

# MICB Genetic Variants and Its Protein Soluble Level Are Associated with the Risk of Chronic GvHD and CMV Infection after Allogeneic HSCT

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## Abstract

The aim of the present study was to determine the associations between the *MICB* genetic variability and the expression and the risk of development of post-transplant complications after allogeneic hematopoietic stem cell transplantation (HSCT). HSCT recipients and their donors were genotyped for two *MICB* polymorphisms (rs1065075, rs3828903). Moreover, the expression of a soluble form of MICB was determined in the recipients' serum samples after transplantation using the Luminex assay. Our results revealed a favorable role of the *MICB* rs1065075 G allele. Recipients with donors carrying this genetic variant were less prone to developing chronic graft-versus-host disease (cGvHD) when compared to recipients without any symptoms of this disease (41.41% vs. 65.38%,  $p = 0.046$ ). Moreover, the *MICB* rs1065075 G allele was associated with a lower incidence of cytomegalovirus (CMV) reactivation, both as a donor ( $p = 0.015$ ) and as a recipient allele ( $p = 0.039$ ). The *MICB* rs1065075 G variant was also found to be associated with decreased serum soluble MICB (sMICB) levels, whereas serum sMICB levels were significantly higher in recipients diagnosed with CMV infection ( $p = 0.0386$ ) and cGvHD ( $p = 0.0008$ ) compared to recipients without those complications. A protective role of the G allele was also observed for the rs3828903 polymorphism, as it was more frequently detected among donors of recipients without cGvHD (89.90% vs. 69.23%;  $p = 0.013$ ). *MICB* genetic variants, as well as serum levels of sMICB, may serve as prognostic factors for the risk of developing cGvHD and CMV infection after allogeneic HSCT.

## Keywords

MICB · sMICB · NK cells · HSCT · Post-transplant complications

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## 1. Introduction

According to the European Society for Blood and Marrow Transplantation registry, a total of 47,412 hematopoietic stem cell transplantation (HSCT) procedures were performed in 2021. Allogeneic HSCT constituted 42% of them (19,806) (Passweg et al. 2023). Although HSCT has a high success rate, it may still lead to some post-transplant complications, including graft-versus-host-disease (GvHD) and cytomegalovirus (CMV) infection (Eberhardt et al. 2023; González-Cruz et al. 2023; Holtick et al. 2024; Sulaiman et al. 2024). Natural killer (NK) cells are the first cell subset to reconstitute after HSCT. They belong to the innate lymphoid cells family

and are one of the most important parts of the human innate immunity (Peterson and Barry 2021; Blunt and Khakoo 2023; Prokopeva et al. 2023). The NK cells are regulated by their activating and inhibitory receptors, i.e., killer Ig-like receptors, natural cytotoxicity receptors, or C-type lectin-like proteins, with the activating NKG2D being one of the most studied receptors (Patil and Schwarzer 2009; Bogunia-Kubik and Łacina 2021; Siemaszko et al. 2023).

In humans, ligands for the NKG2D receptor are the MHC class I chain-related A and B molecules (MICA and MICB) and the UL-16 binding proteins (Siemaszko et al. 2021). These molecules serve as natural biomarkers, as they are typically not expressed on normal cells but are often overexpressed on stress-induced or transformed cells (Goulding et al. 2023; Sánchez-Cerrillo et al. 2023). Both MICA and MICB can be expressed in serum in their soluble forms (Nagai et al. 2022). It was reported that the soluble MICA (sMICA) levels were decreased in healthy individuals compared with its elevated levels in patients suffering from various diseases and malignancies, e.g., ankylosing spondylitis, non-small cell carcinoma, pancreatic cancer, breast cancer as well as SARS-CoV-2 infection (Wang et al. 2015; Onyeaghala et al. 2017; Wang et al. 2021; Farzad et al. 2022; Kshersagar et al. 2022). The impact of the serum soluble MICB (sMICB) molecule has

