Ratio between IgG against the spike protein of seasonal coronaviruses and SARS-CoV-2 is associated with a fatal outcome of hospitalized COVID-19 patients

Wouter Smit (wouter.vriend@gmail.com)
University Medical Center Utrecht

Sophie van Tol
National Institute for Public Health and the Environment (RIVM)

Lenneke van Lelyveld
Diakonessenhuis Utrecht

Gijs Limonard
Diakonessenhuis Utrecht

Ailko Bossink
Diakonessenhuis Utrecht

Chantal Reusken
National Institute for Public Health and the Environment (RIVM)

Michiel Heron
Diakonessenhuis Utrecht

Steven Thijsen
Diakonessenhuis Utrecht

Short Report

Keywords:

Posted Date: December 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2331119/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

SARS-CoV-2 infection elicits a cross-reactive and back boosting immune response from prior exposure to any of the human seasonal coronaviruses (HCoVs), but it has yet to be determined whether this response is associated with fatal outcome of severe COVID-19 disease. In a cohort of 58 hospitalized patients, we have shown that heterologous immune responses are associated with increased disease severity. Here, we report that a lower anti-spike IgG ratio of SARS-CoV-2 to HCoVs, in particular to the human coronavirus 229E, correlates with decreased neutralizing antibody titers, and is associated with a fatal disease course. These results support the hypothesis that pre-existing humoral immunity to spike antigens of HCoVs may impair an efficient immune response during COVID-19.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a clinical spectrum ranging from asymptomatic infections to severe COVID-19 pneumonia that requires hospitalization and admission to the pulmonary or intensive care unit (ICU). A proportion of critically ill patients, predominated by elderly or patients with pre-existing comorbidities\(^1\), exhibit a dysregulated immune response characterized by impaired type I interferon activity\(^2,3\), delayed neutralizing antibody production\(^4\), and excessive cytokine release (cytokine storm) which can be blocked to mitigate severe disease\(^5\). This indicates a maladapted immune response that contributes to a fatal disease course, the mechanism of which is poorly understood.

One factor that may alter the disease course in COVID-19 is back boosting of humoral responses due to immunological recall resulting from previous exposure to endemic human coronaviruses (HCoVs; HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63). Recent evidence suggests there is cross-reactivity of antibodies directed at the spike S2-subunit of coronaviruses, although this does not confer cross-protection\(^6,7\). Accordingly, memory B cell clones directed to HCoVs boosted during severe COVID-19 indeed poorly neutralize SARS-CoV-2\(^8\). In our cohort, we have also reported a back boost of HCoV-related anti-spike IgG in patients with severe disease, with the strongest correlation of anti-spike IgG to SARS-CoV-2 and to the Betacoronaviruses (OC43 and HKU1) seen in severely ill patients\(^9\). Following up on this work, we assessed neutralizing immunity and evaluated the anti-spike IgG ratio of SARS-CoV-2 to HCoVs in the same cohort, focusing on the subgroup of deceased patients in order to identify markers of a fatal disease course.

Results

Anti-spike IgG ratio between SARS-CoV-2 and HCoVs is altered in patients with fatal compared to non-fatal COVID-19

58 patients admitted to the intensive care unit (ICU) or the pulmonary ward during the first or second wave of infections in 2020 in the Netherlands with a severe form of COVID-19 pneumonia were enrolled
as part of the SARS-CoV-2 immune response (SIR) study, and sera were collected upon confirmation of SARS-CoV-2 infection with a validated in-house PCR test. Concurrently, a group of 62 healthy volunteers was included, of which 27 reported a history of asymptomatic or mild COVID-19 based on serostatus. Severe disease was divided into individuals who recovered during follow-up (termed non-fatal disease) and individuals who deceased during hospitalization or up to one week after discharge (termed fatal disease). Baseline characteristics of each of these cohort groups have previously been published. We then assessed the proportion of IgG that was directed against the spike protein (S-protein) of HCoVs compared to SARS-CoV-2 using a protein array that allows for parallel and quantitatively assessment of antigen-specific IgG. Spike epitopes spanned the full S-protein (Fig. 1A), with modifications shown in Fig. 1A. We found that patients with fatal disease had a lower anti-spike IgG ratio of SARS-CoV-2 to the HCoVs, when compared to non-fatal disease and seropositive healthy controls (Fig. 1B). A significant predominance of HCoV-specific antibodies was observed in the case of 229E (as indicated by a median IgG ratio in the gray area of the graph in Fig. 1B). Because we hypothesized that these differences arose due to interference of heterologous responses directed against the S2 subunit of spike, we also assessed IgG responses specifically directed against the S1 subunit. Importantly, here we did not find a difference between the ratio of SARS-CoV-2 to the HCoVs (figure S1).

