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Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Prevalence of COVID-19-associated symptoms during acute infection in relation to SARS-CoV-2-directed humoral and cellular immune responses in a mild-diseased convalescent cohort[☆]

Christian M. Tegeler^{a,b}, Tatjana Bilich^{a,c,d}, Yacine Maringer^{a,c,d}, Helmut R. Salih^{a,d},
Juliane S. Walz^{a,c,d,e}, Annika Nelde^{a,c,d,*}, Jonas S. Heitmann^{a,d}

^a Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), Department of Internal Medicine, University Hospital Tübingen, Otfried-Müller-Str. 10, 72076 Tübingen, Germany

^b Department of Obstetrics and Gynecology, University Hospital of Tübingen, Calwerstraße 7, 72076 Tübingen, Germany

^c Institute for Cell Biology, Department of Immunology, University of Tübingen, Auf der Morgenstelle 15, 72076 Tübingen, Germany

^d Cluster of Excellence iFIT (EXC2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, Röntgenweg 11, 72076 Tübingen, Germany

^e Robert Bosch Center for Tumor Diseases (RBCT), Auerbachstraße 110, 70367 Stuttgart, Germany

ARTICLE INFO

Article history:

Received 8 February 2022

Revised 7 April 2022

Accepted 8 April 2022

Keywords:

SARS-CoV-2

T cells

antibody

immune response

symptoms

ABSTRACT

Objectives: Besides SARS-CoV-2-directed humoral immune responses, T cell responses are indispensable for effective antiviral immunity. Recent data have shown a correlation between COVID-19 symptoms and humoral immune response, but so far, little is known about the association of SARS-CoV-2-directed T cell responses and disease severity. Herein, we evaluated the prevalence of different clinical COVID-19 symptoms in relation to SARS-CoV-2-directed humoral and cellular immune responses.

Methods: The severity of eight different symptoms during acute infection were assessed using questionnaires from 193 convalescent individuals and were evaluated in relation to SARS-CoV-2 antibody levels and intensity of SARS-CoV-2-specific T cell responses 2–8 weeks after positive polymerase chain reaction. **Results:** Although increased IgG serum levels could be associated with severity of most symptoms, no difference in T cell response intensity between different symptom severities was observed for the majority of COVID-19 symptoms. However, when analyzing loss of smell or taste and cough, awareness of more severe symptoms was associated with reduced T cell response intensities.

Conclusions: These data suggest that rapid virus clearance mediated by SARS-CoV-2-specific T cells prevents severe symptoms of COVID-19.

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Introduction

SARS-CoV-2 induces highly variable clinical manifestations of COVID-19, ranging from asymptomatic infections or mild disease characteristics to severe and even life-threatening courses of disease (García, 2020). Infected individuals present with a variety of different disease symptoms, comprising fever, fatigue, loss of smell and taste, cough, headache, and/or dyspnea (Li et al., 2020). On the basis of the manifestations of these symptoms, patients are categorized into different disease severity groups (Son et al., 2021) with

most patients experiencing mild symptoms that do not require medical intervention or hospitalization (Nakamichi et al., 2021). After relief of symptoms, patients are considered to be protected from reinfection for several months, mediated by the immunological memory of the humoral and cellular immune system (Tan et al., 2020). The humoral immune response (ie, SARS-CoV-2-specific antibodies) detected in convalescent individuals has led to the approval of immune therapeutics like the REGN-COV2 antibody cocktail (Weinreich et al., 2021) and prophylactic vaccines, which aim to induce a protective immune response (Polack et al., 2020). Although most infected individuals remain asymptomatic or show mild symptoms and mount a potent immune response, others suffer from a severe course of disease with a dysregulated immune reaction (Kowalik et al., 2020; Krämer et al., 2021; Qin et al., 2020).

[☆] Short title: Clinical symptoms and immune responses in COVID-19 convalescent individuals

* Corresponding author.

E-mail address: annika.nelde@uni-tuebingen.de (A. Nelde).

A plethora of data are available regarding the characteristics of humoral and cellular immune responses in SARS-CoV-2-infected individuals (Altmann and Boyton, 2020; Bilich et al., 2021a; Bilich et al., 2021b; Brunk et al., 2021; Jarjour et al., 2021; Kared et al., 2021; Karlsson et al., 2020; Nelde et al., 2021; Sekine et al., 2020; Woldemeskel et al., 2020; Woodruff et al., 2020). Although antibody titers have been reported to be increased in patients with more severe COVID-19 (Woodruff et al., 2020), so far, little is known about the correlation of T cell responses with clinical symptoms. T cells are not only central for immune modulation and for guiding B cells to produce antibodies, but they also play an important role in virus clearance by direct killing of SARS-CoV-2-infected cells (Grifoni et al., 2020; Kared et al., 2021).

Here, we studied the COVID-19 course of 193 individuals during asymptomatic to moderate SARS-CoV-2 infection and their association with SARS-CoV-2-specific antibody and T cell responses up to 59 days after acute infection. The documented differences in symptom severity and immune response provide insights into the role of T cell immunity against SARS-CoV-2 for the occurrence and severity of clinical symptoms.