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been, however, less studied. A study conducted on cancer patients (individuals diagnosed with various malignancies, including prostate cancer, gastrointestinal cancers, breast cancer, or lung cancer) revealed that they had increased sMICB levels in the serum when compared with healthy individuals. A significant association was also observed between the sMICB level and metastasis (Holdenrieder et al. 2006b). As MICA is the most polymorphic of all nonclassical MHC and MHC-like molecules, another frequently studied factor is its genetic variability. One of the most-frequently studied *MICA* single nucleotide polymorphisms (SNPs) is the methionine (Met)-to-valine (Val) amino acid exchange at position 129. Contrary to the Val isoform, the Met variant is associated with higher NKG2D receptor signaling, resulting in not only more efficient NK cell cytotoxicity but also with faster NKG2D downregulation on NK and T cells (Isernhagen et al. 2015). The impact of MICA Met129Val polymorphism was described in many studies, indicating the protective effects of the Met allele and describing the Val variant (and especially the Val/Val genotype) as a risk factor (Ouni et al. 2017; Zingoni et al. 2018; Ouni et al. 2020). The other significant factor is *MICA* Met129Val compatibility between the recipient and the donor. It was reported that in unrelated transplantations, a MICA Met129Val mismatch is a risk factor for acute graft-versus-host disease (aGvHD) (Parmar et al. 2009; Fuerst et al. 2016). MICB polymorphisms are less studied. It was reported that in acute myeloid leukemia patients receiving HSCT, the MICB-58 (Lys58Glu) polymorphism had a negative effect on relapse-free survival (Machuldova et al. 2021). The *MICB* rs1051788 polymorphism was associated with reduced risk of primary graft dysfunction after lung transplantation (Aguilar et al. 2024). The impact of MICB polymorphisms was also investigated in different diseases, e.g., leukemia, dengue fever, rheumatoid arthritis, or systemic lupus erythematosus (Yu et al. 2017; Baek et al. 2018; Wang et al. 2019; Faridah et al. 2023).

## 2. Materials and Methods

### 2.1. Study population

For this study, 232 allogeneic HSCT recipients from five Polish transplantation centers and their 124 donors were enrolled. Recipients were 18- to 73-years-old with the median age of 50. There were 135 males and 97 females. Patients approved for HSCT were diagnosed with various hematological disorders, including blood cancers. The most common type of donor was matched sibling. Myeloablative conditioning was applied to 53.88% of all the recipients. After transplantation, the most common complications were aGvHD and CMV infection (39.22% and 38.79%, respectively). Detailed characteristics of patients can be seen in Table 1. The study complied with the Declaration of Helsinki and was approved

**Table 1.** Patients' characteristics

N = 232	
Age (years, median, range)	50, 18–73
Sex (M/F)	135 (58.19%)/97 (41.81%)
Type of donor	
MSD	107 (46.12%)
MUD	54 (23.28%)
Haploidentical	53 (22.84%)
MMSD	17 (7.33%)
Diagnosis	
AML	92 (39.66%)
ALL	29 (14.50%)
MDS	25 (12.50%)
NHL	18 (9%)
MPN	17 (8.50%)
HL	10 (5%)
PCM	8 (4%)
Other	33 (16.50%)
Conditioning	
RIC/MAC/NMA	104 (44.83%)/125 (53.88%)/3 (1.29%)
Post-transplant complications	
aGvHD (I–IV)	91 (39.22%)
aGvHD (II–IV)	48 (20.69%)
cGvHD	46 (19.83%)
cGvHD de novo/progression of aGvHD to cGvHD/after aGvHD remission	17 (36.96%)/8 (17.39%)/20 (43.48%)
CMV	90 (38.79%)
Relapse	31 (13.36%)
Death	30 (12.93%)
No complications <sup>a</sup>	83 (35.78%)

<sup>a</sup>Recipients without GvHD and CMV infection.

aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; CMV, cytomegalovirus; GvHD, graft-versus-host disease; MMSD, mismatched sibling donor; MPN, myeloproliferative neoplasm; MSD, matched sibling donor; MUD, matched unrelated donor; PCM, plasma cell myeloma; RIC, reduced intensity conditioning; MAC, myeloablative conditioning.

by the Wrocław Medical University Ethics Committee (identification code KB-561/2019).

