Asymptomatic SARS-CoV-2 infection in recipients of hematopoietic stem cells in the Omicron period

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ABSTRACT

Aim: We aimed to determine the prevalence of SARS-CoV-2 infection, including both symptomatic and asymptomatic courses, and to identify predictors of asymptomatic or symptomatic SARS-CoV-2 infection in patients within seven months after allo-HSCT (allogenic hematopoietic stem cell transplantation) in the Omicron period.

Methods: Prevalence of the past SARS-CoV-2 infection was determined in patients within seven months after allo-HSCT in the Omicron period using the cellular and humoral immune response against the SARS-CoV-2 nucleoprotein (NCP).

Results: Positive markers of past infection were identified in 45.2% of patients (n = 42). The infection was asymptomatic in 68.4% of anti-NCP positive patients. The search for risk factors for symptomatic SARS-CoV-2 infection in allo-HSCT recipients revealed that a low level of B cell reconstitution was the only significantly associated risk factor.

Conclusion: A high proportion of allo-HSCT recipients who were asymptomatically infected within up to seven months after transplantation from 2022 to 2023 despite being immunosuppressed and unvaccinated indicates an attenuation of the circulating virus and may signal less risk for transplanted patients from SARS-CoV-2 infection in the Omicron period. Vaccination of these patients against SARS-CoV-2 was shown to be associated with a low but significant risk of exacerbation of cured chronic GVHD (graft versus host disease) and the risk of de novo GVHD. The low level of B-cell reconstitution was the only significant risk factor for symptomatic SARS-CoV-2 infection in HSCT recipients.

KEY WORDS

asymptomatic SARS-CoV-2 infection – allogenic hematopoietic stem cell transplantation – Omicron variant – T cell response – nucleoprotein

SOUHRN

Šťastná-Marková M., Roubalová K., Hainz P., Kryštofová J., Labská K., Vosáhlová T., Němečková Š.: Asymptomatická infekce SARS-CoV-2 u příjemců hematopoetických kmenových buněk v období cirkulace varianty omikron

Cíl: Zaměřili jsme se na stanovení prevalence infekce SARS-CoV-2 se symptomatickým nebo asymptomatickým průběhem a na identifikaci prediktorů symptomatické nebo asymptomatické infekce SARS-CoV-2 u pacientů během sedmi měsíců následujících po transplantaci alogenních hematopoetických kmenových buněk (alo-HSCT) v období cirkulace varianty omikron.

Metody: Prevalence proběhlé infekce SARS-CoV-2 byla detekována u pacientů během sedmi měsíců po allo-HSCT v omikronovém období pomocí buněčné a humorální imunitní odpovědi proti nukleoproteinu SARS-CoV-2 (NCP).

Výsledky: Pozitivní markery prodělané infekce byly identifikovány u 45,2 % pacientů (n = 42). Infekce byla asymptomatická u 68,4 % pacientů s anti-NCP pozitivitou. Hledání rizikových faktorů pro symptomatickou infekci SARS-CoV-2 u příjemců alo-HSCT odhalilo, že nízká úroveň rekonstituce B buněk byla jediným signifikantně souvisejícím rizikovým faktorem.

Závěr: Vysoký podíl příjemců alo-HSCT, kteří byli asymptomaticky infikováni do sedmi měsíců po transplantaci v letech 2022–2023, přestože byli imunokompromitovaní a neočkovaní, ukazuje na oslabení cirkulujícího viru a může signalizovat pro pacienty po transplantaci menší riziko onemocnění SARS-CoV-2 v omikronovém období. Ukázalo se, že očkování těchto pacientů proti SARS-CoV-2 je spojeno s nízkým, ale významným rizikem exacerbace vyléčené chronické reakce štěpu proti hostiteli (GVHD – Graft Versus Host Disease) a s rizikem de novo GVHD. Nízká úroveň rekonstituce B-buněk byla jediným významným rizikovým faktorem pro symptomatickou infekci SARS-CoV-2 u příjemců alo-HSCT.