Methods

Convalescents and blood samples

Blood and serum samples as well as questionnaire-based assessment of donor characteristics and disease symptoms from convalescent volunteers after asymptomatic to moderate symptomatic SARS-CoV-2 infection (n = 193) were collected at the University Hospital Tübingen from April to July 2020. Informed consent was obtained in accordance with the Declaration of Helsinki protocol. The study was approved by and performed according to the guidelines of the local ethics committees (179/2020/BO2). SARS-CoV-2 infection was confirmed by polymerase chain reaction (PCR) test after nasopharyngeal swab. Donor recruitment was performed by online and paper-based advertising (homepage, flyer). Sample collection in terms of peripheral blood mononuclear cells (PBMCs) and serum was performed approximately 3–8 weeks after the end of symptoms and/or negative virus smear. Symptom categories were determined by subjective disease symptoms (no, mild, moderate, and severe; reported by questionnaire) of individual donors. Detailed donor characteristics and distribution of symptom severity are provided in Tables 1 and 2.

T cell and antibody responses

Data on SARS-CoV-2-specific T cell responses assessed by interferon- γ (IFN- γ) enzyme-linked immunospot (ELISpot) assay, anti-SARS-CoV-2 nucleocapsid antibody titers assessed by Eleysys® anti-SARS-CoV-2 immunoassay (Roche Diagnostics), and anti-SARS-CoV-2 spike antibody titers assessed by Euroline Anti-SARS-CoV-2® (Euroimmune) were retrieved from a previous publication

Table 1

Donor characteristics of COVID-19 convalescent donors.

	COVID-19 convalescent donors
Number of donors	193
Age (years)	
Range	18 - 79
Mean	43.3
SD	14
Sex (n [%])	
Female	91 (47)
Male	102 (53)
Time PCR to sample collection (d)	
Range	16 - 59
Median	41

n: number of donors. %: percentage of donors. SD: standard deviation. PCR: polymerase chain reaction. d: days.

(Nelde et al., 2021). For this analysis, we considered SARS-CoV-2-specific T cell response intensities against the previously described SARS-CoV-2-specific epitope compositions for human leukocyte antigen (HLA) class I and HLA-DR. These SARS-CoV-2-specific epitope compositions were designed from immunogenic SARS-CoV-2-derived T cell epitopes, derived from different open reading frames, including spike, nucleocapsid, and membrane proteins, and recognized exclusively in convalescent patients after SARS-CoV-2 infection and not in SARS-CoV-2 unexposed individuals. The HLA class I and HLA-DR epitope compositions cover several different HLA class I and HLA-DR allotypes, respectively, to allow for standardized evaluation and determination of intensities of SARS-CoV-2-specific T cell responses. The intensity of T cell responses was measured as mean spot counts of duplicates in the ELISpot assay normalized to 5×10^5 cells minus the normalized mean spot count of the respective negative control.

Software and statistical analysis

Data are displayed as mean with standard deviation (SD), box plots as median with 25th or 75th quantiles and min/max whiskers. Continuous data were tested for distribution and individual groups were tested using an unpaired Mann-Whitney *U* test or Kruskal-Wallis test and corrected for multiple comparison, if applicable. Missing data were included in tables and in descriptive analysis. Graphs were plotted using GraphPad Prism v.9.1.2. Statistical analyses were conducted using JMP Pro (SAS Institute, v.15) software. P-values < 0.05 were considered statistically significant.

Results

Clinical characteristics of COVID-19 convalescent cohort

For this study, we analyzed clinical symptoms and SARS-CoV-2-specific antibody and T cell response data of 193 convalescent

Table 2

Distribution of symptom severity in COVID-19 convalescent donors.

Symptom	n	Symptom severity - n (%)			
		Asymptomatic	Mild	Moderate	Severe
Feel sick	193	20 (10)	39 (20)	86 (45)	48 (25)
Cough	193	65 (34)	63 (33)	42 (22)	23 (12)
Fever	193	88 (46)	32 (16)	52 (27)	21 (11)
Sore throat	193	102 (53)	49 (25)	28 (15)	14 (7)
Headache	192	46 (24)	47 (24)	55 (29)	44 (23)
Loss of smell or taste	193	70 (36)	22 (11)	15 (8)	86 (45)
Shortness of breath	192	132 (69)	32 (17)	26 (13)	2 (1)
Fatigue	192	32 (17)	40 (21)	50 (26)	70 (36)

n: number of donors. %: percentage of donors.