### 2.2. Samples

Peripheral blood of HSCT recipients and donors was collected on ethylenediaminetetraacetic acid (EDTA) tubes before transplantation. Genomic DNA extraction was performed by a column method using the NucleoSpin Blood kit (MACHEREY-NAGEL, Germany), according to the manufacturer's protocol. Briefly, 200 mL of whole blood was used. Isolated DNA was stored at –20°C for genetic studies. Serum

was isolated directly after blood collection and stored at  $-80^{\circ}\text{C}$  for further use.

### 2.3. SNP genotyping

Two SNPs associated with the *MICB* gene were selected. An SNP resulting in an amino acid change at position 48 (Lys/Glu), rs1065075, is localized in exon 2, which encodes the alpha1 region of the MICB protein. Another SNP, rs3828903, is an intronic variant, localized between the leader sequence and the alpha1 region of MICB. Both polymorphisms were selected based on the on-line SNP Function Prediction tool (Xu and Taylor 2009), and the frequency of the minor allele was higher than 0.30 in the European populations. The SNPs were determined using LightSNiP (TIB MOLBIOL, Berlin, Germany) assays, and real-time PCR was performed on the LightCycler 480 II instrument (Roche Diagnostics, Rotkreuz, Switzerland).

### 2.4. Serum sMICB concentration

The serum level of sMICB was determined using the Luminex Discovery Assay premixed kit (R&D Systems, bio-technie, Minneapolis, Minnesota, USA) according to the manufacturer's protocol. In total, serum from 82 HSCT recipients that represents the patients having and lacking various post-transplant complications (aged 20–73 years, 28.57% diagnosed with chronic graft-versus-host disease (cGvHD), 36.59% diagnosed with aGvHD, and 43.90% diagnosed with CMV) collected 30 days after transplantation was used. For each experiment, a series of three-fold diluted standards was prepared to create the standard curve. All samples were prepared in two-fold dilution and measured in duplicates in

a Luminex 200 instrument (Luminex Corp., Austin, Texas, USA). The median fluorescence intensity was calculated using the xPonent 4.2 software (Diasorin, Saluggia, Italy).

