Presence of Parvovirus B19 but Not Herpesvirus Genome in Acute Skin Rash after Allogeneic Stem Cell Transplantation Correlates with Outcome

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Keywords

Stem cell transplantation · Skin rash · Graft-versus-host disease · Polymerase chain reaction · Herpes viruses · Parvovirus B19

Abstract

Introduction: Skin rash is a first symptom of acute graft-versus-host disease (GvHD) after allogeneic stem cell transplantation (ASCT) but can also be caused by viruses. The relevance of virus DNA analyses in skin rash for diagnosis and clinical outcome is unknown. **Objectives:** To record the frequencies of detection of herpes and parvovirus B19 (ParvoB19) DNA in skin rash within 100 days after ASCT and to analyze their relevance for diagnosis, clinical course, and non-relapse mortality (NRM). **Methods:** We retrospectively identified 55 patients with virus DNA analysis for CMV, EBV, HHV6, HHV8, HSV, VZV, or ParvoB19. We assessed the rate of virus DNA detection and studied associations with histological diagnosis, virus DNA from concomitantly analyzed blood, clinical presentation, exanthema treatment, and NRM. **Results:** CMV, EBV, HHV6, HHV8, HSV, VZV and ParvoB19 DNA were detected in 12.5, 11.8, 10, 0, 0, 2.9, and

26.7% of exanthemas. Histopathological diagnosis was not associated with virus polymerase chain reaction (PCR) results. Detection of CMV, EBV, or HHV6 DNA but not ParvoB19 in skin and blood was associated with PCR results ($p = 0.016$; $p < 0.001$; $p = 0.067$; $p = \text{n.a.}$). Detection of CMV, EBV, HHV6, or ParvoB19 DNA in the skin was not significantly associated with patient, ASCT, or GvHD characteristics. Detection of ParvoB19 but not herpes virus DNA was associated with less immunosuppressive treatment ($p = 0.015$) and lower NRM ($p = 0.041$). In multivariate analyses, detection of ParvoB19 was associated with a lower NRM. **Conclusions:** Detection of ParvoB19 DNA in exanthema after ASCT might be associated with lower NRM.

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Introduction

Despite many improvements over the last decades, transplant-related non-relapse mortality (NRM) after allogeneic stem cell transplantation (ASCT) is still around 15% for matched unrelated donor transplants [1, 2]. In the first months after ASCT, acute graft-versus-host disease

(aGvHD) has the highest impact on NRM with a mortality rate of 40–50% [3]. In most cases, skin rash is the first symptom of aGvHD [4, 5]. However, skin rashes until day 100 after ASCT can have very different causes, such as viral infections, drug allergies, radiation dermatitis due to total body irradiation, or engraftment syndrome [6].

Skin rash can be the first and even the only clinical sign of viral reactivation or primary infection, especially due to herpes virus such as CMV, EBV, HHV6, HHV8, HSV, and VZV [7–9]. These reactivations are associated with increased NRM after ASCT [10–12]. In particular, reactivation of CMV is the leading cause amongst virus-induced morbidity and mortality after ASCT. In transplantations with CMV-seropositive donor or recipient, 60–70% of patients will develop CMV viremia, and 20–30% will develop symptomatic CMV disease without preemptive treatment [13], and CMV-IgG-positive recipients have an absolute increase in NRM of as much as 40% [14, 15].

In addition to herpes virus infections, human parvovirus B19 (ParvoB19) can persist in several tissues [16] and reactivate after ASCT [17]. ParvoB19 has a pronounced tropism toward erythroid progenitors [18] and most commonly causes anemia. However, in some patients the only sign of reactivation is a cutaneous rash [19, 20] also reported to present with a GvHD-like erythema in 2 stem cell recipients [21].

In contrast to GvHD, where treatment is immunosuppressive therapy (IST) [22–24], treatment of viral infection comprises reduction of immunosuppression to support antiviral immune response and in some cases virostatics. Increased immunosuppression is paralleled by an increased risk of simultaneous viral exacerbation with a higher risk to develop a fatal course [25]. Thus, exact delineation of the etiology of a skin rash is important to establish correct treatment of skin rash after ASCT and may lead to reduced NRM.

However, making a distinction between aGvHD of the skin and the competing diagnoses is difficult and mainly based on clinical findings, as no specific diagnostic markers for aGvHD are established [6]. The diagnostic value of skin histopathology is limited by the lack of specific diagnostic criteria [26]. Histopathology was not associated with clinical diagnosis, clinical severity, and outcome of aGvHD in several studies [27–29]. Experts estimate sensitivity and specificity of histopathological examination for diagnosis of acute GvHD to only reach 0.73 and 0.66, respectively [30].

The detection of virus genome in affected skin after ASCT might help to reveal the underlying etiology of the rash. It also might be associated with the clinical course

and NRM in patients with skin rash after ASCT. Polymerase chain reaction (PCR)-based assays are able to detect virus genome in skin affected by herpes and parvoB19 viruses [31–33].