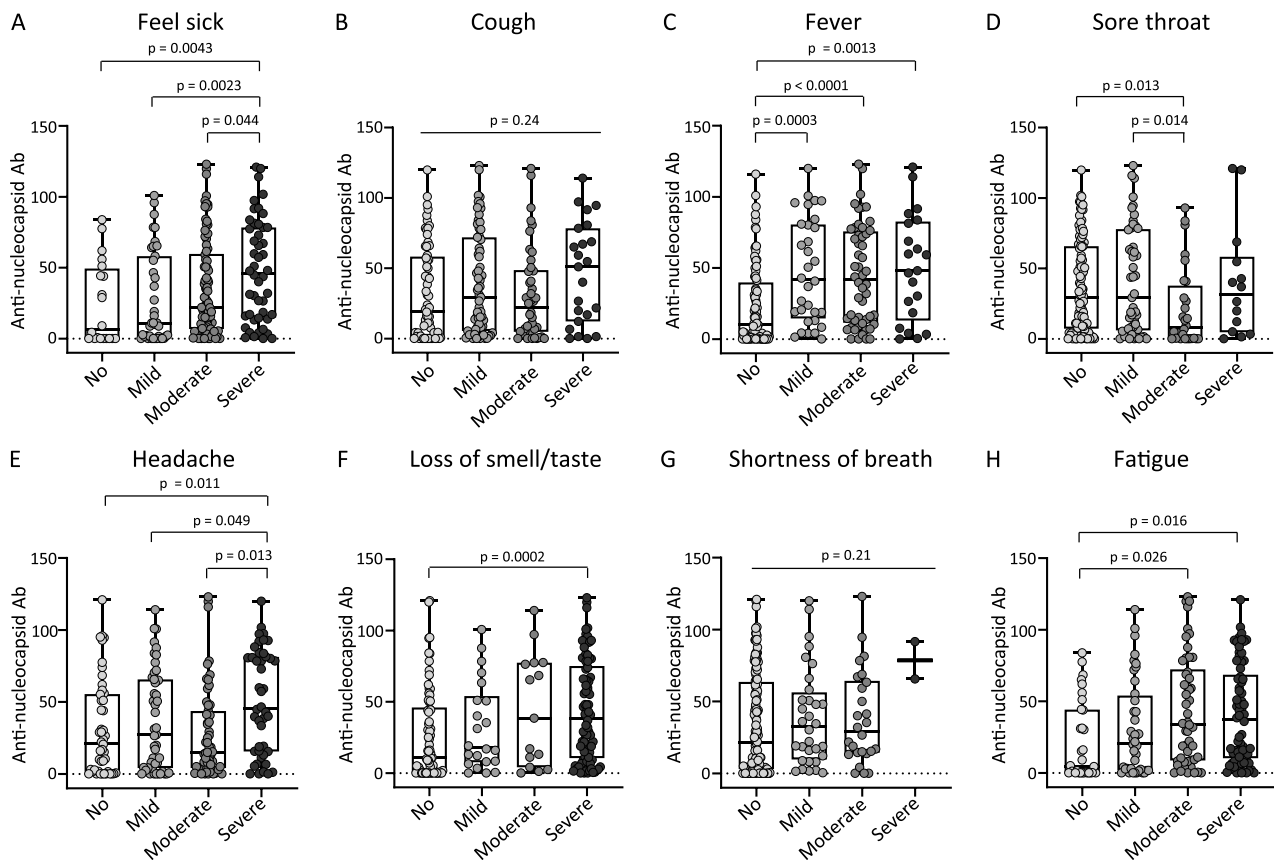


Fig. 1. Symptom severity and antibody response in COVID-19. Antinucleocapsid antibody (Ab) response (Elecys® Anti-SARS-CoV-2 S-test) assessed in COVID-19 convalescent donors ($n = 193$) with different COVID-19 symptoms grouped according to respective symptom severities. Symptoms during COVID-19 (ie, [A] feel sick, [B] cough, [C] fever, [D] sore throat, [E] headache, [F] loss of smell or taste, [G] shortness of breath, and [H] fatigue) were assessed by questionnaire and grouped according to self-reported severity (no, mild, moderate, severe symptoms). p: p-value. Brackets mark significant p-values between two categories (Kruskal-Wallis test with Dunn correction), continuous lines indicate p-values between all categories (Kruskal-Wallis test). Box plots with min/max whiskers.

donors with an asymptomatic to moderate COVID-19 course. None of the donors were hospitalized, had severe/life-threatening symptoms according to the World Health Organization (WHO) criteria (Son et al., 2021), or were vaccinated before SARS-CoV-2 infection. It is assumed that all infections were caused by the wild type of SARS-CoV-2, although samples were not sequenced. Antibody and T cell responses were assessed 16–59 days (median 40.5 days) after positive PCR (Nelde et al., 2021). Mean age of convalescents was 43.3 (SD \pm 14) years. The male : female ratio was nearly equally distributed with 1 : 1.2. As expected, subjective symptom severity was heterogeneously distributed. The most frequent symptoms were “feel sick” (93%), followed by “fatigue” (86%). A total of 6% of study participants had reported no symptoms. Individuals experiencing “loss of smell or taste” or “fatigue” graded these symptoms mostly severe (45% and 36% of all convalescents, respectively). Interestingly, only two convalescents reported severe dyspnea according to questionnaire. Clinical data and details regarding distribution of severity of the respective symptom groups are presented in Tables 1 and 2.