KLÍČOVÁ SLOVA

asymptomatická infekce SARS-CoV-2 – alogenní transplantace hematopoetických kmenových buněk – varianta omikron – odpověď T buněk – nukleoprotein

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INTRODUCTION

Studies on COVID-19 infection initiated by the European Society for Blood and Marrow Transplantation (EBMT) have demonstrated high mortality in immunocompromised allogeneic hematopoietic stem cell transplant recipients (allo-HSCT) during the initial period of the pandemic [1, 2]. Over time the outcomes of the COVID-19 patients have improved in the association both with newly emerged Omicron variants and with advancements in therapeutic management for hospitalized patients [3, 4]. The twelve-week overall survival of infected patients increased between 2020 and 2022 from 77.3% to 95.3% [5].

In meta-analysis the median of the earliest detection of IgG response in patients with SARS-CoV-2 infection from general population is shown to be at day 12, peak of seroprevalence at day 25, start of titer decline at day 60, and persistence of IgG has a median of 120 days [6]. The IgG positivity against the nucleocapsid protein (NCP) can be used for determining previous SARS-CoV-2 infection. However, in some subjects, precise estimation of seropositivity is complicated by the waning antibody levels during recovery and requires the using a highly sensitive immunoassay [7] such as dual antibody assay [8]. The NCP-specific T cell response in recovered patients appears to be more long-lived and stable compared to the circulating antibody levels [9, 10].

The present study aimed to determine the true prevalence of SARS-CoV-2 infection including both symptomatic and asymptomatic course and to identify predictors of asymptomatic or symptomatic SARS-CoV-2 infection in patients within seven months after allogeneic transplantation of hematopoietic stem cells.

MATERIAL AND METHODS

Study participants

Sixty-three adult patients who underwent allo-HSCT at the Institute of Hematology and Blood Transfusion, Prague, between March 2020 and April 2023, were recruited for this study. All transplanted patients obtained peripheral blood stem cells as a graft. They were categorized into three cohorts. In cohort I there were patients with haematological malignancies who were transplanted between April 2020 and July 2021 (n = 21), and with the exception of three patients, were not vaccinated against SARS-CoV-2 during the follow-up period. Cohort II was intended to be a control group of patients transplanted between April 2021 and April 2022 for clinical morbidity of Covid-19 evaluation (n = 71). The patients in the cohort III were transplanted between February 2022 and April 2023. They were not vaccinated during the follow-up period, some of them (64%) received one dose of EvusheldTM(100 mg) (mix of monoclonal antibodies against SARS-CoV-2) on day + 30 after HSCT (n = 42). Completed vaccination before transplantation was reported in 18 patients of cohorts I and III. The follow-up period for Covid-19 was seven months when patients donated a blood sample for the analysis of SARS-CoV-2-specific T cell and antibody responses. The plasma and peripheral blood mononuclear cells (PBMCs) were separated and frozen stored for further analyses. Frozen PBMCs isolated from 13 blood donors between 2011 and 2018 were used as SARS-CoV-2 negative controls. All the participants provided written informed consent. This study was approved by the institutional ethical board. The patients' residences were dispersed across the country.

Measurement of SARS-CoV-2-specific antibodies

The presence of antibodies against SARS-CoV-2 spike (S) and nucleoprotein (NCP) antigens was detected in plasma samples by Anti-SARS-CoV2 ELISA (IgG) assay or Anti-SARS-CoV-2 NCP ELISA (IgG) assay (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) as described previously [11].

Detection of a SARS-CoV-2-specific T cell response

T-cell responses against S1, S2, and NCP SARS-CoV2 antigens were measured after *ex vivo* stimulation with the pools of 15-amino-acid-long overlapping peptides (PepMix™) using an in-house ELISPOT-IFNγ assay described previously [11]. The mean values of the spot-forming units (SFUs) in peripheral blood mononuclear cells (PBMCs) cultivated without the peptides were subtracted from those in stimulated cultures. Frozen PBMCs collected from blood donors between 2011 and 2018 were used to establish the cut-off for a positive cellular response. The ELISPOT method was validated using a Quan-T-cell ELISA (Euroimmun, Lübeck, Germany). Results of both methods (Table 1) correlated significantly mutualy and with the incidence of Covid in transplanted recipients.

