

Original article

A whole blood test to measure SARS-CoV-2-specific response in COVID-19 patients

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ABSTRACT

Objectives: To examine whether specific T cell responses to SARS CoV 2 peptides can be detected in COVID 19 using a whole blood experimental setting, which may be further explored as a potential diagnostic tool.

Methods: We evaluated interferon (IFN) γ levels after stimulating whole blood with spike and remainder antigens peptides megapools (MP) derived from SARS CoV 2 sequences; interleukin (IL) 1 β , IL 1RA, IL 2, IL 4, IL 5, IL 6, IL 7, IL 8, IL 9, IL 10, IL 12p70, IL 13, IL 15, IL 17A, eotaxin, basic fibroblast growth factor (FGF), granulocyte colony stimulating factor (G CSF), granulocyte macrophage colony stimulating factor (GM CSF), IFN γ , Interferon gamma induced protein 10 (IP 10), monocyte chemoattractant protein 1 (MCP 1), macrophage inflammatory protein (MIP) 1 α , MIP 1 β , Platelet derived growth factor (PDGF), RANTES (regulated on activation, normal T cell expressed and secreted), tumour necrosis factor alpha (TNF α), vascular endothelial growth factor (VEGF) were also evaluated.

Results: IFN γ response to spike and remainder antigens MPs was significantly increased in 35 COVID 19 patients compared with 29 'no COVID 19' individuals (medians spike MP: 0.26 vs 0, $p = 0.0002$; medians remainder antigens MP: 0.07 vs 0.02; $p = 0.02$).

This response was detected independently of patients' clinical parameters. IFN γ response to SARS CoV 2 unrelated antigens cytomegalovirus (CMV) and Staphylococcal Enterotoxin B (SEB) was similar in COVID 19 compared with 'no COVID 19' individuals (median CMV: 3.46 vs 5.28, $p = 0.16$; median SEB: 12.68 vs 15.05; $p = 0.1$). In response to spike MPs in COVID 19 compared with 'no COVID 19' individuals, we found significant higher median of IL 2 (50.08 vs 0, $p = 0.0018$), IFN γ (90.16 vs 0, $p = 0.01$), IL 4 (0.52 vs 0, $p = 0.03$), IL 13 (0.84 vs 0, $p = 0.007$) and MCP 1 (4602 vs 359.2, $p = 0.05$).

Conclusions: Immune response to SARS CoV 2 peptides in a whole blood assay is associated with COVID 19 and it is characterized by both Th1 and Th2 profile. This experimental approach may be useful for

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Introduction

COVID 19 is an emerging respiratory infection caused by SARS CoV 2 with an estimated global burden of more than 30 million [1]. Diagnosis is based on RT PCR targeting one or more viral genes using nasopharyngeal swabs or other respiratory specimens [2]. High SARS CoV 2 specific IgG levels correlate with viral neutralizing antibodies [3]. However, IgM/IgG based tests may show cross reactions with other coronaviruses [2].

SARS CoV 2 infection is characterized by at least three main clinical presentations: absence of symptoms, mild/moderate disease, severe and critical disease [4–6]. Critically ill patients show a proinflammatory cytokine/chemokine storm [5] that contributes to the respiratory distress exacerbation; hence, several clinical studies aim to block the cytokine release. Increased production of inflammatory cytokines correlates with an impairment of both innate and adaptive cytotoxic antiviral functions [7]. Lymphopenia, another important feature of COVID 19, correlates to disease severity [8]. Moreover, transiently increased activated T cells have been identified prior to symptoms resolution in non severe diseased patients [9]. Regulatory and exhausted T cells, as well as alterations in the memory T cell subsets have also been recently reported in COVID 19 patients [10], as well as T cell SARS CoV 2 specific responses [11–14]. Both CD4 and CD8 T cells recognize peptides of SARS CoV 2 antigens, such as the spike (S), membrane and nucleocapsid antigens. Responses were noted to large pools of peptides encompassing the sequence of the spike protein, or the remainder genome encoded proteins. These responses were detected during the recovery [11] and the acute phase of disease [13].

T cell based tests have been explored in several infectious diseases including viral infections [15–20] and cytokine release based tests in whole blood are routinely or experimentally used for cytomegalovirus (CMV) infection monitoring [16], tuberculosis infection diagnosis [18] and have been explored for hepatitis B virus [15], toxoplasmosis [17] and cystic echinococcosis [19,20] diagnosis. This approach has not been scouted yet for SARS CoV 2 infection.