Antibody levels and COVID-19 symptoms

As a first step, we compared antinucleocapsid-SARS-CoV-2 antibody titers of convalescent donors (Nelde et al., 2021) with the severity of subjective COVID-19 symptoms. In the case of the symptom “feel sick”, antibody titers were significantly higher in severe than asymptomatic, mild, and moderate cases (p-value 0.0043, 0.0023, and 0.044, respectively; Fig 1A). Regarding “cough”, there was a tendency for higher IgG titers in convalescents exper-

riencing severe symptoms than other severity grades but without reaching statistical significance (Fig 1B). Antibody titers were significantly higher in convalescents reporting mild, moderate, and severe “fever” than asymptomatic cases (p-value 0.0003, <0.0001, and 0.0013, respectively; Fig 1C). Individuals with no and mild “sore throat” had significantly higher antibody titers than individuals with moderate symptoms (p-value 0.013 and 0.014, respectively; Fig 1D). Convalescents with severe “headache” showed significantly higher antibody levels than convalescents with no, mild, or moderate symptoms (p-value 0.011, 0.049, and 0.013, respectively; Fig 1E). In individuals reporting “loss of smell or taste”, IgG levels were significantly higher for convalescents with severe than for those with no symptoms (p-value 0.0002; Fig 1F). Considering “shortness of breath”, no significant difference between asymptomatic, mild, moderate, and severe symptoms was observed (Fig 1G). Convalescents experiencing severe or moderate “fatigue” had significantly higher IgG levels than convalescents without fatigue (p-value 0.016 and 0.026, respectively; Fig 1H). In addition, we compared antispikes-SARS-CoV-2 antibody titers of convalescent donors (Nelde et al., 2021) with severity of subjective COVID-19 symptoms. Antibody titers were significantly higher in convalescents reporting severe manifestation of all assessed symptoms than asymptomatic individuals (Supplementary Fig 1). Taken together, antinucleocapsid IgG levels were significantly higher in individuals reporting severe manifestation of the symptoms “feel sick”, “fever”, “headache”, “loss of smell or taste”, and “fatigue” than in individuals without these symptoms, whereas antispikes antibody titers differed for all assessed symptoms.

Table 3
Analysis of symptoms (asymptomatic vs symptomatic convalescents) and antinucleocapsid antibody levels.

Symptom	Asymptomatic COVID-19 convalescent donors			Symptomatic COVID-19 convalescent donors			p-value
	n	Mean level of antibodies	SD	n	Mean level of antibodies	SD	
Feel sick	20	24.70	29.26	173	37.38	35.03	0.048
Cough	65	32.61	32.99	128	37.82	35.43	0.25
Fever	88	23.05	28.26	105	46.97	35.81	< 0.0001
Sore throat	102	37.21	33.12	91	34.78	36.39	0.38
Headache	46	31.61	33.41	146	37.72	34.98	0.28
Loss of smell/taste	70	26.38	31.88	123	41.58	35.04	0.0003
Shortness of breath	132	34.60	35.2	60	39.88	33.32	0.11
Fatigue	32	21.92	27.59	160	39.06	35.30	0.0023

Symptomatic convalescent individuals reported mild, moderate or severe symptoms. n: number of donors. SD: standard deviation; p-values calculated with Mann-Whitney-U test.

T cell immunity and COVID-19 symptoms

We compared the severity of COVID-19 symptoms with the intensity of SARS-CoV-2-specific T cell responses for HLA class I- and HLA-DR-restricted T cell epitopes, assessed by IFN- γ ELISpot assay using SARS-CoV-2-specific epitope compositions in PBMC samples of SARS-CoV-2 convalescents (Nelde et al., 2021). Intensity of HLA class I and HLA-DR T cell responses did not differ for the severity of the symptom “feel sick” (Fig 2A). Convalescent donors with moderate “cough” had a significantly decreased intensity of T cell responses to HLA class I epitope compositions than convalescents experiencing mild or no symptoms (p-value 0.0049 and 0.031, respectively; Fig 2B). Of note, convalescents with mild “cough” displayed significantly increased intensity of HLA-DR T cell responses compared with asymptomatic convalescents or convalescents experiencing moderate “cough” (p-value 0.014 or 0.041, respectively; Fig 2B). Regarding the severity of “fever”, T cell responses to HLA class I epitope compositions were found to be significantly decreased in severe compared with mild cases (p-value 0.031; Fig 2C). This was not observed for T cell responses to HLA-DR epitope compositions (Fig 2C). In individuals experiencing “sore throat” and “headache”, the intensity of T cell response did not differ between symptom grades for HLA class I and HLA-DR epitope compositions (Fig 2D and E). When grouped according to the severity of “loss of smell or taste”, T cell responses to HLA class I epitope composition were significantly decreased in severe compared with mild cases (p-value 0.031; Fig 2F). In contrast, T cell responses to HLA-DR epitope composition were not different among the four categories of severity (Fig 2F). Intensity of HLA class I and HLA-DR T cell responses showed no significant difference between symptom severity categories for “shortness of breath” and “fatigue” (Fig 2G and H). Of note, when dividing the study population according to the time of sample collection (cut-off median: 42 days) into two groups, we did not observe higher HLA class I nor HLA-DR T cell response at the earlier time point (Supplementary Fig 2). In summary, no difference in T cell response intensity between different symptom severities was observed for the majority of COVID-19 symptoms. However, for “loss of smell or taste” and “cough”, awareness of more severe symptoms was associated with lower HLA class I-restricted T cell response intensities.

Classification of symptoms and immune response

When symptom severity was grouped in asymptomatic versus symptomatic (mild/moderate/severe), antinucleocapsid antibody titers differed significantly for “feel sick”, “fever”, “loss of smell or taste” and “fatigue” (p-value 0.048, <0.0001, 0.0003, and 0.0023, respectively; Table 3). When analyzing the humoral response in symptomatic convalescent donors (mild versus moderate/severe symptoms), no significant difference was observed for

most symptoms except for “feel sick”, showing increased antinucleocapsid antibody titers in moderate/severe cases (p-value 0.013; Table 4).

