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Guidelines

Humoral and cellular immune response levels at a 1-year follow-up after mild COVID-19

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ABSTRACT

The primary objective of this study was to establish a 1-year follow-up of patients after mild COVID-19 with no or only short-term detection of antibodies shortly after disease. At 1 year after disease, cellular memory against SARS-CoV-2, as measured by IFN-γ release by T cells, was detected in 76% (38/50) of participants. The data suggest that even if antibody levels decline after the primary infection has resolved, a cellular immune response may be detectable for longer.

1. Introduction

More than a year ago, the pandemic with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), began. How long a person vaccinated against or recovered from COVID-19 retains immunity to the virus is not yet well understood [1]. An important mechanism for defense against the virus is the formation of antibodies. However, it is already known that after a few months, a proportion of recovered individuals after COVID-19 no longer show detectable antibodies against SARS-CoV-2 [2, 3]. The cellular immune response has become an increasing focus of research. It has been shown during the disease, that there is an increased activity of T-cells [4].

In this study, we included individuals with short-term humoral immune response who were diagnosed with COVID-19 approximately one year ago. We investigated whether the T-cells are still stimulable when re-exposed to SARS-CoV-2 peptides.

2. Methods

2.1. Study design

For this study, we included individuals who were diagnosed with COVID-19 approximately one year ago (range: 291 – 380 days). We took the current 50 study participants from a collective we have already examined in the context of convalescence plasma donations [5] (Fig. 1).

Inclusion criteria were a positive virus detection by RT-PCR between March 8th and April 8th 2020 and no detectable anti-SARS-CoV-2 IgG/IgA antibodies (EUROIMMUN AG) (82% (41/50) of the participants) or lose of detectable anti-SARS-CoV-2 IgG/IgA antibodies (EUROIMMUN AG) until August 2020 (18% (9/50) of the participants). Individuals with vaccination against SARS-CoV-2 were not included. By including only individuals who no longer showed antibodies after COVID-19, we aim to investigate whether a cellular immune response is independently present. Institutional Review Board approval was obtained from the ethics committee of Bad Oeynhausen (Reg.-No. 670_2/2020).

Subjects after mild COVID-19 progression were studied in a 1-year follow-up. Various antibody tests were used, as well as an interferon-γ (IFN-γ) release assay.

2.2. Detection of anti-SARS-CoV-2 antibodies

Six different assays were performed for determination of antibodies against SARS-CoV-2. "Anti-SARS-CoV-2 ELISA (IgG)" (cut-off: ≥1.1 ratio) and "Anti-SARS-CoV-2 ELISA (IgA)" (cut-off: ≥1.1 ratio) from EUROIMMUN AG (Lübeck, Germany), "SARS-CoV-2 IgG II Quant assay" (cut-off: ≥7 BAU/mL) from Abbott (Abbott Park, Illinois, U.S.A.) (all three detecting antibodies against the S1 domain (spike protein) and receptor binding domain) and "LIAISON® SARS-CoV-2 S1/S2 IgG" (cut-off: ≥15 AU/mL) from Diasorin (S.p.A Via Crescentino SNC, 13,040 Saluggia (VC) – Italy) (detecting antibodies against the S1 and S2 domain (spike protein)). To measure neutralizing antibodies the

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surrogate virus neutralization test (sVNT) from GenScript (cut-off: ≥20% inhibition) (GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, Genscript, The Netherlands) (detecting antibodies against the ACE2 receptor) was used. Measurement of total antibodies against SARS-CoV-2 was performed using the "SARS-CoV-2 Total Assay" (cut-off: ≥1 U/mL) from Siemens Healthineers (Erlangen, Germany) (detecting antibodies against the S1 domain (spike protein), receptor binding domain and N-protein).

2.3. Interferon-γ release assay

The QuantiFERON Monitor ELISA (Qiagen, Hilden, Germany) was used for the analysis of the Interferon-γ (IFN-γ) release of the T-cells as described by Fischer et al. [6]. Peptide-stimulated (SARS-CoV protein S, N, and M, 6 nmol each, Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany) and unstimulated samples (negative control) were measured. All samples were transferred to a 96-well plate coated with anti-human IFN-γ monoclonal antibodies.

2.4. Statistical analysis

All continuous data are presented as the mean ± standard deviation (SD) or median ± interquartile range (IQR). Normality testing was performed using the Shapiro–Wilk test. Categorical data are presented as numbers and percentages. T-tests were conducted for analysis of the continuous variables.