Next, we performed virus neutralization assays to measure the neutralization capacity. We found that a higher proportion of back boosted antibodies against HCoVs correlated with a decrease in virus neutralization, as demonstrated by a good correlation between neutralization and the SARS-CoV-2/HCoV anti-spike IgG ratio (Fig. 2A). Sera from patients with fatal disease had significantly lower neutralization capacity compared to non-fatal disease (IC50 titer = 98 versus 22; p = 0.04) (Fig. 2B). This shows that predominance of IgG directed against the spike protein of HCoVs at the moment of hospital admission is associated with a fatal disease course. This supports the notion that a back boost of humoral immunity derived from previous exposure to HCoVs may interfere with efficient virus neutralization of SARS-CoV-2, thereby contributing to the immunopathogenic response that underlies fatal COVID-19.

**Discussion**

Presence of heterologous immunity against HCoVs is detectable in patients with severe COVID-19 and is considered to arise due to substantial homologous epitopes embedded in the spike protein of these coronaviruses. Here, using a protein microarray, we show that a fatal outcome is associated with an increased proportion of anti-spike IgG against HCoVs, at the time of hospital admission. Furthermore, the anti-spike IgG ratio of SARS-CoV-2 to HCoVs correlated with virus neutralization titers, which was reduced at the onset of a fatal disease course. These findings point towards a disadvantageous role for the observed back boost of spike-specific humoral immune responses arising from previous exposure to HCoVs. Additional prospective research is needed to show that the IgG ratio at hospital admission may be used as a predictor for a poorer disease outcome.
Exposure to epitopes from antigen of a novel pathogen that closely resembles epitopes of a previous pathogen, either due to antigenic drift or vaccine-derived, is well-described phenomenon first observed in influenza\textsuperscript{11}. Coronaviruses also share a substantial degree of homogeneity, predominantly in the more conserved S2-subunit of the spike protein. On exposure to a novel coronavirus this can initiate back boosting of memory B cell responses to previous HCoVs, and this occurs prior to development and maturation of the novel humoral response against the SARS-CoV-2. Whether these cross-reactive antibodies are effectively neutralizing the virus is an important question that may relate to the immunopathogenesis of severe COVID-19. Our data tallies with findings of several recently published studies, that have suggesting that immunity to HCoVs might impede or potentially delayed the mounting of an effective humoral immunity after a first encounter with the novel SARS-CoV-2 virus\textsuperscript{8,12–14}.

Several outstanding questions are still to be addressed with regard to this hypothesis. What mechanism may cause back boosted, high affinity antibodies to limit the development of neutralizing antibodies? Is it delayed or disturbed development of specific potent high-affinity anti-S1 subunit antibodies, which would be more in line with an antigenic sin theory\textsuperscript{15}, or could the presence of non-neutralizing antibodies facilitate viral entry, as has been proposed to occur due to antigen-dependent enhancement\textsuperscript{16}. Finally, the role of vaccinations should be explored, since vaccine-induced immunity might “reset” the memory B cell response, removing the aforementioned constrains of immune imprinting\textsuperscript{17}. If so, this underscores the importance of aiming for optimal vaccine coverage.

**Methods**

**Study cohort**

Patients who were admitted to the Diakonessenhuis hospital from May 2020 with COVID-19 disease were enrolled in the SIR study once SARS-CoV-2 infection was confirmed with PCR on nasal mucosa or bronchial excrete. Patients were either included from the pulmonary ward or directly from the ICU, and no exclusion criteria were used. Medical history was retrieved from the medical records. Healthy controls consisted of hospital personnel, without a medical history of a condition that could be expected to influence their immune status or the natural history of COVID-19 disease, who voluntarily participated in the study. Individuals who had already been vaccinated were excluded.