T cell responses did not differ significantly between asymptomatic and symptomatic (mild/moderate/severe) participants (Tables 5 and 6). Analyzing T cell responses in symptomatic convalescents (mild versus moderate/severe symptoms) revealed no significant difference for most symptoms (Fig 3), except for “cough” and “loss of smell or taste” where the intensity of HLA class I T cell responses was significantly decreased in moderate/severe cases (p-value 0.0095 and 0.025, respectively; Fig 3B and F). Similar results were observed for the symptom “cough” regarding T cell responses to HLA-DR epitope compositions but failed to reach statistical significance (Fig 3B).

Discussion

Patients with COVID-19 present with a variety of symptoms, ranging from asymptomatic infections or mild to severe courses of disease, potentially being also life-threatening and lethal (Li et al., 2020; Miller and Yan, 2021; Qin et al., 2020; Zhu et al., 2020). Patients with severe COVID-19 according to the WHO grading scale (Son et al., 2021) have been reported to develop a potent humoral immune response with high antibody titers against SARS-CoV-2 (Horton et al., 2021; Qin et al., 2020). In this study, to the best of our knowledge, we are the first to report the association between symptom severity of subjective symptoms in 193 individuals with asymptomatic SARS-CoV-2 infection or mild to moderate COVID-19 (according to WHO) (Son et al., 2021) and humoral and cellular immune responses to SARS-CoV-2 by assessing both serum antibody titers as well as SARS-CoV-2-specific T cell responses. “Feel sick” and “fatigue” were the most reported symptoms in our cohort, which had also been reported in other large cohort studies (Bakilan et al., 2021). In line with recent data, regarding elevated antispikes antibody levels in severe/life-threatening COVID-19 in hospitalized patients (Horton et al., 2021; Woodruff et al., 2020), we found serum antinucleocapsid antibody levels to increase with more severe COVID-19 for most symptoms and serum antispikes antibody levels to increase with more severe COVID-19 for all symptoms. In contrast, T cell immune responses decreased or showed no difference between the severity of COVID-19 cases (Toor et al., 2021). These results indicate that the extent of the humoral immune response also appears to be associated with the severity of subjective symptoms in mild COVID-19 cases. Assessment of humoral immune responses to SARS-CoV-2 could therefore allow for the identification of asymptomatic individuals or patients with mild COVID-19, who would profit from early vaccination after recovery. This is supported by further data on SARS-CoV-2 antibodies in convalescent individuals, showing a decline in antibody titers a few weeks to months after infection (Bilich et al., 2021a;