Therefore, in this study we examined whether a whole blood based assay could be expanded to the detection of the SARS CoV 2 specific T cell responses in COVID 19 patients.

Material and method

Study population

Ethical Committee of Lazzaro Spallanzani National Institute of Infectious Diseases (INMI) approved the study (58/2020) that was conducted between 8 April 2020 and 1 July 2020. Informed, written consent was required to consecutively enroll patients and controls by physicians. The 35 COVID 19 patients (all with positive nasopharyngeal swab for SARS CoV 2) were classified as mild, moderate, severe and critical, according to WHO [4]. For controls, 29 ‘no COVID 19’ individuals were enrolled and were healthy donors (HDs) ($n = 11$) volunteers from our laboratory or a convenience sample of consecutively patients hospitalized for other diseases as bacterial pneumonia ($n = 3$), latent tuberculosis infection ($n = 10$), active tuberculosis under therapy ($n = 4$), echinococcosis ($n = 1$). Demographic and clinical information were collected at enrollment.

Stimuli

SARS CoV 2 peptide pools (MegaPools, MPs) have been designed and validated [12,13,24]. The megapool design was carried out on the Wuhan Hu 1 reference isolated (GenBank ID:MN908947). To ensure a comprehensive assessment of spike specific reactivity, the main target of vaccine candidates, overlapping 15mers by 10 spanning the entire protein were synthesized and pooled separately (spike; $n = 253$). The remainder of the SARS CoV 2 proteome was filtered applying the seven allele method CD4 T cell prediction [22] with a cutoff of ≤ 20 , aiming to predict promiscuous epitopes with the capability to bind across the most common HLA class II specificities (remainder antigens; $n = 221$).

Peptides were synthesized as crude material (A&A, San Diego, CA, USA), resuspended in dimethyl sulphoxide and pooled according to spike or remainder antigens MP composition followed by sequential lyophilization steps [23].

A CMV MP ($n = 180$) previously described [23], and Staphylococcal Enterotoxin B (SEB) positive control were included.

IFN γ whole blood assay

Six hundred microlitres of whole blood were stimulated or not with spike or remainder antigens MP, CMV MP as unrelated antigen and SEB as positive control. Plasma was harvested after 6/24 h of stimulation at 37°C (5% CO₂) and stored at -80°C. IFN γ levels were evaluated by ELISA, according to manufacturer's instructions (www.quantiferon.com). IFN γ values were subtracted from the unstimulated or dimethyl sulphoxide control. The detection limit of the test was 0.065 IU/mL.

IgG serology

SARS CoV 2 IgG levels were measured by ELISA according to manufacturer's instructions (DIESSE Diagnostica Senese S.p.a., Monteriggioni, Italy). The ratio between the optical density of the sample and that of the cut off reagent (index) has been calculated. The samples were scored positive (index >1.1), doubtful (index between 1.1 and 0.9), negative (index is < 0.9). In the ‘no COVID 19’ group, ELISA positive samples were tested with an indirect immunofluorescence assay, using home made slides prepared with SARS CoV 2 infected Vero E6 cells.

Multiplex analysis

Cytokines, chemokines and growth factors [Interleukin (IL) 1 β , IL 1RA, IL 2, IL 4, IL 5, IL 6, IL 7, IL 8, IL 9, IL 10, IL 12p70, IL 13, IL 15, IL 17A, eotaxin, basic fibroblast growth factor (FGF), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), IFN γ , Interferon gamma induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP) 1 α , MIP 1 β , Platelet derived growth factor (PDGF), RANTES (regulated on activation, normal T cell expressed and secreted), tumour necrosis factor alpha (TNF α), vascular endothelial growth factor (VEGF)] were evaluated in harvested plasma using Bio Plex Pro Human Cytokine 27 plex Assay panel and the MagPix system (all from Bio Rad, Hercules, CA, USA), according to manufacturer's

instructions. Raw data were generated using the Bio Plex Manager software. Concentrations below the detection range were considered as zero. Concentrations above the detection range were converted to the highest value of the standard curve. Analyte levels were subtracted from the unstimulated control. Samples with acquired beads count <50 were excluded from the final analysis.