Table 1. Correlation analysis of ELISPOT-IFNγ and QuanT-cell ELISA results and prevalence of SARS-CoV-2 infection

	COVID-19 after Tx			NCP-ELISPOT		
	Spearman r	95% CI	p value	Spearman r	95% CI	p value
NCP-ELISPOT	0.7086	0.3627–0.8829	**0.0006**			
Quan-T-cell ELISA	0.5730	0.1613–0.8149	**0.0082**	0.6457	0.2576–0.8545	**0.0028**

Flow cytometric evaluation of immune system reconstitution after HSCT

For the quantitative and qualitative analysis of the major immune cell populations in HSCT recipients at the 7th month post HSCT a 9-colour panel of antibodies: CD8-alexa700, CD4- pacific blue (Exbio, Prague); CD3-APC-Cy7, CD56-BV510 (BioLegend); CD45-BV605, CD16-FITC (Sony); CD19-BV786 (BD Biosciences); LIVE/DEAD™ for UV-blue (Invitrogen) was employed. Flow cytometry was performed using a BD LSR Fortessa 5 L flow cytometer (BD Biosciences) equipped with five lasers. The obtained data were analyzed using the FlowJo 10.5 software (TreeStar, Ashland, OR, USA). We evaluated the frequency of B cells, monocytes, αβ T cells, NK cells, and NKT cells.

Collection of epidemiological data

A cumulative overview of newly confirmed cases of COVID-19 infection reported daily to the Information System of Infectious Diseases (ISIN) by the Regional Public Health Authority and laboratories was downloaded from an official Czech website maintained by the Ministry of Health (COVID-19 in the Czech Republic: Open datasets and datasets for download are available at https://onemocneni-aktualne.mzcr.cz/api/v2/covid-19) [12]. The file containing the information about the rate of COVID-19 sequencing along with the absolute numbers and percentage distributions of the variants of concern (VOC) by week and country was downloaded from the ECDC website (www.ecdc.europa.eu/en/publications-data/data-virus-variants-covid-19-eueea).

Statistical analysis

All statistical tests were conducted at a significance level of > 0.05. All calculations and data plots were performed using the GraphPad Prism software, version 10.0.2 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

SARS-CoV-2 specific immune response in HSCT recipients

The immune response against SARS-CoV-2 antigens in HSCT recipients from cohorts I+III was measured at a median of 6.7 months (range 6.1–15.1) after HSCT (Figure 1). Anti-NCP T cell response was related (Table 2) to SARS-CoV-2 infection within seven months after HSCT, anti-S1 and S2 T cell responses, and levels of anti-NCP IgG**.** No association was found between anti-NCP T cell response and anti-S IgG or the transplantation conditions (diagnosis, recipient's age, gender, conditioning regimen, extent of HL-A matching between donor and recipient, or the incidence and treatment of acute or chronic graft versus host disease (GVHD), or recipient's pre-transplant COVID-19 infection and SARS-CoV-2 vaccination status. No relationship between the recipient's anti-NCP immune response and donor' previous COVID-19 disease or vaccination status was found.