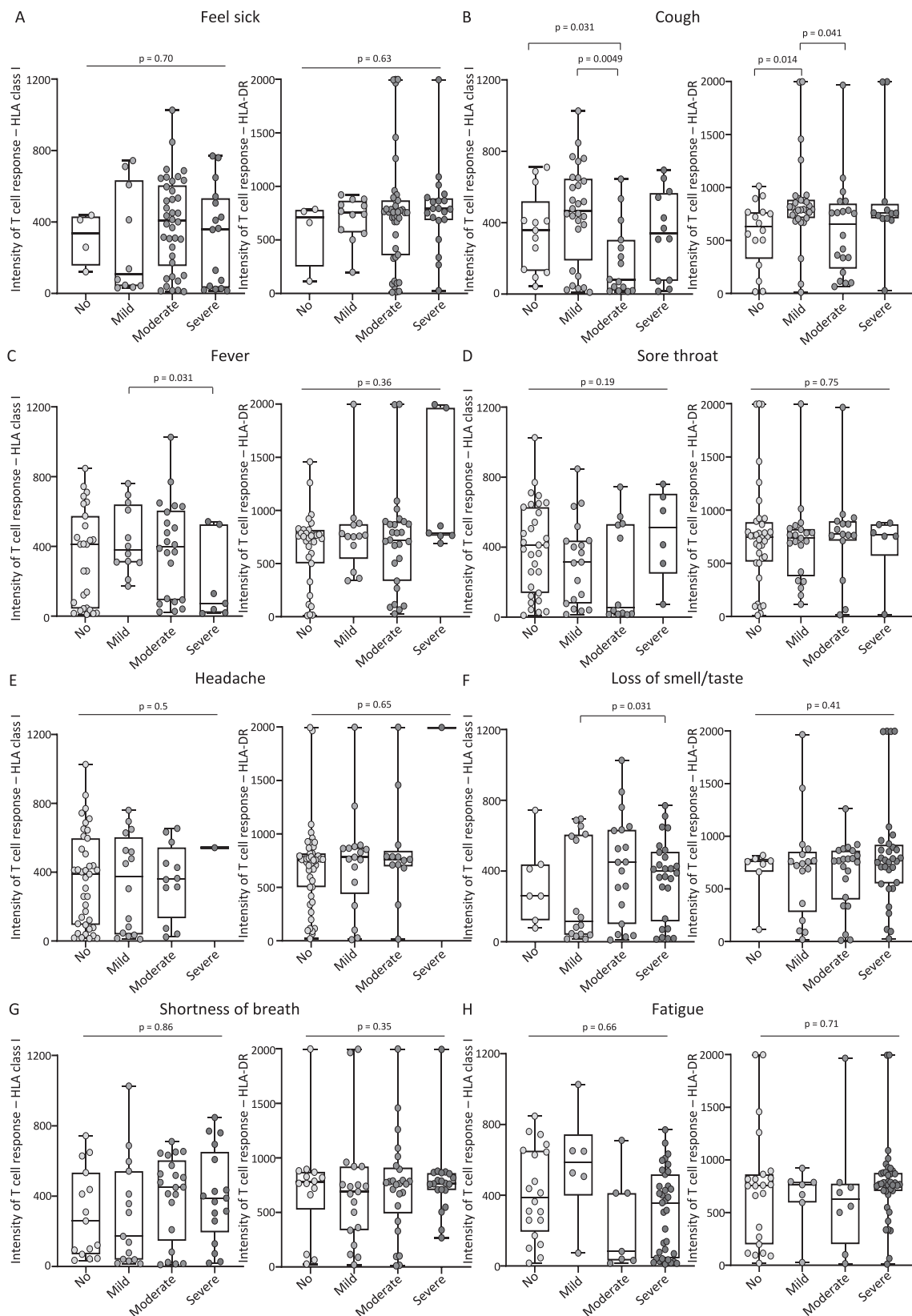


Fig. 2. Symptom severity and intensity of SARS-CoV-2-specific T cell response in COVID-19. Intensity of SARS-CoV-2-specific HLA class I (left) and HLA-DR (right) T cell response in COVID-19 convalescent donors (n = 68 for HLA class I, n = 78 for HLA-DR) with different symptom severities in the course of COVID-19. Symptoms during COVID-19 (ie, [A] feel sick, [B] cough, [C] fever, [D] sore throat, [E] headache, [F] loss of smell or taste, [G] shortness of breath, and [H] fatigue) were assessed by questionnaire and grouped into four grades according to self-reported severity (no, mild, moderate, severe symptoms). p: p-value. Brackets mark significant p-values between two categories (Kruskal-Wallis test with Dunn correction), continuous lines indicate p-values between all categories (Kruskal-Wallis test). Box plots with min/max whiskers.