3. Results

The mean age of study participants was 49 years (interquartile range (IQR): 16.35) and 52% of participants were female. The mean duration of COVID-19 was 12.5 days (standard deviation (sd): 7.6), with no participant requiring hospitalization. The median time between symptom onset and readmission for the 1-year follow-up was 321.5 days (IQR: 49.3). In the 41 participants in whom anti-SARS-Cov-2 antibodies were never measured, the time between symptom onset and antibody measurement was 55.8 days in mean (sd 31.4 days). In the remaining 9 participants, the time between symptom onset and first antibody detection was 39.2 days in mean (sd 22.1 days) and no antibodies were detectable after 75.2 / 97.9 days (sd 48.2 / 68.2).

Measurement of IFN-γ release by T-cells induced with SARS-CoV-2 peptides showed increased IFN-γ release in 76% of patients (Fig. 2D). A positive response was attributed to participants in whom the difference between unstimulated and stimulated samples was ≥0.43 U/mL IFN-γ. This value was determined from measurements of individuals who had no history of infection with SARS-CoV-2 (data not shown).

Subjects with positive IFN-γ release assays (mean 12.5 days, sd 7.7) showed no significantly longer duration of COVID-19 than in subjects with negative results (mean 12.3 days, sd 7.7) (p.value > 0.05) (Fig. 2E).

The different antibody tests showed partly large differences in the detection of antibodies against SARS-CoV-2. In the EUROIMMUN IgG test, 8% of the subjects were positive. Also performed were the Siemens test with 66%, the Abbott test with 54%, the EUROIMMUN IgA test with 30%, the DiaSorin IgG test with 32%, and the neutralizing antibody assay with 30% positive subjects.