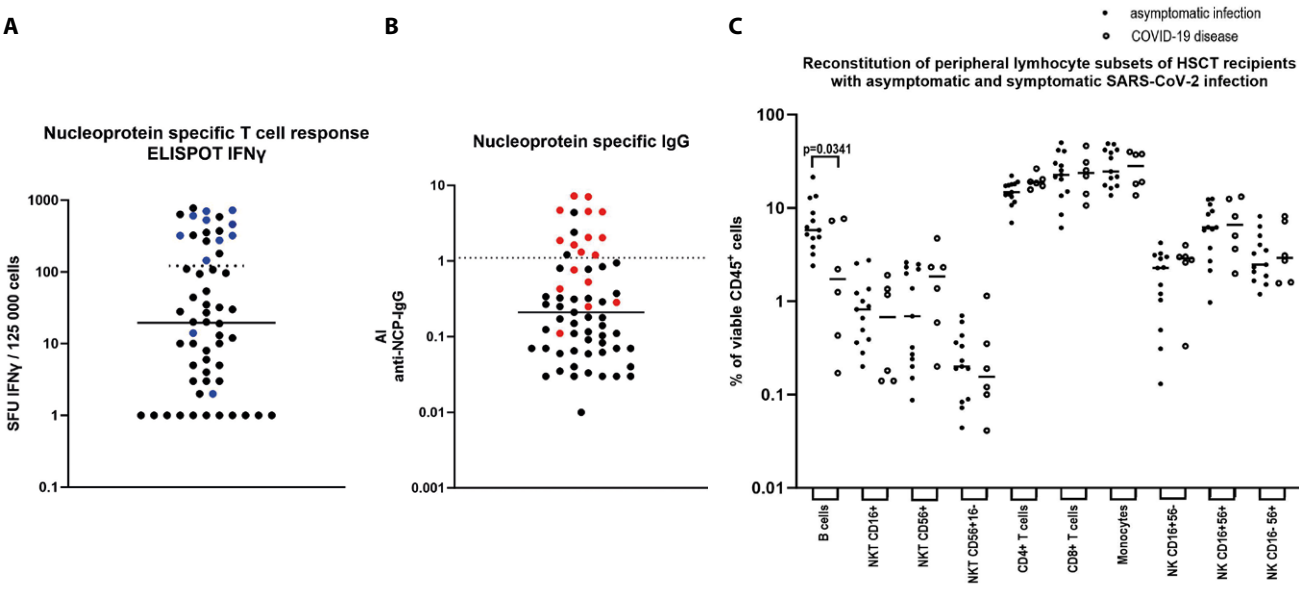

Figure 1. Immune system of SARS-CoV2- infected HSCT recipients
Nucleoprotein specific immune response **(A, B):** T cell response was detected by ELISPOT-IFNγ in patients after follow-up period. Cut-off value shown as dotted line was determined as mean + 2 s.d. of the response in healthy donors. The SFU values that were simultaneously positive for anti- NCP IgG antibodies are shown in blue symbols **(A):** NCP- specific IgG. Dotted line stands for ELISA Antibody Index (AI) cut-off value. Anti-NCP IgG values that were positive in ELISPOT-IFNγ assay are shown in red symbols **(B):** Reconstitution of peripheral lymphocyte subsets **(C):** Lymphocyte subsets in the patients from cohort III with asymptomatic (n = 36) or symptomatic (n = 6) SARS-CoV-2 infection were quantified by flow cytometry using the NKBMT panel at month 7 after transplantation. Statistical significance of the differences was tested by the Kolmogorov-Smirnov test.

Table 2. Univariable simple linear regression models for NCP-specific T cell response and symptomatic COVID-19 disease

Reference category	NCP IFNg (SFU/125 000)			Symptomatic COVID-19 disease (n=134)[d]		
Variable	**Estimate (slope)**	**95% CI**	**P value**	**Estimate (slope)**	**95% CI**	**P value**
Age[a]	0.001847	-0.01233–0.01602	0.7952	2.881	-1.876–7.637	0.2331
Female Gender[a]	-0.0002082	-0.0008044–0.0003880	0.4873	-0.1823	-0.3834–0.01887	0.0753
Diagnosis (myeloid or lymphoid)[a]	-0.0001491	-0.0006008–0.0003025	0.5111	-0.03333	-0.2348–0.1681	0.7439
Conditioning regimen[a]	-0.0000735	-0.0005388–0.0003918	0.753	-0.08143	-0.2510–0.08819	0.344
HLA match (MUD or MRD)[a]	0.0003999	-0.0002977–0.001098	0.2558	-0.08957	-0.5087–0.3295	0.6731
anti-NCP IgG[a]	0.003078	0.001564–0.004592	**0.0001**			
COVID-19 after HSCT[a]	0.0009328	0.0006678–0.001198	**< 0.0001**			
S1 IFNg (SFU/125 000)[b]	0.2884	0.04666–0.5302	**0.0207**			
S2 IFNg (SFU/125 000)[b]	0.9573	0.7112–1.203	**< 0.0001**			
anti-S-IgG[b]	0.003976	-0.0003893–0.008341	0.073			
Vaccination before HSCT (number of doses)[b]	0.000575	-0.001251–0.002401	0.514			
COVID-19 before HSCT[b]	-0.0002385	-0.0009317–0.0004546	0.490			
Vaccination of HSC donor [c]	0.0002901	-0.001612–0.002192	0.756			
COVID-19 of HSC donor[c]	-0.0001286	-0.001039–0.0007820	0.7713			
aGVHD grade	0.0005205[a]	-0.001218–0.0001770	0.1407	0.01034	-0.2838–0.3045	0.9447
cGVHD grade	0.00005562[a]	-0.0006459–0.0007572	0.8745	0.1701	-0.04882–0.3889	0.1267