Statistical analysis

Data were analysed using SPSS software (Version 19 for Windows, Italy SRL, Bologna, Italy), and Graph Pad (GraphPad Prism 8 XML Project). Medians and interquartile ranges (IQRs) were calculated; the following tests were used: Kruskal–Wallis test for comparisons among groups, Mann–Whitney *U* test with Bonferroni correction for pairwise comparisons, Chi squared test for categorical variables; receiver operator characteristic (ROC) analysis for evaluating diagnostic performance; Spearman Rank Correlation for correlations and $r_s > 0.7$ was considered high correlation, $0.7 < r_s < 0.5$ moderate correlation and $r_s < 0.5$ low correlation.

Results

Description of the studied population

The COVID 19 group showed a significantly higher median age ($p = 0.006$) and a higher number of females compared with the 'no COVID 19' group ($p = 0.23$) (Table 1). COVID 19 patients were classified as mild ($n = 9$, 25.7%), moderate ($n = 15$, 42.9%), severe ($n = 2$, 5.7%) and critical ($n = 9$, 25.7%). SARS CoV 2 IgG serology results were available for 29 COVID 19 (83%) and for all 'no COVID 19' individuals (100%). Within the COVID 19 group, 20 (69%) scored SARS CoV 2 serology positive. One (HD) in the 'no COVID 19' group had a positive IgG serology by ELISA not confirmed by immunofluorescence testing which showed an antibody pattern not SARS CoV 2 specific. Therefore, all 'no COVID 19' individuals were considered SARS CoV 2 serology negative. IgG serology testing and whole blood test were performed concomitantly (or within a week).

Table 1
Demographical and clinical characteristics of the enrolled subjects

	COVID-19	No COVID-19	<i>p</i>
<i>n</i> (%)	35	29	
Age median (IQR)	61 (51–76)	50 (39–61)	0.006
Male, <i>n</i> (%)	14 (40.0)	16 (55.2)	0.23
Origin, <i>n</i> (%)			0.78
Western Europe	26 (74.3)	19 (65.6)	
Eastern Europe	2 (5.7)	2 (6.9)	
Asia	3 (8.6)	3 (10.3)	
Africa	3 (8.6)	2 (6.9)	
North America	0 (0)	0 (0)	
South America	1 (2.8)	3 (10.3)	
Swab positive results, <i>n</i> (%) [*]	35 (100.0)	0 (0)	<0.0001
Serology results, <i>n</i> (%) [§]			0.0001
Positive	20 (69.0)	0 (0)	
Negative	9 (31.0)	29 (100.0)	
Severity, <i>n</i> (%)			
Mild	9 (25.7)		
Moderate	15 (42.9)		
Severe	2 (5.7)		
Critical	9 (25.7)		

Origin refers to country of birth. IQR, interquartile range.

^{*} Information available from 35 COVID-19 (100%) and seven (24%) 'no-COVID-19' individuals.

[§] Information available from 29 COVID-19 (83%) and 29 'no COVID-19' (100%) individuals.

Whole blood IFN γ response to SARS CoV 2 peptides is increased in COVID 19 compared with 'no COVID 19' individuals

Two different concentrations of spike and remainder antigens MPs and two stimulation time points were tested (Supplementary Figs. S1 and S2). No significant differences were found in response to both spike or remainder antigens MPs at the concentrations tested (0.1–1 $\mu\text{g}/\text{mL}$) neither in COVID 19 (Supplementary Fig. S1(a),(b)) nor in 'no COVID 19' individuals (Supplementary Fig. S1(c), (d)). A higher IFN γ response after 24 h of stimulation for all stimuli compared with after 6 h was found (Supplementary Fig. S2). Following experiments were performed testing SARS CoV 2 peptides at 0.1 $\mu\text{g}/\text{mL}$ for 24 h stimulation.

IFN γ response to spike MP (median: 0.26, IQR: 0.01–1.37) was higher compared with remainder antigens MP (median: 0.07, IQR 0–0.62) ($p = 0.18$) in COVID 19 patients; 'no COVID 19' subjects showed a similar trend (spike MP median: 0, IQR: 0–0.07; remainder antigens MP median: 0.02, IQR: 0–0.05, $p = 0.53$). Interestingly, IFN γ levels in response to spike and to remainder antigens MPs stimulations were significantly higher in COVID 19 compared with 'no COVID 19' individuals ($p = 0.0002$ and $p = 0.02$, respectively) (Fig. 1(a), (b)). Importantly, no significant differences among the COVID 19 and 'no COVID 19' groups were found using SEB (median: 12.68, IQR 11.22–15.03 vs median 15.05, IQR 12.58–17.50; $p = 0.1$) and CMV stimulus (median: 3.46, IQR 0.14–8.47 vs median: 5.28, IQR 1.71–12.9; $p = 0.16$), employed as non specific and unrelated stimulation, respectively (Fig. 1(c), (d)). Within the 'no COVID 19' group, no significant differences were found comparing the IFN γ response with all stimuli between HDs and the controls ($p \geq 0.32$).