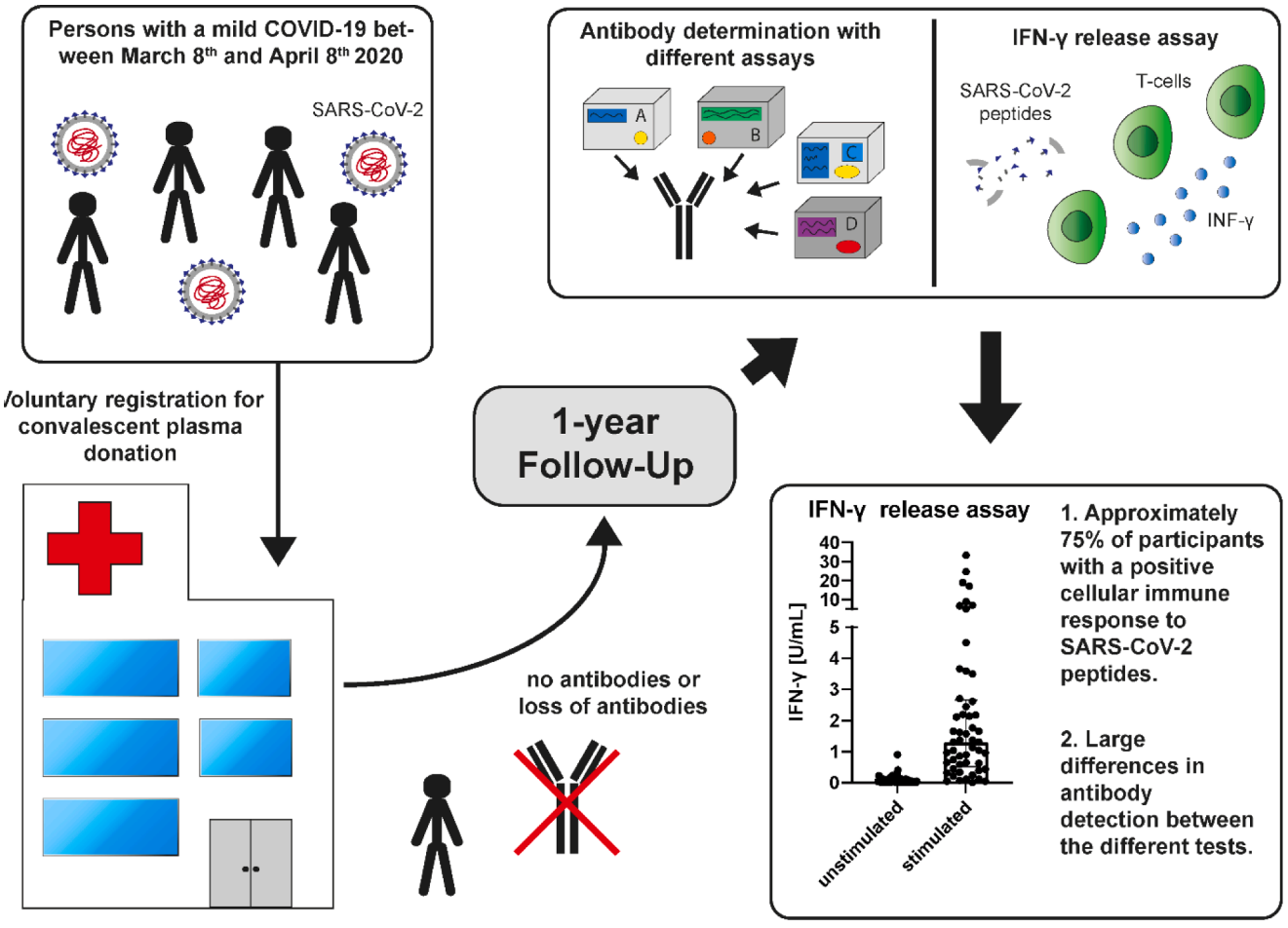

Fig. 1. Flowchart as an overview of the study procedure.