[a]Evaluation was performed using samples of patients in cohort I and III (n = 63).
[b]Evaluation was performed using samples of patients from cohort III (n = 42).
[c]Evaluation was performed using available information from donors for patients in cohort III (n = 23).
[d]Evaluation was performed using available clinical data of patients in cohorts I, II and III (n = 134).

Prevalence of IgG antibodies against NCP at seven months post HSCT (22%) was lower than the prevalence of NCP-specific T cell responses (28%) – Figure 1AB. The positivity detected by T cells was confirmed by anti-NCP-IgG positivity in 41.2% of the cases only (see Figure 1A). Whereas anti-NCP-IgG positivity was confirmed in 78% of the cases by NCP-specific ELISPOT-IFNγ (Figure 1B). Our results corroborate the previous findings that the waning of NCP specific IgG is faster than that of the cellular response [13]. For further analysis of SARS-CoV-2 infection in HSCT recipients, the NCP-specific immune response (IgG and T cell) was used as a marker of recent virus infection.

Prevalence of SARS-CoV-2 infection during seven months post HSCT

Symptomatic infection was defined based on the respiratory symptoms going along with the detection of laboratory markers of SARS-CoV-2 infection (the virus antigen or RNA positivity in oropharyngeal or nasal swab). Asymptomatic infection was defined by NCP-specific T-cell or antibody positivity in the absence of clinical respiratory symptoms during the observational period. The highest prevalence of COVID-19 in HSCT recipients was found in cohort II (23.9%) and cohort III (14.3%) (Table 3). The difference between the cohorts was not significant (Fisher's exact test; Odds ratio 1.889; 95% CI 0.7203–5.232; p = 0.2392). The number of COVID-19 cases in cohort I was too low for analysis.

Using immune response analysis, we found that the proportion of asymptomatic SARS-CoV-2 infection in cohort III was 68.4%. No association was observed between Evusheld and the prevalence of asymptomatic infection ($\chi 2$ test; OR 0.9091; 95% CI = 0.08923–15.57; p = 0.9432).

Table 3. COVID-19 disease and SARS-CoV-2 infection in HSCT recipients

Period	Circulating SARS-CoV-2 variant	N = 140	COVID-19 (%)	Median of interval of COVID-19 positivity after HSCT	Infected patients anti NCP positive (%)	Asymptomatic among infected (%)	Comparison of asympt. Infection rate in cohorts I and III p value
Cohort I. 29.4.20 - 28.7.21	**Other VOC, Alpha, Delta**	21	1 (9.5%)	208 (202–214)	3 (14.3%)	3 (75.0%)	
Cohort II. 9.4.21 - 29.4.22	**Alpha, Delta, Omicron**	71	17 (23.9%)	167.5 (17–210)	n.d.	n.d.	n.d.
Cohort III. 27.2.22 - 21.4.23	**Omicron**	42	6 (14.3%)	41.5 (5–202)	19 (45.2%)	13 (68.4%)	n.s.

Further analysis revealed that reconstitution of the immune system affects the outcome of the infection. The proportions of the main lymphocyte subpopulations in PBMCs were determined using flow cytometry in cohort III. Compared to the asymptomatic infection, the symptomatic infection was associated with a lower frequency of B cells (see Figure 1C) (p = 0.0341, Kolgomorov-Smirnov D 0.6667). The differences in other lymphocyte subpopulations were not statistically significant.