An ROC analysis was performed to evaluate the accuracy of the test for the diagnosis of COVID 19 (Supplementary Fig. S3(a), (b)). Significant Area Under the Curve (AUC) results were obtained for both spike (AUC 0.76, 95% confidence interval (CI) 0.64–0.88; $p = 0.0004$) and remainder antigens MP (AUC: 0.66, 95% CI 0.53–0.79; $p = 0.03$).

Based on the likelihood ratio, we defined the cut off for scoring purposes (0.16 IU/mL for the IFN γ response to spike MP and 0.095 IU/mL for the remainder antigens MP) identifying, respectively, 60% and 46% as responders within the COVID 19 patients.

Whole blood IFN γ response to SARS CoV 2 peptides is increased in COVID 19 patients with a positive SARS CoV 2 IgG serology

IFN γ levels in response to both spike and remainder antigens MPs were increased in COVID 19 patients with a positive serology compared with those with a negative serology and the difference was significant for remainder antigens MP ($p = 0.11$ and $p = 0.04$, respectively) whereas no differences were found in response to SEB or CMV ($p = 0.32$ and $p = 0.81$, respectively) (Supplementary Fig. S4 (a)–(d)). COVID 19 patients with a positive or negative serology showed similar clinical characteristics as disease severity, lymphocyte counts, therapy ($p \geq 0.18$) (data not shown). The correlation between IFN γ levels in response to spike and remainder antigens and the serology index was performed in 28 COVID 19 and 26 'no COVID 19' individuals. A significant low correlation was found for both spike ($r_s = 0.45$, $p = 0.0006$) and remainder antigens MPs ($r_s = 0.37$, $p = 0.006$) (data not shown).

Whole blood IFN γ response to SARS CoV 2 peptides is detected independently of the disease severity, symptoms onset, and lymphocytes counts

Stratifying the COVID 19 patients based on disease severity, no significantly different IFN γ levels were observed among patients

