1 **Less severe course of COVID-19 is associated with elevated levels of antibodies against seasonal**
2 **human coronaviruses OC43 and HKU1 (HCoV OC43, HCoV HKU1)**

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10 **Abstract**
11 The clinical course of COVID-19 is very heterogeneous: Most infected individuals can be managed in an
12 outpatient setting, but a substantial proportion of patients requires intensive care, resulting in a high rate of
13 fatalities. Recently, an association between contact to small children and mild course of COVID-19 was
14 reported. We performed an observational study to assess the impact of previous infections with seasonal
15 coronaviruses on COVID-19 severity. 60 patients with confirmed COVID-19 infections were included (age
16 30 - 82 years; 52 males, 8 females): 19 inpatients with critical disease, 16 inpatients with severe or
17 moderate disease and 25 outpatients (age and gender matched to inpatients). Patients with critical disease
18 had significantly lower levels of HCoV OC43- (p=0.016) and HCoV HKU1-specific (p=0.023) antibodies at
19 the first encounter compared to other COVID-19 patients. Our results indicate that previous infections with
20 seasonal coronaviruses might protect against a severe course of disease. This finding should be validated
21 in other settings and could contribute to identify persons at risk before an infection.

22 **Introduction**

23 At present, approximately 10 to 20 percent of COVID-19 patients need medical treatment in hospitals and
24 about 5% need long-term treatment on intensive care units (ICU). In contrast, the majority of COVID-19
25 patients can be managed in an outpatient setting. Known important risk factors are age, male gender, high
26 body mass index and pre-existing chronic conditions [1]. However, also young and seemingly healthy
27 individuals are at risk to die from COVID-19 infections. At present, this heterogeneity of the disease course
28 is not well understood. Partial immunity against SARS-CoV-2 might contribute to this phenomenon, as
29 recently discussed in reports about cross-reactivity against SARS-CoV-2: Grifoni [2] and Le Bert [3]
30 described T cell responses to SARS-CoV-2 in unexposed human individuals. In a recent survey, patients
31 with mild course of COVID-19 reported frequent contact to small children [4]; therefore, exposure to
32 childhood-related infections might modify the disease severity of COVID-19. This corresponds to the low
33 incidence of severe COVID-19 infections in small children [5]. Seasonal coronaviruses mainly cause mild
34 respiratory tract infections and are frequently found in children. Like SARS-CoV-2, those viruses belong to
35 the subfamily of Orthocoronavirinae. Hence, the objective of this work is to assess if previous infections
36 with a seasonal coronavirus – as indicated by antibody levels – modify the disease course of COVID-19.

37 **Material and Methods**

38 Patient cohort
39 Serum samples from 60 Patients with COVID-19 infections confirmed by RT-qPCR were analyzed in the
40 context of the Coronaplasma Project (local ethics committee approval: AZ 2020-220-f-S) and COVID-19
41 biomarker study (local ethics committee approval: AZ 2020-210-f-S) at the University Hospital of Münster,
42 Germany. Median age of patients was 58 years (range 30 - 82 years). 52 males and 8 females were
43 included. Outpatients were manually selected to match age and gender of inpatients. After informed
44 consent, serum was collected at the first patient contact. Median age was 58 years for outpatients (22
45 male, 3 female), 58 years for inpatients with critical disease (ICU group: 17 male, 2 female) and 55 years
46 for inpatients with severe or moderate disease (non-ICU group: 13 male, 3 female), respectively. Critical

47 disease was defined by invasive ventilation or ECMO therapy; severe disease by oxygen insufflation;
48 moderate disease by hospitalization for other reasons without oxygen treatment. Median length of stay
49 (LoS) for all inpatients was 10 days (range 2 - 55); 3 fatalities occurred.

50 Antibody measurement
51 Antibodies against seasonal coronaviruses and SARS-CoV-2 were measured with the immunostrip assay
52 recomLine SARS-CoV-2 IgG from Mikrogen GmbH, Neuried, Germany. Regarding seasonal coronaviruses,
53 this assay measures IgG antibodies directed against the nucleocapsid protein (NP) of HCoV 229E, NL63,
54 OC43 and HKU1. With respect to SARS-CoV-2, NP-specific and spike protein (S)-specific antibodies
55 directed against the S1 subunit and the receptor binding domain (RBD) were determined. Measurements
56 were performed at the Institute of Virology/Department of Clinical Virology of the University Hospital
57 Münster according to the guidelines of the manufacturer. To test precision and reliability, a dilution series
58 and repetitive antibody measurements were performed. Negative and positive controls were analyzed.