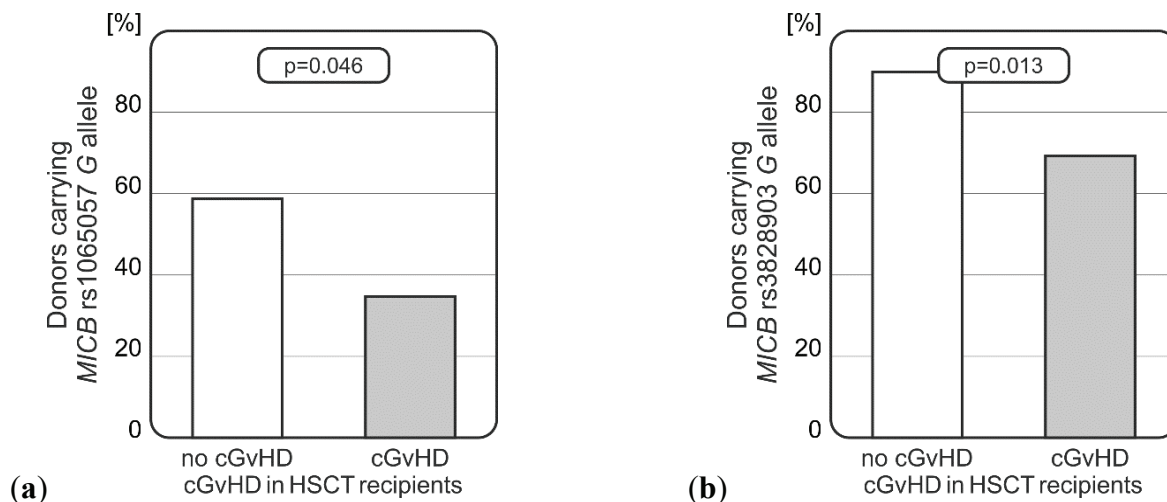
### 2.5. Statistical analysis

The Fisher exact test and the Mann–Whitney  $U$  test were used for the statistical analysis of the obtained results. The concentration of serum sMICB was calculated as a mean value with standard deviation (SD). For data visualization and calculations, GraphPad Prism (Dotmatics, Boston, Massachusetts, USA) was used. Multivariate logistic regression analysis was performed using the RStudio software (v. 2022.12.0, posit, Boston, Massachusetts) to determine the risk factors for post-transplant CMV infection. In this analysis, we used a number of factors (recipients' age, donor–recipient HLA compatibility, donors' and recipients' CMV IgG sero-status, donors' sex and the occurrence of *MICB* rs1065075  $G$  allele) to point out those that are of major significance.  $P$  value at 0.05 was considered as statistically significant.

## 3. Results

### 3.1. Donor *MICB* polymorphisms and the risk of cGvHD incidence

The SNPs genotyping revealed that the presence of the donor *MICB* rs1065075  $G$  allele was less common among patients who had developed cGvHD after HSCT. This genetic variant was detected in 34.62% of the donors whose recipients developed cGvHD and in 58.59% of the donors of the remaining recipients ( $p = 0.046$ , Figure 1a). Similarly, the donor *MICB* rs3828903  $G$  allele was less prevalent among patients who



**Fig 1.** *MICB* genetic variants and development of cGvHD. (a) Donor rs1065075  $G$  allele was less common among patients who developed cGvHD. (b) Donor rs3828903  $G$  allele was more prevalent among recipients lacking cGvHD post-transplantation. cGvHD, chronic graft-versus-host disease; HSCT, hematopoietic stem cell transplantation.

developed cGvHD when compared to patients free of that disease (69.23% vs. 89.90%,  $p = 0.013$ , Figure 1b).

These relationships were also apparent when the patients who developed cGvHD *de novo*, as progression of aGvHD or after remission of aGvHD was considered separately in relation to the donor *MICB* rs1065057 genotype. However, they did not reach statistical significance. As compared to patients without cGvHD symptoms transplanted from donors carrying the *MICB* rs1065075 G allele (58.9%), there were 50.00% of the patients with *de novo* cGvHD and 33.33% with cGvHD after aGvHD remission received HSC from donors with the *MICB* rs1065075 G allele. None of the patients who developed cGvHD as a progression from aGvHD were transplanted from the *MICB* rs1065075 G positive donor.

### 3.2. MICB polymorphism and risk of CMV infection

CMV infection was detected in 38.79% (90/232) of the HSCT recipients after transplantation. It was observed that the presence of the *MICB* rs1065075 G allele in either the donor or recipient may be associated with a decreased risk of infection. This genetic variant was detected among 41.57% of the recipients who developed CMV infection after HSCT (Figure 2a). Similarly, the donor *MICB* rs1065075 G allele was more commonly detected among patients who did not develop CMV infection (Figure 2b). This favorable role of the *MICB* rs1065075 G variant was confirmed in a multivariate analysis. Multivariate logistic regression analysis showed that the recipient IgG status constitutes an independent risk factor for CMV infection, while the HLA compatibility and presence of the *MICB* rs1065057 G allele in recipients significantly protect