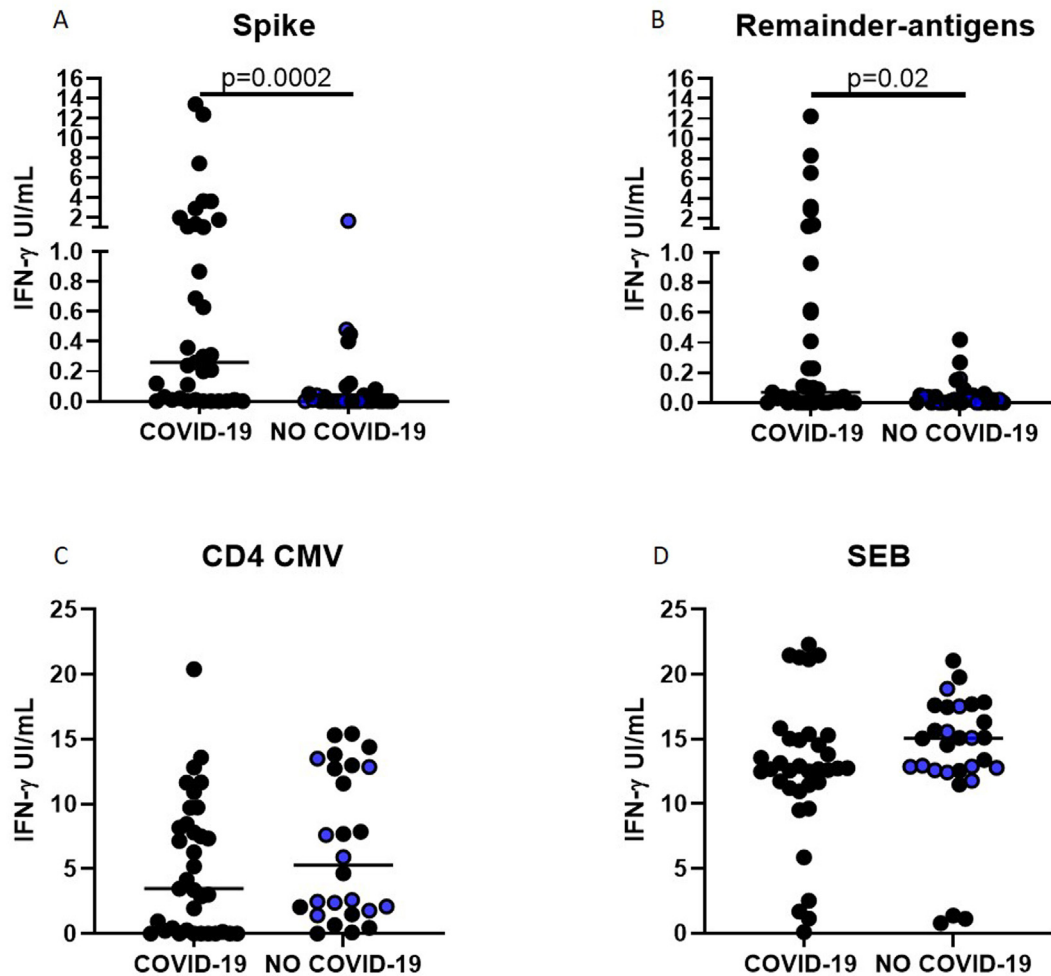


Fig. 1. Increased interferon (IFN)- γ response to SARS-CoV-2 peptides is associated with COVID-19. IFN- γ levels are significantly increased in COVID-19 patients compared with 'no COVID-19' patients after stimulating whole-blood with spike- (a) or remainder-antigens- megapools (MPs) (b). IFN- γ levels in response to the unrelated antigens CMV (c) and SEB (d) are similar in COVID-19 and 'no COVID-19' groups. IFN- γ was measured by ELISA in stimulated plasma. The horizontal lines represent the median; statistical analysis was performed using the Mann-Whitney test, and p-value was considered significant when ≤ 0.05 . Blue dots highlight healthy donors. CD, cluster of differentiation; CMV, cytomegalovirus; SEB, staphylococcal enterotoxin B.

with mild or moderate or severe/critical disease in response to both spike (p = 0.27) (Fig. 2(a)) and remainder antigens MPs (p = 0.72) (Fig. 2(b)). The impact of symptoms onset on the SARS-CoV-2 response was evaluated in 22/35 (62.9%) COVID-19 patients. IFN- γ levels were stratified considering the COVID-19 symptoms onset within 15 days, 16–30 days or more than 31 days and no significant differences were found neither for spike (Fig. 2(c)) (p = 0.51) nor for remainder antigens MPs (Fig. 2(d)) responses (p = 0.78).

COVID-19 patients (29/35) were stratified based on ranges of lymphocyte absolute number: $< 1 \times 10^3/\mu\text{L}$; $\geq 1 \times 10^3/\mu\text{L} < 2 \times 10^3/\mu\text{L}$; $> 2 \times 10^3/\mu\text{L}$ (Fig. 3(a)–(c)) and neither significant differences in the IFN- γ response to spike (p = 0.55) and remainder antigens MPs (p = 0.70) nor correlations with lymphocyte counts were found (Fig. 3(d), (e)). The SEB response was robust especially in patients with more than $1 \times 10^3/\mu\text{L}$ lymphocytes and a significant low correlation between IFN- γ levels and lymphocyte counts was found ($r_s = 0.37$, p = 0.05) (Fig. 3(f)).

Therapy was evaluated as a potential factor impacting the IFN- γ response to SARS-CoV-2 peptides; however, the studied population was too small to perform statistical analysis.