The impact of the SARS-CoV-2 epidemiological situation in the Czech Republic on the prevalence of Covid-19 post HSCT

The circulation of SARS-CoV-2 variants changed during the study. Fluctuations in the seasonal incidence of new cases are shown in Figure 2A, and the local prevalence of the sequenced variants of concern is presented in Figure 2B. While cohort I patients may have been exposed to the Alpha variant or ancestral variants, patients in cohort II may have come into contact with the Alpha, Delta, and Omicron variants. The cohort III patients were enrolled during the wave of Omicron variants. Incidences of Covid-19 in cohorts I, II, and III (9.5%, 23.9%, and 14.3%, respectively, see Table 3) seem to reflect the ascending and descending course of the epidemic at particular intervals. Patients in cohorts I and II were more likely to become infected in later months after transplantation than those in cohort III. The median intervals from HSCT to SARS-CoV-2 diagnosis were 208, 167.5, and 41.5 days in cohorts I, II, and III, respectively.

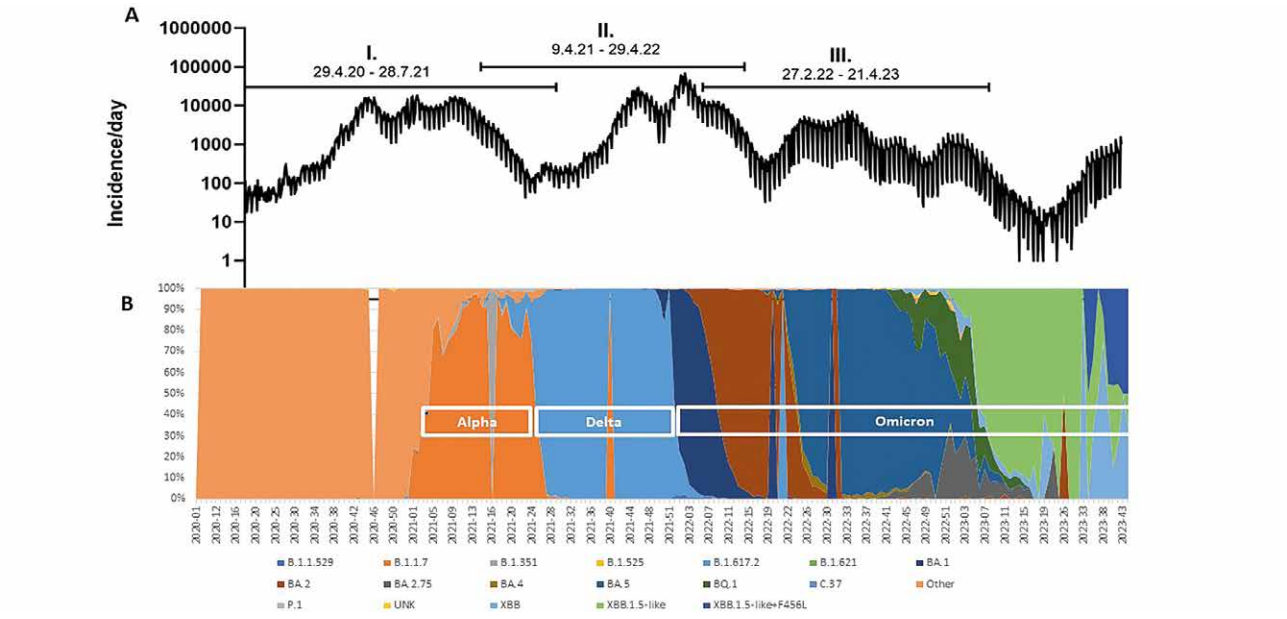

Figure 2. COVID-19 in the Czech Republic and study cohorts I–III
(A) Daily reported cases of SARS-CoV-2 infection between 29. 4. 2020 and 31. 10.2 023. Horizontal lines depict follow-up periods of study cohorts I-III.
(B) Time distribution of sequenced cases of variants circulating in the Czech Republic during the pandemic [year-week].

DISCUSSION

The quantification of the both arms of adaptive immunity to identify patients who were infected with SARS-CoV-2 within seven months after HSCT was used in this study. The assay to detect SARS-CoV-2-specific cellular immunity was based on the observation that the virus-specific T cells can persist for months after infection [13]. Although some assays (QuanT cell ELISA) available for the diagnosis of COVID-19 aim at the detection of T cells specific for the SARS-CoV-2 spike antigen, we decided to measure the T cell response against nucleoprotein. This allowed to detect past infection in vaccinated patients in cohort I. To improve the sensitivity of the determination of the immune status, anti-NCP antibodies were also assessed. Detection of NCP-IgG was not affected by anti-S-Mab from Evusheld in patients in cohort III.