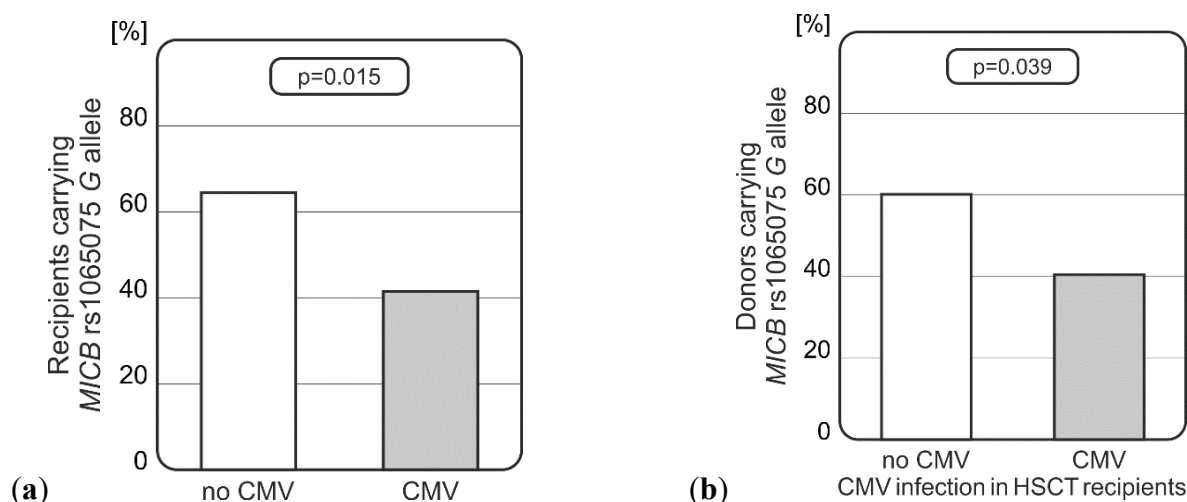
from CMV infection ( $p = 0.0142$  and  $p = 0.0238$ , respectively; Table 2).

### 3.3. Serum sMICB concentrations

Concentration of sMICB was measured in serum collected 30 days after HSCT. The mean level of serum sMICB was 78.79 pg/mL in all samples. Recipients with CMV infection after HSCT were characterized as having an increased level of serum sMICB when compared to recipients without post-transplant CMV infection. The mean value of sMICB was 67.13 pg/mL in individuals without CMV infection and 96.85 pg/mL in recipients diagnosed with CMV infection ( $p = 0.0386$ ; Figure 3a). The sMICB serum concentration was also found to be associated with cGvHD incidence. Recipients who developed cGvHD characterized with increased sMICB levels when compared with patients without cGvHD (62.47 pg/mL in recipients without cGvHD vs. 116.2 pg/mL in recipients with cGvHD,  $p = 0.0008$ ; Figure 3b). Detailed results of serum sMICB concentration measurements are shown in Table 3.

### 3.4. Serum sMICB concentration in relation to MICB polymorphisms

The sMICB level in the recipients' serum seems to be associated with the *MICB* genetic variants (Table 4). *MICB* rs1065057 GG carriers were observed to have the lowest sMICB levels. Homozygous patients with *MICB* rs1065057 GG genotype had decreased sMICB concentration compared with both the AG heterozygous ( $p = 0.0215$ ) and AA homozygous patients ( $p = 0.0155$ ; Figure 4a). A similar association,



**Fig 2.** Associations between the *MICB* genotype and the risk of CMV infection development. (a) CMV infection was less frequent in recipients carrying the rs1065075 G allele. (b) Lower incidence of CMV infection in patients transplanted from donors with rs1065075 G allele. CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation.

although not statistically significant, was observed for *MICB* rs3828903 polymorphism. Recipients carrying the *GG* genotype had lower sMICB concentration when compared with the *A* allele carriers (63.49 pg/mL vs. 90.45 pg/mL,  $p = 0.0730$ ; Figure 4b).

#### 4. Discussion

In our present study, HSCT recipients and donors were genotyped for two *MICB* SNPs (rs1065075; *G* to *A* substitution resulting in Lys48Glu amino acid exchange and rs3828903; *G* to *A* nucleotide substitution within intronic sequence). In addition, serum sMICB levels were determined in patients' sera 30 days after transplantation. Our findings showed significant associations between the *MICB* polymorphisms and sMICB serum levels and risk for development of CMV infection or cGvHD.