The whole blood response to SARS-CoV-2 response is characterized by Th1 but also Th2 cytokines

The production of several cytokines, chemokines and growth factors was evaluated in 13 COVID-19 and 13 'no COVID-19' individuals for spike MP and 14 COVID-19 and 13 'no COVID-19' individuals for remainder antigens MP enrolled within a week from swab results (median 4 days, IQR: 3–20 days). In response to spike MP, significantly higher levels of: IL-2 (median 50.08, IQR 3.765–111.8 vs median 0, IQR 0–6.98, p = 0.0018), IFN- γ (median 90.16, IQR 5.7–222.5 vs median 0, IQR 0–66.68, p = 0.01), IL-4 (median 0.52, IQR 0.14–1.84 vs median 0, IQR 0–0.76, p = 0.03), IL-13 (median 0.84, IQR 0–4.720 vs median 0, IQR 0–0.1, p = 0.007), and MCP-1 (median 4602, IQR 65.66–6692 vs median 359.2, IQR 0–818.8, p = 0.05) were found in COVID-19 compared with 'NO COVID-19' individuals (Supplementary Fig. S5(a)–(e)). A trend of high IL-17, IL-10 and GM-CSF levels was also found in COVID-19 patients (Supplementary Fig. S5(f)–(h)) compared with controls (p = 0.06, p = 0.054 and p = 0.06, respectively). Regarding remainder antigens MP, an increased IL-2 response in the COVID-

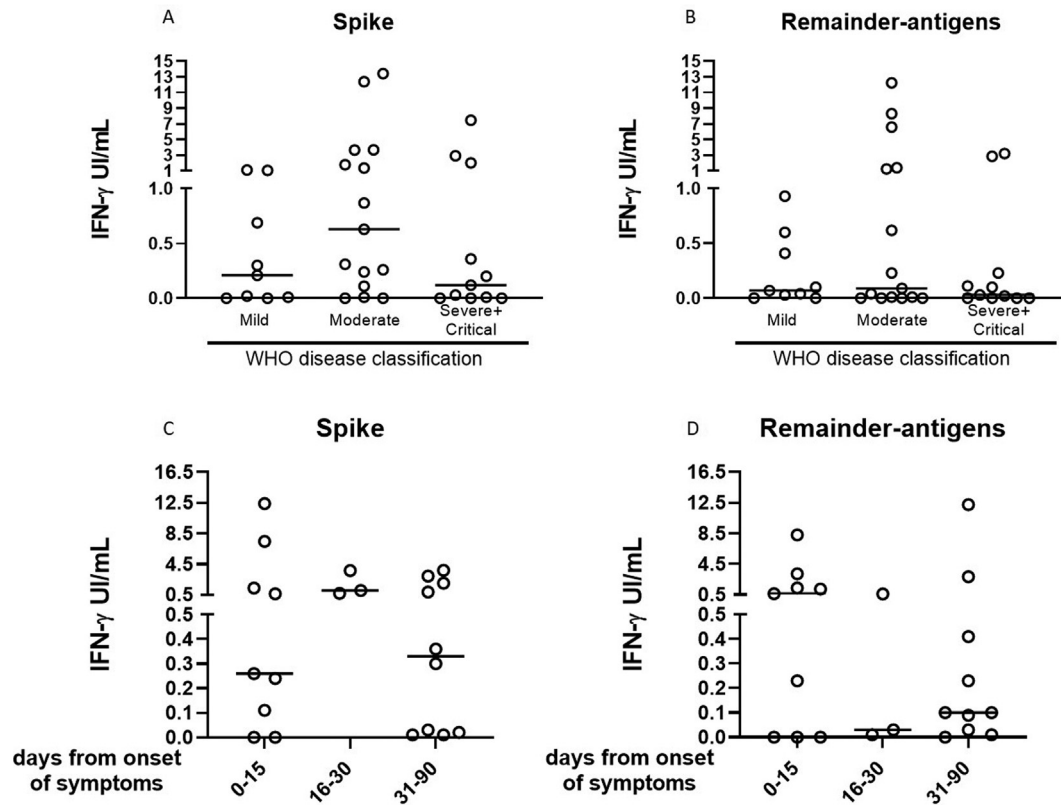


Fig. 2. The interferon (IFN)- γ response to SARS-CoV-2 peptides can be detected independently of the disease severity and symptoms onset. Evaluation of the IFN- γ response to spike- (a) and to remainder-antigens-megapools (MPs) (b) in COVID-19 patients according to the severity of the disease. No significant differences in the IFN- γ levels were found comparing patients with mild or moderate or severe/critical illness. Evaluation of the IFN- γ response to spike- (c) and to remainder-antigens- MP (d) in COVID-19 patients according to symptoms onset. No significant differences were found in the IFN- γ levels stratifying patients based on symptoms onset in respect to IFN- γ testing. IFN- γ was measured by ELISA in stimulated plasma. The horizontal lines represent the median; statistical analysis was performed using the Mann-Whitney test and $p \leq 0.016$ was considered significant.