59 Data processing and analysis
60 Antibody levels were visually determined according to the guidelines of the manufacturer as ordinal values
61 using the cutoff band of immunstrips as internal reference. Results of individual coronavirus-specific bands
62 were rated on an ordinal scale as non-detectable (-), below cutoff (+/-), with cutoff intensity (+), above cutoff
63 (++), and very strong intensity (+++). In addition, relative antibody levels were quantitatively determined
64 with ImageJ (version 153, 64bit-Version for windows) [6] using the signal intensity of the cutoff band of
65 individual immunstrips as internal reference. Results were expressed as ratio signal intensity HCoV-specific
66 band to signal intensity cutoff band. Standardized photographs from immunostrip assays were used for this
67 analysis. Demographical data, type of treatment and length of stay were extracted by the Medical Data
68 Integration Center (MeDIC) from the hospital information system of the University Hospital Münster (ORBIS
69 from Dedalus Healthcare Group). Descriptive statistics and statistical tests were performed with R (version
70 3.6.1). Ordinal and numerical values were analyzed with Wilcoxon tests. Association of numerical values
71 was assessed with Spearman correlation. A two-sided p-value of 0.05 was considered significant.

72 **Results**

73 According to the visual determination of band intensities, levels of HCoV OC43- and HKU1-specific IgG
74 antibodies were significantly lower for COVID-19 inpatients with critical disease compared to all other
75 patients (p=0.016 for OC43; p=0.023 for HKU1; Wilcoxon test with ordinal measurement data). Figure 1
76 presents ordinal IgG antibody levels against HCoV for COVID-19 patients with critical disease compared to
77 other COVID-19 patients. This finding was confirmed by densitometric determination of relative antibody
78 levels (Figure 2).

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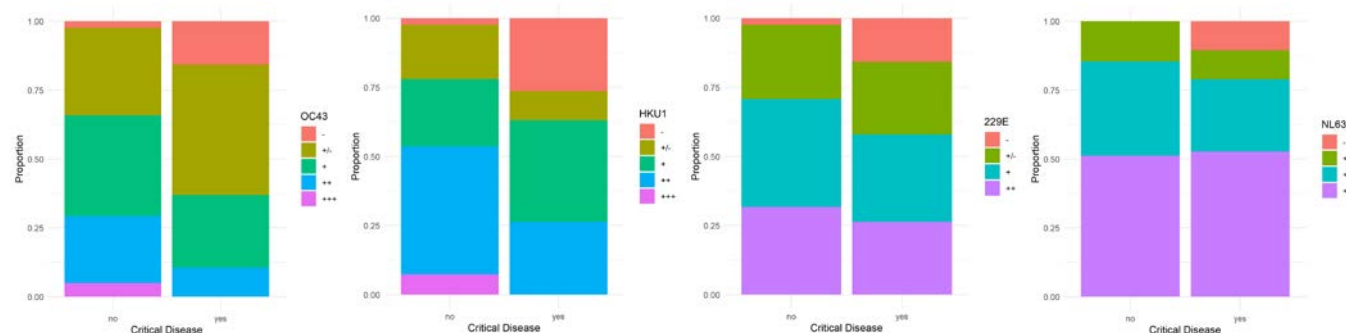
80 *Figure 1: Proportion of ordinal HCoV antibody levels from COVID-19 patients with and without critical*
81 *disease. a) OC43 (p=0.016) b) HKU1 (p=0.023) c) 229E (p=0.30) d) NL63 (p=0.82). COVID-19 patients*
82 *with critical disease present low antibody levels more frequently than patients without critical disease. This*
83 *difference is significant for OC43 and HKU1.*