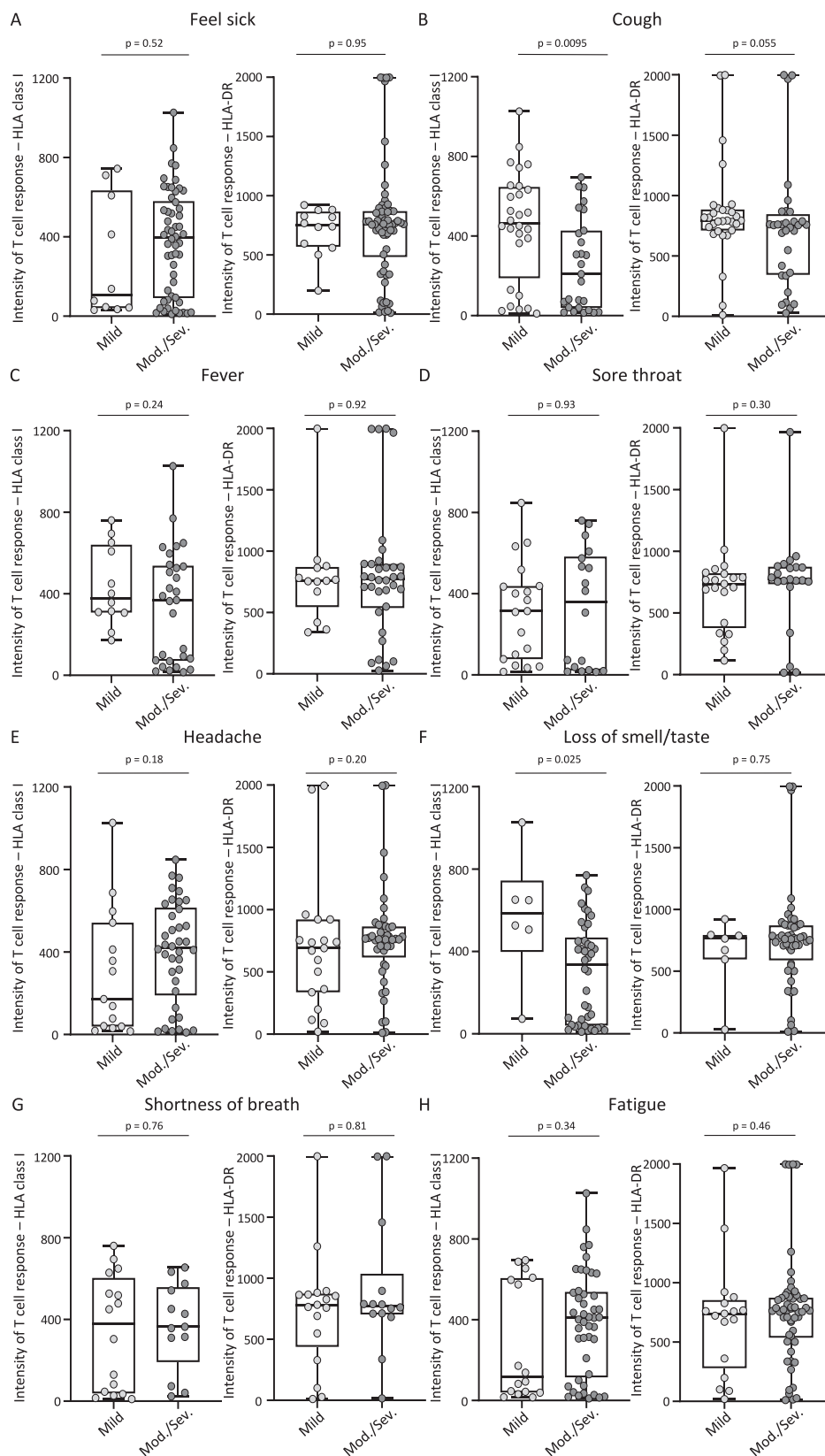


Fig. 3. Grouped symptom severity and intensity of SARS-CoV-2-specific T cell response in COVID-19. Intensity of SARS-CoV-2-specific HLA class I (left) and HLA-DR (right) T cell response in symptomatic COVID-19 convalescent donors with different symptom severities (mild versus moderate/severe) in the course of COVID-19. Symptoms during COVID-19, such as (A) feel sick (HLA class I: mild n = 10, moderate/severe n = 54; HLA-DR: mild n = 12, moderate/severe n = 62), (B) cough (HLA class I: mild n = 28, moderate/severe n = 27; HLA-DR: mild n = 30, moderate/severe n = 32), (C) fever (HLA class I: mild n = 12, moderate/severe n = 29; HLA-DR: mild n = 13, moderate/severe n = 34), (D) sore throat (HLA class I: mild n = 19, moderate/severe n = 18; HLA-DR: mild n = 21, moderate/severe n = 21), (E) headache (HLA class I: mild n = 15, moderate/severe n = 38; HLA-DR: mild n = 19, moderate/severe n = 45), (F) loss of smell/taste (HLA class I: mild n = 6, moderate/severe n = 42; HLA-DR: mild n = 7, moderate/severe n = 48), (G) shortness of breath (HLA class I: mild n = 16, moderate/severe n = 13; HLA-DR: mild n = 17, moderate/severe n = 14), and (H) fatigue (HLA class I: mild n = 16, moderate/severe n = 45; HLA-DR: mild n = 17, moderate/severe n = 54) were assessed by questionnaire. Mod./Sev.: Moderate/severe; p: p-value; continues lines indicate p-values between all categories (Mann-Whitney U-test). Box plots with min/max whiskers.

Table 4
Analysis of symptoms and antinucleocapsid antibody levels in symptomatic convalescents (mild versus moderate/severe).

Symptom	COVID-19 convalescent donors with mild symptoms			COVID-19 convalescent donors with moderate/severe symptoms			p-value
	n	Mean level of antibodies	SD	n	Mean level of antibodies	SD	
Feel sick	39	27.78	32.64	134	40.17	35.33	0.013
Cough	63	39.14	36.46	65	36.55	34.64	0.65
Fever	32	47.75	36.55	73	46.63	35.74	0.74
Sore throat	49	40.92	37.97	42	27.62	33.48	0.053
Headache	47	37.15	34.93	99	37.98	35.18	0.67
Loss of smell/taste	22	32.43	31.04	101	43.57	35.68	0.19
Shortness of breath	32	38.73	33.87	28	41.19	33.26	0.78
Fatigue	40	31.00	33.44	120	41.74	35.63	0.054

Symptomatic convalescent individuals reported mild, moderate or severe symptoms. n: number of donors. SD: standard deviation; p-values calculated with Mann-Whitney-U test.

Table 5
Analysis of symptoms (asymptomatic versus symptomatic convalescents) and HLA class I T cell response.

Symptom	Asymptomatic COVID-19 convalescent donors			Symptomatic COVID-19 convalescent donors			p-value
	n	Mean T cell intensity	SD	n	Mean T cell intensity	SD	
Feel sick	4	307.54	146.83	64	357.51	268.06	0.82
Cough	13	352.01	223.15	55	355.17	272.28	0.90
Fever	27	333.67	274.35	41	368.33	256.15	0.61
Sore throat	31	395.25	259.97	37	320.48	262.43	0.24
Headache	15	298.81	250.99	53	370.35	265.28	0.55
Loss of smell/taste	20	412.42	257.64	48	330.47	262.77	0.20
Shortness of breath	39	358.69	274.22	29	349.02	249.44	0.97
Fatigue	7	329.99	226.01	61	357.39	267.39	0.92

Symptomatic convalescent individuals reported mild, moderate or severe symptoms. n: number of donors. SD: standard deviation; p-values calculated with Mann-Whitney U test. The intensity of T cell responses is depicted as mean spot counts of duplicates in the ELISpot assay normalized to 5×10^5 cells minus the normalized mean spot count of the respective negative control.

Table 6
Analysis of symptoms (asymptomatic versus symptomatic convalescents) and HLA-DR T cell response.