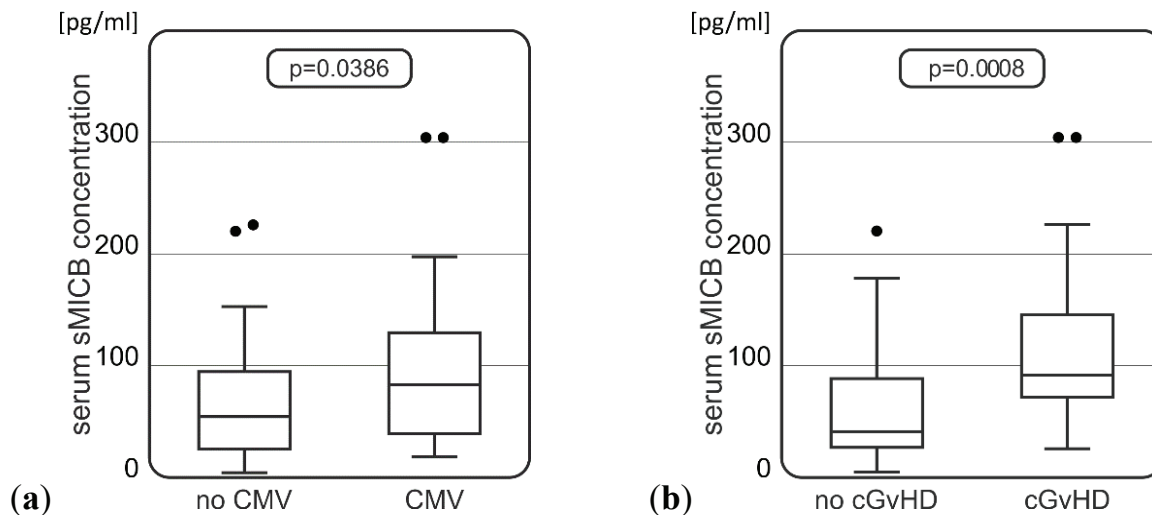
**Table 2.** Results of the multivariate analysis for CMV risk factors

Variables	P value	OR	95% CI
Age	0.5988	0.9937	0.9702–1.0176
D/R HLA compatibility	0.0142	0.4276	0.2144–0.8385
Recipient CMV IgG status	<0.0001	16.2592	4.8663–76.9165
Donor CMV IgG status	0.1884	0.5834	0.2570–1.2944
Donor sex	0.1606	1.6562	0.8250–3.3949
<i>MICB</i> rs1065057 <i>G</i> allele	0.0238	0.4701	0.2417–0.8988

CI, confidence interval; CMV, cytomegalovirus; D, donor; HLA compatibility, HLA-A, B, C, DRB1 and DQB1 match at a high resolution level; OR, odds ratio; R, recipient.

The *MICA* molecule is the most polymorphic of all non-classical HLA and HLA-like molecules, whereas *MICB* is characterized by more limited genetic variability. Its polymorphic variants were reported to have an impact on relapse-free survival or mortality in HSCT recipients, dengue severity, and immunosurveillance in oral squamous cell carcinoma (Ivanova et al. 2021; Machuldova et al. 2021; Faridah et al. 2023; Petersdorf et al. 2023). An interesting *MICB* polymorphism is the Ile/Met amino acid exchange at position 98. Carapito et al. (2020) reported that the donor/recipient mismatch for Ile98Met was associated with an increased risk of developing severe aGvHD grades II–IV or cGvHD. Here we focused on two other SNPs, not previously investigated in the context of HSCT, to study their potential associations with the risk of post-transplant complications. Both SNPs chosen for this study might be related to the ligand–receptor interactions and are located in a similar region of the *MICB* gene. The rs1065057 polymorphism (exon 2) is localized within the region responsible for encoding of the  $\alpha 1$  domain of *MICB* protein, which is exposed to the corresponding NKG2D receptor. The other polymorphism (rs3828903) is localized in intron 1, which is placed between the leader sequence and exon 1.