19 group compared with the 'no COVID 19' group was found ($p = 0.07$) (Supplementary Fig. S5). No other differences neither in response to spike nor to remainder antigens MP (Supplementary Figs. S6 and S7) were found. No significant differences were found stratifying the cytokines levels based on SARS CoV 2 IgG serology scores ($p \geq 0.5$). A significant moderate correlation was found between IgG index and the levels of IL 2 in response to spike MP ($r_s = 0.58$, $p = 0.009$) (data not shown).

Discussion

In this study, we showed the ability to detect T cell response to SARS CoV 2 specific peptides using a whole blood cytokine release assay. In particular, COVID 19 patients showed a significant high IFN γ response, although both Th1 and Th2 cytokines were detected. Unrelated SARS CoV 2 antigens induced a cytokine response independently of COVID 19 status, thus supporting the specificity of the immune response to viral peptides. Moreover, the SARS CoV 2 specific response was independent of the severity of the disease, symptoms onset and lymphocytes count. These results suggest that the whole blood test for evaluating the T cell response to SARS CoV 2 may be a potential additional tool for further diagnostic and clinical deeper evaluations.

These immunological data are supported by recent findings showing a predominant Th1 response and by a concomitant Th2 and Th17 compartment in COVID 19 [10,13]. Th1 profile has been associated with disease resolution [24], whereas the Th17 cells may be crucial for immune mediated tissue damage, mediating

neutrophils recruitment and their activation in the lungs [10,25]. In COVID 19, we showed increased levels of IL 17 (almost significant) and MCP 1, both involved in the immune cells recruitment. These results suggest that COVID 19 is characterized by a broad immune activation with both inflammatory and regulatory arms involved, similar to bacterial sepsis [10].

Cytokine responses after MP stimulations were found in few 'no COVID 19' individuals. These data are in agreement with previous studies demonstrating a cross reactivity of SARS CoV 2 antigens in 'no COVID 19' individuals induced by a past exposure to seasonal cold coronaviruses [11–13,26]. Therefore, a more species specific peptide selection is needed to fully understand the immune response to SARS CoV 2.

A correlation between the B cell and T cell responses to SARS CoV 2 antigens was found. Indeed, COVID 19 patients with negative SARS CoV 2 serology showed a low IFN γ response to the viral peptides. This result underlines the strict relationship between the two immune compartments in COVID 19 as in other viral diseases [18].

Limitations of this work are related to the low number of the patients evaluated within an observational designed study preventing the full evaluation of the potentials of this immune based approach for supporting COVID 19 diagnosis. A prospective study design including exposed individuals followed over time and/or the enrollment of a large cohort of COVID 19 patients at different stages, will undoubtedly help addressing these issues. Also, we did not evaluate by cytometry or ELISPOT the cells responsible of the IFN γ response to the SARS CoV 2 MP. These peptides were