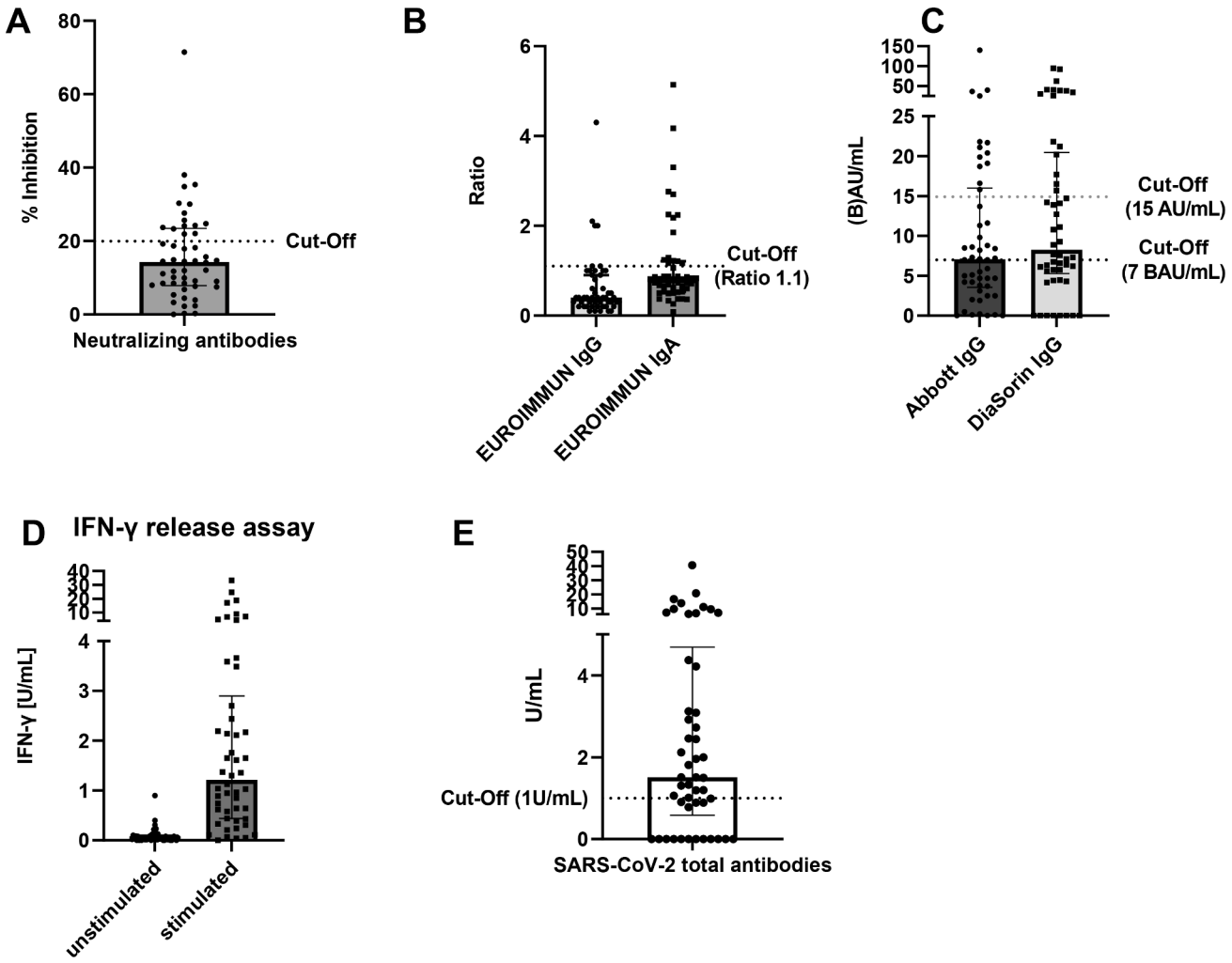

Fig. 2. Results of the different antibody detection assays and the IFN-γ release assay. A) Results of neutralizing antibody assay (median, IQR); 32% of participants were above the cut-off of 20% inhibition. B) The cut-off for the IgG and IgA test of EUROIMMUN is at a ratio of ≥1.1, and 8% of the participants showed a positive result in the IgG determination, and 32% in the IgA determination (median, IQR). C) IgG measurement using the Abbott and Diasorin assays. The cut-off for the Abbott assay is ≥7 BAU/mL, and there were 32% with a positive result (median, IQR). For the DiaSorin assay, the cut-off is ≥15 AU/mL, and there were 54% of participants with a positive result. D) Interferon-γ (IFN-γ) release after incubation with SARS-CoV-2 peptides (stimulated) or without incubation with these peptides (unstimulated) (median, IQR). A delta of ≥0.43 U/mL IFN- γ release was defined as a positive response to the SARS-CoV-2 peptides. There were 76% of participants showing a positive result. E) SARS-CoV-2 total antibodies showed 33 (66%) participants with positive results (cut-off ≥1 U/mL) (mean, IQR).

4. Discussion

In this study, we performed a follow-up after approximately 1 year in 50 subjects who had recovered from mild COVID-19. In our cohort, cellular immunity, as measured by the reactivity of T cells to SARS-CoV-2 peptides, and the detection of antibodies as markers of the humoral immune response were examined. Included in this study were only those subjects who did not have detectable antibodies (EUROIMMUN AG) to SARS-CoV-2 after recovery in spring 2020 (March 8th and April 8th) (82%), or lost the antibodies shortly after the initial examination (18%).

Using the IFN-γ release assay to determine T-cell activity, we demonstrated that a positive response could still be elicited in 76% of individuals. The values of the stimulated samples were lower than described by Fischer et al. [6], though, who used the same IFN-γ release assay in his study. However, the time between infection and measurement described in the population of Fischer et al. was 28–228 days, which was much shorter than in our study. Petrone et al. who used the QuantiFERON Monitor ELISA as we did could not find a correlation between the duration of COVID-19 and the amount of IFN-γ released [7], which we could confirm in our collective.

Surprisingly, it was observed that, depending on the test performed, between 8% and 66% of participants now tested positive for antibodies to SARS-CoV-2. However, in most cases the results were only just above the cut-off (Fig. 2A–C). This may be due to further contact with the virus or fluctuations in the measurement of the antibody titer. Additionally, it is possible that the results were false-positive or false-negative during the first measurement. At follow-up, we were still able to measure a positive IFN-γ release assay in 6 subjects (12%) who showed a negative result in each of the six used antibody assay. In one study participant without previous antibody detection, the values in all antibody tests were clearly positive and the IFN-γ delta value was also >10 U/mL. This suggests that a new, possibly unnoticed, infection with the virus has occurred.

In summary, we demonstrated that 76% of the subjects studied showed a cellular immune response after stimulation with SARS-CoV-2 peptides almost 1-year after SARS-CoV-2 infection, although they did not show specific antibodies as markers of a humoral immune response shortly after illness. This is a very encouraging result with regard to a long especially cellular immunity even in a mild course of COVID-19, and it will be interesting to see whether this can also be confirmed with regard to protection after vaccination.

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CRediT authorship contribution statement

Conceptualization and data collection and analysis were performed by Tobias Flieder and Ingvild Birschmann. Laboratory measurements were organized and performed by Tobias Flieder, Bastian Fischer, Andreas Peter and Katharina von Bargen. The first draft of the manuscript was written by Tobias Flieder. Tobias Flieder, Bastian Fischer, Katharina von Bargen, Andreas Peter, Cornelius Knabbe and Ingvild Birschmann read and approved the final manuscript.

Ethics approval

Institutional Review Board approval was obtained from the ethics committee of Bad Oeynhausen (Reg.-No. 670_2/2020).

Consent to participate

No informed consent was required because the additional analyses were performed from residual material and all other data were analyzed anonymously.

Consent for publication

All authors approved the publication of the manuscript.

Declaration of Competing Interests

Ingvild Birschmann received speaker's honoraria from Aspen Germany GmbH, Bristol-Myers Squibb/Pfizer, Siemens Healthcare and CSL Behring and reimbursement for congress traveling and accommodation from aspen and performed contract research for Siemens Healthcare. Ingvild Birschmann is a member of the advisory board of LFB biomedicaments, Siemens Healthcare, CSL Behring and Alexion Pharma Germany GmbH. Tobias Flieder, Bastian Fischer, Katharina von Bargen, Andreas Peter and Cornelius Knabbe have nothing to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105236.

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