Our results on the genetic distribution of two *MICB* polymorphisms revealed that in both rs1065057 (Lys48Glu) and rs3828903 (intronic substitution) SNPs, the *G* allele plays a favorable role as it was associated with a decreased incidence of cGvHD development after HSCT. Additionally, we showed that the presence of the *MICB* rs1065075 *G* allele in recipients and donors may play a protective role against CMV infection after HSCT. The effect of recipient genotype



**Fig 3.** Serum sMICB levels in recipients diagnosed with various post-transplant complications. (a) Increased sMICB concentration in recipients with CMV infection. (b) Higher sMICB level in patients who developed chronic form of GvHD. cGvHD, chronic graft-versus-host disease; CMV, cytomegalovirus; GvHD, graft-versus-host-disease; sMICB, soluble MICB.



was confirmed in a multivariate analyses together with HLA compatibility and lack of pre-transplant anti-CMV IgG antibodies as protective factors.

Both MICA and MICB molecules can be shed in their soluble forms from the cell surface, indicating immune evasion and escape from detection by NK cells (Chitadze et al. 2013; Suresh 2016). Being one of the most characteristic tumor immune escape mechanisms, shedding of these two molecules is possible due to metalloproteinases (ADAMs and

MMPs families) and disintegrins (Zocchi et al. 2015). When expressed on the surface of target cells, MICA and MICB serve as ligands for NKG2D activating receptor, allowing their recognition by NK cells. Blocking this ligand–receptor interaction compromises cytotoxic properties of the NK cells. Increased levels of soluble NK cell ligands (sMICA, sMICB, and sULBPs) had been associated with poor prognosis in cancer patients (Groh et al. 2002; Doubrovina et al. 2003; Wu et al. 2004; Holdenrieder et al. 2006a; Nuckel et al. 2010; Vela-Ojeda et al. 2021). Interestingly, the MICB molecule can be found in its soluble form in the tumor microenvironment but is not expressed directly on the surface of tumor cells (Raffaghello et al. 2004; Holdenrieder et al. 2006b; Boutet et al. 2009; Kaidun et al. 2023). NKG2D ligands are overexpressed during CMV infection, which helps with the recognition and clearance of the infected cells. It was reported that the human CMV-encoded UL16 protein binds specifically to MICB, competing with NKG2D. This leads to decreased binding to the activating receptor and, as a result, decreased NK cell activity (Spree et al. 2006).

In accordance with these observations, we detected higher sMICB serum concentrations in patients who developed CMV after HSCT. Moreover, increased sMICB serum levels were found in patients who suffered from cGvHD. This is a novel observation that has not been previously described. Furthermore, our study has also revealed associations between the *MICB* polymorphism and sMICB concentrations in patients' sera. Patients homozygous for rs1065057 AA and rs3828903 AA were characterized with higher sMICB levels compared to recipients carrying at least one G allele, further confirming the protective role of those genetic variants. Since soluble forms of non-classical HLA and HLA-like molecules