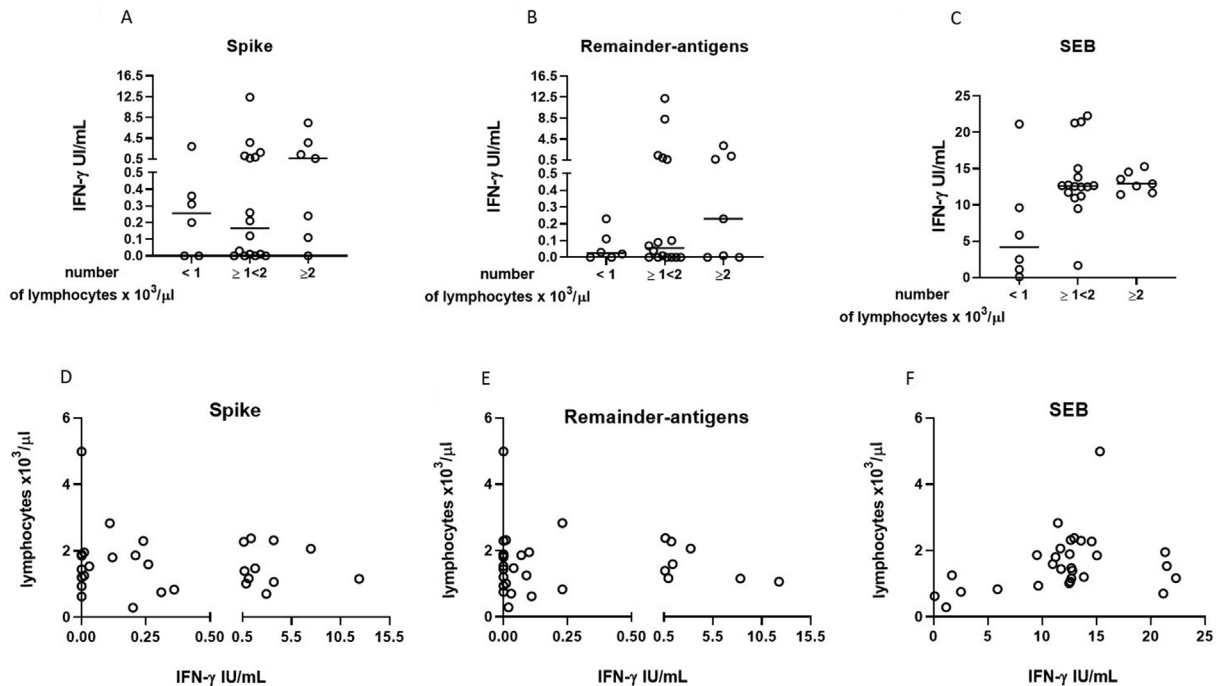


Fig. 3. The interferon (IFN)- γ response to SARS-Cov-2 peptides can be detected independently on the number of lymphocytes. No significant differences were found in the IFN- γ levels in response spike- (a) and to remainder-antigens- megapools (MPs) (b) according to lymphocytes counts. The IFN- γ response to the Staphylococcal Enterotoxin B (SEB) (c) increases in patients with more than $1 \times 10^3/\mu\text{L}$ lymphocytes. Correlation of the lymphocyte counts and IFN- γ levels in response to spike- (d), remainder-antigens-MPs (e) and SEB (f); a significant ($p = 0.05$) but low positive correlation ($r_s = 0.37$) was found for SEB stimulation. IFN- γ was measured by ELISA in stimulated plasma. The horizontal lines represent the median; statistical analysis was performed using the Kruskal–Wallis test, Mann–Whitney test and Spearman's rank correlation test; $p \leq 0.05$ or $p \leq 0.016$ was considered significant.

previously designed to capture SARS CoV 2 specific CD4 T cell response and showed indeed a strong SARS CoV 2 CD4 specific as well as a SARS CoV 2 CD8 specific response in the context of the spike pool protein [11,13,21]. Therefore, this characterization was beyond the scope of this pilot study. Here, we aimed to measure the total T cell response considering that T cells in whole blood are the main source of IFN γ , even if other cell types (e.g., natural killer cells, B lymphocytes) may be involved. Nevertheless, a strength of this study was its verification of the ability of SARS CoV 2 peptides to induce an immune response in a whole blood in vitro system. Thus, this study offers hints for developing rapid T cell based assays for SARS CoV 2 infection. Cytokine release based tests are considered easy and valid tools for the diagnosis of several infectious diseases [15–20]. Moreover, whole blood tests showed a good correlation with other experimental approaches involving peripheral blood mononuclear cell stimulation [28–30] which underlines their analytical robustness. Considering that SARS CoV 2 IgG levels are not constant over time [2,27], the development of a new diagnostic T cell based assay may support COVID 19 diagnosis. Moreover, this approach may be applied in several scenarios including the immune response monitoring in vaccine trials.

In conclusion, our encouraging preliminary results show that the response to SARS CoV 2 peptides can be detected in whole blood and is characterized by Th1 and Th2 profiles.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2020.09.051>.

Author contributions

L.P. analysed and interpreted data, performed the multiplex cytokine analysis and wrote the manuscript; E.P. analysed and interpreted data and wrote the manuscript; V.V. processed blood samples and performed the IFN γ ELISA; G.C. enrolled patients and controls and collected clinical data; S.N.F. performed multiplex cytokine analysis; C.C. performed SARS CoV 2 serology and contributed to the interpretation of the results; T.A. participated in the interpretation of data; F.P., G.G., P.V., E.N., L.L., A.A., A.V. enrolled patients; N.C., F.C., E.G., G.I. participated in the interpretation of data; A.G. provided peptide reagents and participated in the interpretation of data; D.G. designed and wrote the study, coordinated and supervised the project, contributed to the interpretation of the results, wrote the manuscript. All authors approved the final version of the manuscript.

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