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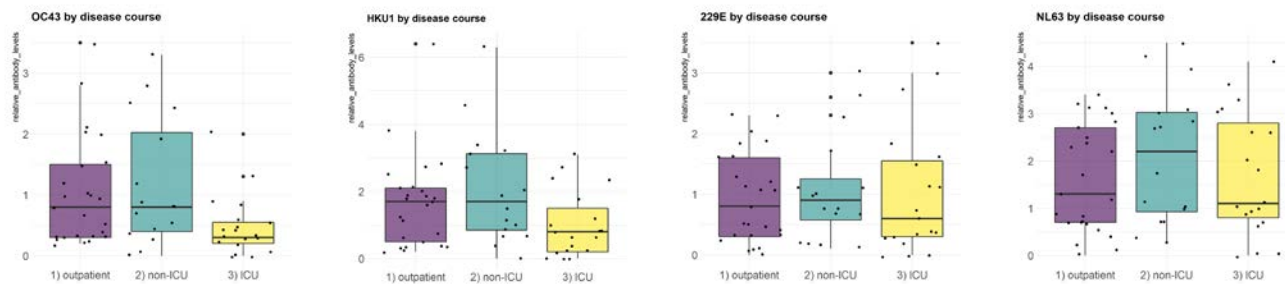
*Figure 2: Relative HCoV antibody levels of OC43 (a), HKU1 (b), 229E (c), NL63 (d). COVID-19 patients
with critical disease (ICU group) present lower values than other groups. This difference is higher for OC43
and HKU1 than for 229E and NL63.*

To further assess potential clinical implications of antibodies against endemic coronaviruses for COVID-19
patients, correlation of LoS and antibody levels against HCoVs OC43 as well as HKU1, respectively, was
analyzed (Figure 3). Long hospitalization periods were predominantly seen in patients with low antibody
levels, but this correlation was not significant.

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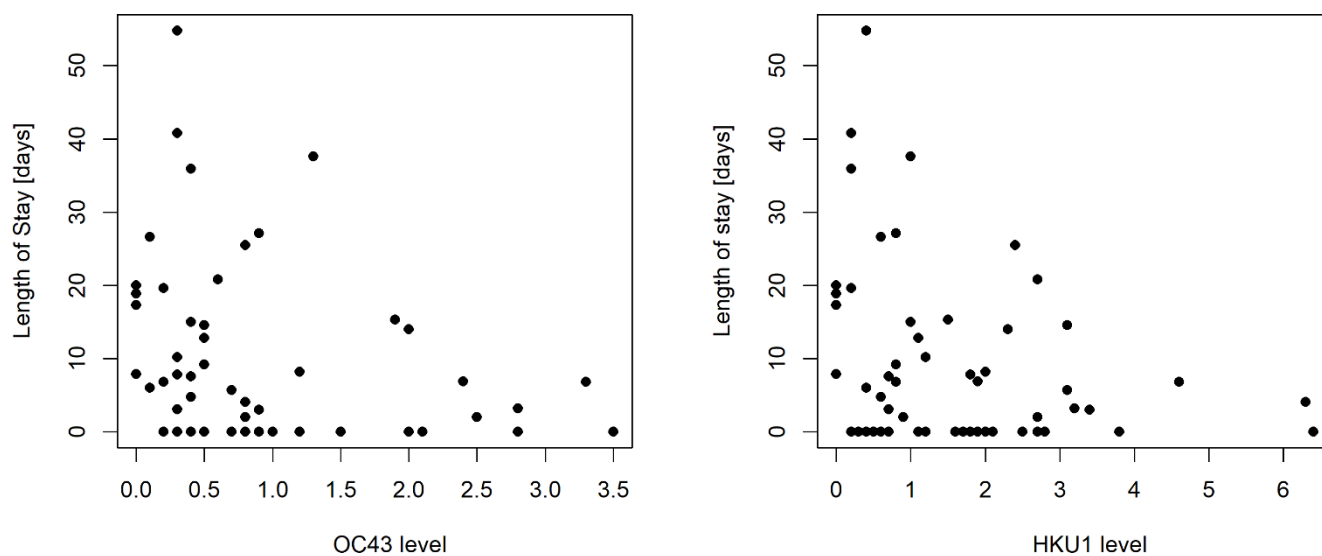
*Figure 3: a) Correlation of length of stay and OC43 antibody levels. A trend is visible, but not significant
(p=0.068).
b) Similar result for correlation of LoS and HKU1 antibody levels. A trend is visible, but not significant
(p=0.083). Higher antibody levels are correlated with reduced duration of hospitalization.*