Initial studies on COVID-19 infection in allo-HSCT recipients reported high case fatality rates ranging from 17% to 32% [1, 2, 14, 15]. At the beginning of the SARS-CoV-2 pandemic, the majority of patients had symptoms while only about 10% were asymptomatic [16]. The poor prognosis of allo-HSCT recipients infected in the first wave improved gradually with the availability of more effective medical care, antivirals, and vaccines along with the emergence of new variant viruses with less severe symptomatology. The SARS-CoV-2 Omicron variant causes milder disease than the earlier variants. As the epidemic progressed, it became clear that the severity of the disease was determined by the virulence of the viral variants as well as the demographic and clinical characteristics of the patients. It is evident that the rate of severe infections is inversely proportional to that of asymptomatic infections. In the initial phase of the epidemic, the rates of asymptomatic infections were 35.1% in general population including elderly asymp. inf. (19.7%) and children asymp. inf. (46.7%) [17]. Vaccination against SARS-CoV-2 is an important contributor to less severe symptoms and a higher proportion of asymptomatic infections. As many as 67.2% (57.3–78.8%) of those who completed a full vaccine series plus one or two booster doses had asymptomatic infection during the Omicron period [18].

Asymptomatic SARS-Cov-2 infection following HSCT was reported in 8.9% of adult recipients in early phase of epidemic [2]. In pediatric HSCT recipients, the proportion of asymptomatic infection was 42%, which was not different from the children general population [17].

Data on asymptomatic SARS-CoV-2 infection in HSCT recipients during the Omicron period have not yet been published. The unusually high representation of asymptomatic SARS-CoV-2 infections of around 70% in our study may be related on the one hand to the change in the virulence of the circulating virus compared to the earlier phase of the epidemic on the other hand to the high sensitivity of the detection method. Using specific immunity testing, more patients infected during the study period could be identified, when compared with the methods for direct demonstration of the virus. At the time of transplantation, existing T cells are eliminated, but in the post-transplantation period, the donor NCP-specific T cells stimulated by viral antigens produced during infection expand. Thus, in this study, only infections occurring during the observation period were detected and the detection efficiency was very high. Unlike this method, the RNA or antigen testing employed by most studies summarized in meta-analyses [17, 19] cannot completely cover the entire observation period, which means that not all asymptomatic infections are detected because the positivity window in the PCR assay or antigen test is short.

It could be argued that the high proportion of recipients responding to the NCP antigen in this study may have resulted from the transfer of SARS-CoV-2-specific antiviral immune cells from HSCT donors who had a history of Covid-19, as described recently [20, 21]. Longitudinal observations [20] have shown that transferred virus-specific T cells expanded only in those recipients who were infected and survived post-transplant COVID-19 infection. However, this expansion has not been observed in patients who were not infected after HSCT. Using the linear regression model, we demonstrated the absence of an association between the level of NCP-specific T lymphocyte response in recipients and previous COVID-19 in graft donors (see Table 1)**,** which may be explained by the fact that the transferred specific T cells, both donor-primed and naïve, cannot expand without antigen stimulation.

CONCLUSIONS

A high proportion of hematopoietic stem cell recipients who were asymptomatically infected within up to seven months after transplantation from 2022 to 2023 despite being immunosuppressed and unvaccinated indicates an attenuation of the circulating virus. This result may signal less risk for transplanted patients from SARS-CoV-2 infection in the Omicron period even if they were not vaccinated. Vaccination of these patients against SARS-CoV-2 was shown to be associated with a low but significant risk of exacerbation of cured chronic GVHD and the risk of de novo GVHD [22, 23]. Asymptomatic patients are known to be a reservoir of infectious viruses in the environment, which could also be true for patients after HSCT.

The search for risk factors for symptomatic SARS-CoV-2 infection in HSCT recipients revealed that a low level of B-cell reconstitution was the only significantly associated risk factor. This result is in line with the studies showing that patients with iatrogenic B cell depletion or various inborn humoral defects have an increased risk of severe symptomatic COVID-19 and death [24, 25].

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