Symptom	Asymptomatic COVID-19 convalescent donors			Symptomatic COVID-19 convalescent donors			p-value
	n	Mean T cell intensity	SD	n	Mean T cell intensity	SD	
Feel sick	4	582.00	316.70	74	745.76	450.41	0.45
Cough	16	567.47	314.74	62	781.20	464.21	0.07
Fever	31	648.12	347.04	47	796.22	493.19	0.24
Sore throat	36	757.99	501.11	42	719.67	394.92	0.67
Headache	14	728.43	482.18	64	739.31	439.84	0.68
Loss of smell/taste	23	720.64	556.49	55	744.35	394.16	0.63
Shortness of breath	47	688.97	392.3	31	810.72	511.72	0.30
Fatigue	7	663.14	246.99	71	744.67	459.87	0.66

Symptomatic convalescent individuals reported mild, moderate, or severe symptoms. n: number of donors. SD: standard deviation; p-values calculated with Mann-Whitney U test. The intensity of T cell responses is depicted as mean spot counts of duplicates in the ELISpot assay normalized to 5×10^5 cells minus the normalized mean spot count of the respective negative control.

Haveri et al., 2021; Van Elslande et al., 2021). Assuming a linear decline, patients with high antibody titers would remain seropositive longer than those with initially lower antibody titers. This suggests that patients with asymptomatic or mild COVID-19 are more likely to be earlier seronegative for SARS-CoV-2 antibodies after infection. Although antibody titers have been shown to rapidly decrease after infection (Marot et al., 2021), T cell responses appear to persist longer (Altmann and Boyton, 2020; Bilich et al., 2021a) and, in the case of SARS-CoV-1, were still detectable up to 17 years after infection (Anderson et al., 2020).

So far, the humoral immune response to SARS-CoV-2 is used to determine previous infection as well as antiviral immunity after vaccination (Polack et al., 2020). The underlying reason for elevated antibody titers in patients with severe COVID-19 is not yet fully understood and is considered to be the result of a more pronounced immune activation. A dysregulated immune response re-

sulting in antibody-dependent enhancement (ADE) has been described for patients infected with SARS-CoV-1 (Karthik et al., 2020; Negro, 2020) and might also contribute to disease severity in COVID-19.

So far, limited data have been provided on the association of T cell responses and COVID-19 symptoms (Kim et al., 2021). Herein, we used previously reported data on SARS-CoV-2-specific T cell responses against HLA class I- and HLA-DR-restricted SARS-CoV-2-derived epitope compositions (Nelde et al., 2021) for a respective correlative study. Interestingly, in contrast to humoral immune responses, intensities of T cell responses were found to be not associated with symptom severity or were even decreased in individuals with worse symptoms in most cases. This held true for both HLA class I- and HLA-DR-restricted T cell responses, indicating that a broad T cell response is induced even after mild disease symptoms. One might speculate that a more symptomatic disease

is triggered and enhanced by negative effects of antibody response (Karthik et al., 2020; Negro, 2020), whereas early viral clearance by T cells might protect against a worse disease course.

A major problem of COVID-19 is the potential persistence of long-term symptoms (Huang et al., 2021; Mendelson et al., 2020), which could be due to a dysregulated or overactive immune response. Whether intensity and characteristics of T cell or antibody responses are associated with occurrence of long-term symptoms is yet unclear but constitutes an important question.

Our study population represents the majority of all COVID-19 cases, including asymptomatic to moderate cases according to WHO classification (Jiang et al., 2020; Liu et al., 2020; Mulchandani et al., 2021; Sattler et al., 2020). The severity of disease symptoms, however, was assessed using a questionnaire, which had to be taken with caution in consideration of nonobjective individual self-assessment (Maddox and Douglass, 1973; Ward et al., 2002). In conclusion, we showed that in contrast to antibody responses, SARS-CoV-2-directed T cell responses are not elevated in severe COVID-19 cases but are equally strong for different disease severities, highlighting the strength of T cell-based immunity for viral clearance.

Author Contributions

H.R.S., J.S.W., A.N., and J.S.H. designed study. C.M.T., T.B., Y.M., and J.S.H. collected convalescent data. C.M.T. and J.S.H. analyzed data and performed statistical analyses. C.M.T., H.R.S., J.S.W., A.N., and J.S.H. drafted the manuscript. All authors revised and approved the manuscript.

Declaration of Competing Interest

A.N., T.B., and J.S.W. hold patents on peptides described in this manuscript, secured under the numbers 20_169_047.6 and 20_190_070.1. The other authors declare no competing interests.

Acknowledgments

We thank all the donors for supporting of our research. We thank all technicians for technical support and project coordination. This work was supported by the Bundesministerium für Bildung und Forschung (FKZ:01KI20130; J.S.W.), the Deutsche Forschungsgemeinschaft (German Research Foundation, grant WA 4608/1-2; J.S.W.), the Deutsche Forschungsgemeinschaft under Germany's Excellence Strategy (grant EXC2180-390900677; J.S.W., and H.R.S.), the German Cancer Consortium (H.R.S.), the Wilhelm Sander Stiftung (grant 2016.177.2; J.S.W.), the José Carreras Leukämie-Stiftung (grant DJCLS 05R/2017; J.S.W.), and the Fortüne Program of the University of Tübingen (Fortüne no. 2451-0-0; J.S.W.).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.04.019.

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