**Table 3.** Serum sMICB concentrations in HSCT recipients

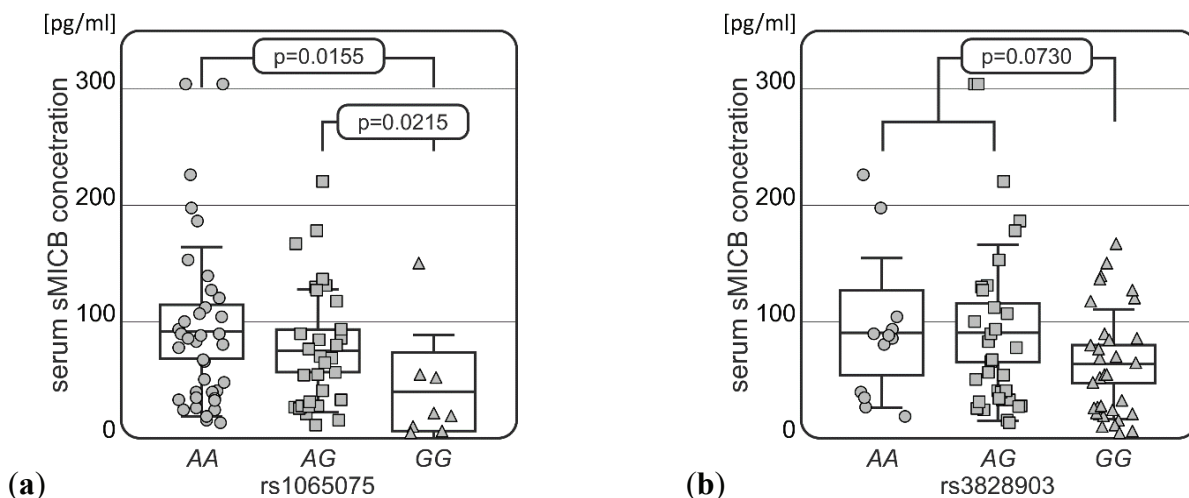
	No CMV (pg/mL)	CMV [pg/mL]	No cGvHD (pg/mL)	cGvHD (pg/mL)
Mean	67.13	96.85	62.47	116.2
SD	54.23	72.04	49.88	77.03
Std. Error	8.47	12.18	6.73	15.72
25–75% percentile	25.38–94.86	39.42–129.8	26.53–88.25	71.60–145.2
95% CI	50.01–84.25	72.10–121.6	48.98–75.95	83.69–148.7

cGvHD, chronic graft-versus-host disease; CI, confidence interval; CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation; SD, standard deviation; sMICB, soluble MICB.

**Table 4.** Mean serum sMICB concentrations of patients with various *MICB* genotypes

Variant	<i>MICB</i> SNP	
	rs1065057	rs3828903
AA	91.47 pg/mL	90.45 pg/mL
AG	74.95 pg/mL	90.48 pg/mL
GG	39.78 pg/mL	63.49 pg/mL

sMICB, soluble MICB; SNP, single nucleotide polymorphism.



**Fig 4.** Relationships between serum sMICB and two *MICB* SNPs. (a) Lower sMICB level in serum samples of *MICB* rs1065057 GG homozygous patients. (b) Differences in sMICB concentration between recipients carrying various *MICB* rs3828903 genotypes. sMICB, soluble MICB; SNPs, single nucleotide polymorphism.

may serve as decoy ligands for NK cell receptors, blocking cytotoxic properties, their increased levels are considered as a negative factor for disease development and worse outcome (Salih et al. 2006; Ribeiro et al. 2016; Siemaszko et al. 2021). Results obtained in this study support these findings. Nevertheless, it should be noted that the number of tested samples is relatively small and a study on a larger cohort could be needed to confirm our findings. Furthermore, the biological material was not available for some of the donors, limiting our observations of the immunogenetic donor–recipient associations.

Taken together, in the present study, we showed significant associations of the *MICB* genetic variants and sMICB serum levels with the development of post-transplant complications in recipients undergoing allogeneic HSCT. We found that the increased serum sMICB concentration is a negative factor for CMV infection and cGvHD development. We showed that both donor and recipient *MICB* variants could be potential prognostic markers of CMV and cGvHD. Our results indicate the emerging role of MICB molecule in the context of HSCT outcome.

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## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Author Contributions

JS performed genotyping studies and statistical analyses, drafted and finalized the manuscript; MD performed the assessment of the sMICB concentration in serum samples; AC, AS, MSK, WF, IS, BNA, PS, MB, AT, GWB, SG, and TW provided patients' clinical samples and clinical data; KBK conceived and designed the study, analyzed the data, drafted and finalized the manuscript and secured funding. All authors approved the final version of the manuscript.

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