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Figure 4 presents results from SARS-CoV-2 IgG antibody measurements at first encounter. In general,
patients with critical disease had higher SARS-CoV-2 IgG antibody levels compared to moderate/severe
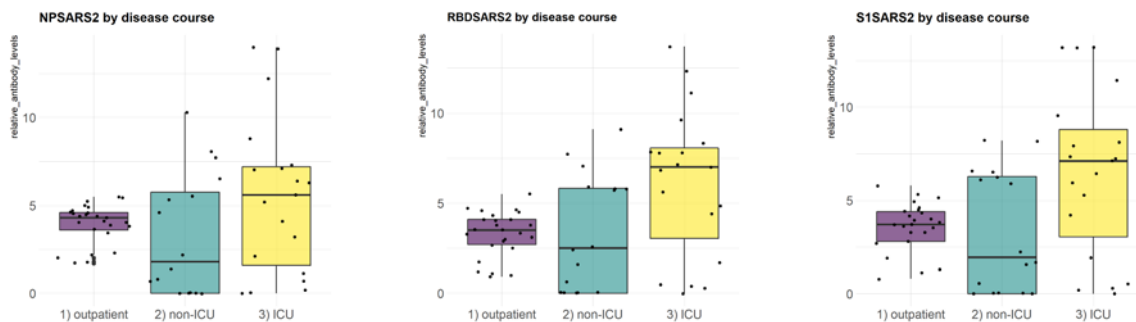inpatients and outpatients.

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Figure 4: Boxplots of SARS-CoV-2 relative antibody levels for outpatients, patients with severe/moderate disease (non-ICU group) and critical disease (ICU group). Median antibody levels are higher for patients with critical disease. a) NP SARS-2 b) RBD SARS2 c) S1 SARS2

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Discussion

Recently, several groups have reported immunological cross reactivity against SARS-CoV-2 in unexposed individuals [2, 3]. From a public health perspective, the relatively high proportion of COVID-19 patients with critical disease poses the key problem of this pandemic: overload of the healthcare system. If a previous infection with a known pathogen modified the course of COVID-19, i.e. decreased the proportion of intensive care patients, this could become a component to overcome the pandemic: Persons at risk for a severe course of disease could be identified *before* a SARS-CoV-2 infection and appropriate protective measures could be taken. Of note, this might also be relevant for vaccination strategies.

This observational study assessed a potential relationship between previous infections with seasonal coronaviruses – measured as antibody levels – and the severity of COVID-19 disease. It was shown that elevated antibody levels for HCoVs OC43 and HKU1 were associated with less need for intensive care therapy. In addition, a clear trend towards a reduced length of hospital stay was observed. One might argue that higher levels of antibodies against seasonal coronaviruses are merely a surrogate marker for a more active immune system. However, HCoVs OC43 and HKU1 are betacoronaviruses and therefore closer related to SARS-CoV-2 than HCoVs 229E or NL63. This is in line with our data that previous exposure to HCoVs OC43 and HKU1 has a stronger association with severity of COVID-19 than HCoV 229E or NL63 infections in the past. A possible explanation might be that previous exposure to seasonal betacoronaviruses facilitates immune response to SARS CoV-2. Further research is needed to assess the molecular mechanism behind our findings. Of note, cross-reactivity between HCoV OC43 and SARS-CoV was already described in 2006 [7]. Kissler [8] developed a simulation model for transmission dynamics of SARS-CoV-2 and reports that even mild cross-immunity from HCoV OC43 and HCoV HKU1 could potentially have a relevant effect on SARS-CoV-2 transmission.

Regarding SARS-CoV-2 antibodies, an inverse pattern was detected: inpatients with critical disease demonstrated higher median antibody levels for NP, RBD and S1 of SARS CoV-2 compared to other patients. Similar results were reported recently [9]. Hence, there is no evidence for a general bias regarding antibody levels and the ability to mount a humoral immune response between the three groups (outpatient, non-ICU, ICU) in our cohort.

This study has important limitations: It is a retrospective single-site study with a limited number of cases and association is not causation. However, it is remarkable that the effect of HCoV OC43 and HKU1-specific antibody levels reached statistical significance regarding the need for intensive care therapy with only 60 patients.

Therefore, these findings should be validated in other sites with larger patient collectives. In a prospective setting (e.g. for risk groups with contacts to many persons like employees of hospitals or supermarkets) it

140 should be tested if the absence of HCoV OC43 and HKU1-specific antibody levels can identify persons at
141 risk for a severe course of COVID-19. Identification of vulnerable individuals is a key priority in the current
142 stage of the pandemic to guide protective measures and to design vaccination strategies.

143 **Conclusion**

144 Elevated levels of pre-existing antibodies against seasonal coronaviruses, specifically HCoV OC43 and
145 HKU1, are associated with less severe course of COVID-19. Further studies should validate this finding
146 and explore the potential to identify persons at risk for severe disease course before a SARS-CoV-2
147 infection.

148 **Acknowledgement**

150 **Data availability statement**

151 Due to data protection regulations, personal identifiable data cannot be published.

152 **Code availability statement**

153 R code is available on request from the authors.

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