because of COVID-19 (23,5%) during 2020 and 2021.

36 patients among those with an indeterminate QFT-P assay were enrolled for reassessing the test after recovery from COVID-19. In 12 patients peripheral blood lymphocyte subsets were also reassessed.

RESULTS

Considering disease severity, 30 were classified as severe and 6 as non-severe; 1 patient was admitted to the Intensive Care Unit (ICU). Median age was 57,5 (interquartile range [IQR]: 49,5-63,8), with a prevalence of male sex (M/F: 24/12); median Charlson Comorbidity Index was 2 (IQR: 1-3).

The second QFT-P assay was performed after at least 1 month from the first assay (median time 7 months, IQR: 5-12 months). All QFT-P assays gave a determined result: 2 positive (5.5%) and 34 negatives (94,4%). A statistically significant difference was observed after comparing the laboratory parameters at the time of the first and the second QFT-P assay. Specifically, the absolute counts of total lymphocyte, total CD3+, CD4+ and CD8+ T-lymphocytes were significantly increased ($p<0.001$) while neutrophil absolute counts, neutrophil to lymphocyte (N/L) ratio, D-dimer, fibrinogen, ferritin, C-reactive protein (CRP) were significantly reduced ($p<0.0001$).

Concerning the QFT-P assay, interferon gamma (INF-γ) production in the Mitogen-Nil, TB1-Nil and TB2-Nil conditions were significantly increased ($p<0.0001$; $p=0.0019$; $p=0.0205$, respectively) (Figure 1 and Table 1).

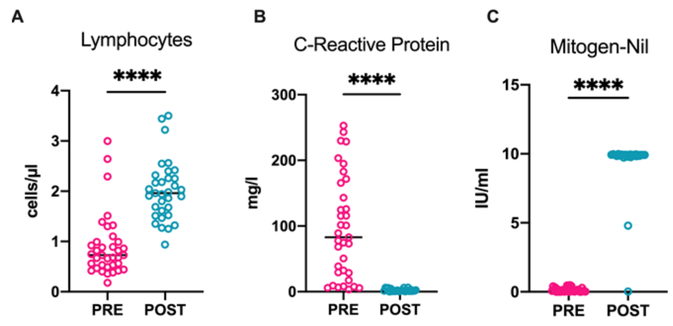

Figure 1. Comparison of laboratory parameter at the time of the first (*PRE*) and the second (*POST*) QFT-P assay. A) Lymphocyte absolute counts; B) C-reactive Protein; C) Mitogen-Nil condition of the QFT-P assay. ****: p<0,0001.

PARAMETER	PRE	POST	P-VALUE
White Blood	7.8 (5.0-11.0)	6.6 (5.8-8.0)	0.0686
Neutrophils	6.0 (4.0-9.2)	3.8 (3.3-4.4)	<0.0001
Lymphocytes	0.7 (0.5-1.0)	2.0 (1.6-2.3)	<0.0001
N/L Ratio	8.6 (4.8-12.5)	1.8 (1.6-2.4)	<0.0001
PCR	82.8 (28.5-165.6)	1.7 (0.8-3.6)	<0.0001
D-dimer	671.5 (449.8-1042.8)	227.0 (179.0-418.5)	<0.0001
Fibrinogen	592.0 (499.0-696.8)	292.5 (263.0-331.3)	<0.0001
Ferritin	916.0 (359.5-1587.0)	97.0 (52.5-201.5)	<0.0001
Mitogen	0.3 (0.1-0.6)	10.0 (10.0-10.0)	<0.0001
TB1	0.1 (0.0-0.2)	0.1 (0.1-0.2)	0.4535
TB2	0.1 (0.0-0.2)	0.1 (0.1-0.2)	0.9516
Mitogen-Nil	0.1 (0.0-0.3)	9.9 (9.9-9.9)	<0.0001
TB1-Nil	0.0 (-0.0-0.0)	0.0 (0.0-0.0)	0.0019
TB2-Nil	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0205
CD3+ Absolute count	397.0 (211.5-626.5)	1332.0 (1168.0-1795.5)	0.0005
CD3+ CD4+ Absolute count	237.0 (125.5-394.5)	859.5 (706.8-1071.3)	0.0005
CD3+ CD8 + Absolute count	107.0 (73.0-203.5)	463.0 (313.0-498.8)	0.0005
CD3+ CD4+ CD8 + Absolute count	5.0 (2.5-7.5)	21.5 (16.0-26.5)	0.0005
CD3+ CD4- CD8- Absolute count	14.0 (6.0-21.5)	47.0 (25.8-66.5)	0.0005
CD19+ Absolute count	89.0 (67.0-147.5)	187.5 (120.3-266.5)	0.0024
CD3-CD16+CD56+ Absolute count	122.0 (75.0-172.0)	216.0 (157.0-243.8)	0.0015
CD4/CD8 Ratio	2.2 (1.5-2.9)	2.1 (1.7-2.8)	0.9097

Table 1. Comparison of laboratory parameter at the time of the first (*PRE*) and the second (*POST*) QFT-P assay. Quantitative variables are presented as median (*interquartile range*). *N/L Ratio*: Neutrphils/Lymphocytes Ratio; *CRP*: C-reaction protein;.

CONCLUSIONS

Once the acute phase of COVID-19 is resolved, inflammatory markers and peripheral blood leucocyte counts tend to normalize with an effective INF-γ production after specific and nonspecific stimulation. The reduction of inflammation and the recovery of total peripheral blood lymphocyte counts is associated to an effective interferon-gamma production after specific mycobacterial peptide and nonspecific phytohemagglutinin stimulation. In our cohort, all the 36 QFT-P reassessed showed a determinate result.

In our cohort, all the 36 QFT-P showed a determinate result. Moreover, we observed 2 positive QFT-P assay (5.5%), supporting the importance of retesting patients with indeterminate result to identify latent tuberculosis infection (LTBI) and monitor patients for possible reactivation because of the immune-suppression associated with COVID-19.

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