**Protein expression**

Secreted recombinant protein were produced in-house by transfection of plasmid DNA in mammalian HEK293F suspension cells as previously described\textsuperscript{18}. The transfected pPPI4 plasmids contained a sequence encoding for the corresponding antigen, followed by a HIS-tag. The following antigens were used: spike protein trimer and S1 subunit of SARS-CoV-2 (GenBank QHD43416.1), HCoV-229E (GenBank JX503061.1), HCoV-HKU1 (GenBank ADN03339.1), HCoV-OC43 (GenBank AiX10763), HCoV-NL63 (GenBank ABE97130.1) and MERS-CoV (GenBank KJ650297.1). In case of spike trimer proteins, the HIS-tag was preceded by a trimerization motif. Here, a prefusion-stabilized S protein ectodomain of SARS-
CoV-2 and HCoVs with a T4 trimerization domain and hexahistidine (His) tag was designed as previously described\textsuperscript{19}. Secreted recombinant protein was purified from the cell suspension by gravity flow chromatography using Ni-Nta beads. For spike trimer proteins, monomers and dimers were excluded from the mixture using size exclusion chromatography. For spike S1, dimerization and complex formation was excluded. Preparation of human coronavirus protein micro-array was based on previously described methodology\textsuperscript{20}.

**Protein microarray**

Sera were tested in 8 3-fold dilutions starting at 1:10, diluted in Blotto buffer containing 0.1% Surfact-Amps\textsuperscript{20} (ThermoFisher). Goat anti-human IgG, F(ab’)\textsuperscript{2} fragment specific, Alexa Fluor 647-conjugated (Jackson Immuno Research, West Grove, USA) was used in a 1:1000 dilution in Blotto buffer containing 0.1% Surfact-Amps\textsuperscript{20}. A pool of SARAS-CoV-2 positive control samples with known titers to eCoVs and SARS-CoV-2, was included in every run to assess low inter-assay variation.

**Statistical analysis**

Statistical values (Spearman’s rank correlations, and \textit{p} values) were calculated with GraphPad Prism version 9.1.0. Groups were compared with unpaired one-way ANOVA or Mann–Whitney U test for comparing two or more groups respectively.

**Declarations**

*Ethics approval and consent to participate, consent for publication*

The regional Medical Research Ethics Committees United approved the study (Nieuwegein, the Netherlands; MEC-U: NL73618.100.20). Inclusion in the study required written informed consent for participation and publication.

*Availability of data and materials*

The datasets generated and/or analyzed during the current study are are available on request from the corresponding author, WLS.

*Competing interests*

None of the authors have a conflict of interest to disclose and all authors have read the current “Instructions to authors” and accept the conditions posed.

This manuscript is original and has not been and will not be submitted elsewhere for publication simultaneously.

*Funding*

This research received no specific grant from any funding agency in the public or commercial sector.
Authors' contributions

S.v.T. performed the protein microarray experiments and neutralization tests, W.L.S. was involved with data analysis and presentation. W.L.S. wrote the manuscript, with support from M.H. and S.T. Each author finally has read and approved the submitted version.

Acknowledgements

This work is supported by the Diakonessenhuis hospital, and the Dutch National Institute for Public Health and the Environment (RIVM). We would also like to express our gratitude to the patients who participated in this study.

References


**Figures**

**Figure 1**

*Ratio of SARS-CoV-2 to HCoV anti-spike antibodies is decreased in patients with fatal disease*
A) the top bar is a linear representation of the design of recombinant stabilized prefusion SARS-CoV-2 Spike ectodomain with the signal peptide shown in blue and the S1 (red) and S2 (yellow), where the furin cleavage site is replaced with a glycine linker (GGGG), two proline mutations are introduced (K986P and V987P), and a trimerization domain (cyan) preceded by a linker (GSGG) is attached. The four recombinant eCoV Spike proteins are shown below, in which the natural furin cleavage site is mutated in the case of HKU1 and OC43. B) IgG levels are plotted as ratio of SARS-CoV-2 to HCoV anti-spike antibodies in different disease groups and healthy controls (HC).

Figure 2

Ratio of SARS-CoV-2 to HCoV anti-spike antibodies correlates to virus neutralization

A) correlation between virus neutralization titer and anti-spike IgG ratio of SARS-CoV-2 to the various HCoVs are shown with Spearman correlation an p-value shown in the adjacent bracket. B) SARS-CoV-2 virus neutralization capacity non-fatal and fatal COVID-19 patients.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.png