This study aimed to evaluate the rate of virus DNA-positive skin biopsies for CMV, EBV, HHV6, HHV8, HSV, VZV, and ParvoB19 in biopsies of acute skin rashes after ASCT. We further investigated, if detection of herpesvirus- or ParvoB19-DNA helps to define the etiology of the exanthema. Associations between skin virus PCR results and clinical presentation at occurrence of exanthema, immunosuppressive and antiviral treatments, further clinical course, and NRM were investigated. We examined the association between histological and DNA test results from skin as well as between DNA test results from skin and blood. These analyses will help to elucidate if there might be a role of virus genome analyses in skin for diagnosis, prediction of the clinical course, and treatment in skin rashes after ASCT.

Methods

Patients and Transplant Procedures

We retrospectively screened all adult patients who underwent ASCT for hematological malignancies or aplastic anemia at the University Hospital Halle between May 2005 and December 2013. We identified and characterized those patients with exanthema occurring 0–100 days after ASCT. All patients received their first ASCT. Transplant procedures and GvHD prophylaxis were performed according to institutional standards. All patients received acyclovir prophylaxis. All data were extracted by reviewing patient charts and physician reports.

Diagnostic and Treatment at the Time of Exanthema Occurrence

In the case of exanthema, skin biopsies were taken by 4 mm punch biopsies including cutis and subcutis. Histopathological analyses were done on formalin-embedded tissue. Multiplex virus PCR analysis of skin was performed from a second punch on tissue stored in 0.9% saline after repetitive washing. Commercially available kits were used for PCR against CMV: Quiagen Artus 4503265, EBV: Quiagen Artus 4501065, HHV6: Tib molbiol 40-0282-32, HHV8: Tib molbiol 05 945 224 001, HSV: Quiagen Artus 4500065, VZV: Quiagen Artus 4502065, Parvovirus B19: Roche 03-246-809-001 according to the manufacturers' guidelines. As quantification is not validated for skin tissue analyses, we used dichotomized positive/negative categories as test results. To analyze whether a viremia at the time of occurrence of exanthema was present, virus PCR analyses for CMV, EBV, HHV6 and ParvoB19 from blood drawn concomitantly with the skin biopsy (+/–10 days) were assessed.

In available cases, immunohistochemistry against CMV (Dako, clone CCH2, EBV LMP1 (Dako, clone CS.1–4) and ParvoB19 (Abcam, clone R92F6) was performed according to diagnostic standard protocols.

Fig. 1. Patient flow. CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV6, herpes virus 6; HHV8, herpes virus 8; HSV, herpes simplex virus; ParvoB19, parvovirus B19; PCR, polymerase chain reaction; VZV, varicella zoster virus.

Treatment decisions regarding immunosuppressive and anti-viral therapy (IST and AVT) were recorded. AVT was administered according to our institutional guidelines: for CMV, HHV6 and HHV8 ganciclovir 5 mg/kg b.i.d. intravenously for 14 days followed by a 14-day maintenance with valganciclovir. HSV and VZV reactivation/infection were treated with intravenous acyclovir 800 mg 5 times per day for 7 days followed by reinitiation of acyclovir prophylaxis. For 2 consecutive EBV viremia above 10,000 copies/mL plasma, Rituximab 375 mg/m² was administered. In addition, all virus reactivations/infections were treated with 30 g intravenous immunoglobulins.

Definitions, End Points, and Statistical Analysis

Positive virus PCR was defined as positive test result according to the manufacturers' definition and negative virus PCR as a sample with negative PCR for all analyzed viruses in the sample. Acute GvHD was staged and graded according to the modified Glucksberg criteria [34, 35]. Recorded additional clinical signs at time of biopsy were fever, fatigue, hepatosplenomegaly, or lymph node enlargement. Modification of IST was defined as increased dose or initiation of IST due to exanthema. The initiation of antiviral agents such as virostatics, intravenous immunoglobulins or B-cell-depleting agents (e.g., rituximab) was recorded and summarized as AVT. NRM was defined as death due to any cause except relapse or disease progression. Patients were censored after death due to the underlying disease or end of follow-up.

Relationships between variables were investigated by bivariate analyses. Nominal or ordinal variables with multiple categories were condensed to binominal variables for comparison. Testing of association of binominal variables was done by χ^2 or Fisher's exact test as appropriate. Pearson *t* test was used to compare metric data

between groups. Kaplan-Meier plots visualized the cumulative incidences of the NRM and statistical comparisons between groups were done by log-rank tests. Cox proportional hazard models were used for multivariate survival analysis regarding NRM. A two-sided *p* value <0.05 was regarded as significant.

Results

Patient Characteristics and Skin Virus PCR Analyses

Ninety-six of the 122 patients (78.7%) who underwent ASCT developed an exanthema after transplantation. Out of these patients, 55 (56.9%) received a skin biopsy with histopathological and concomitant PCR analysis of viral genome for CMV (*n* = 48), EBV (*n* = 51), HHV6 (*n* = 30), HHV8 (*n* = 34), HSV (*n* = 34), VZV (*n* = 23), and ParvoB19 (*n* = 45; Fig. 1). Patient and transplant characteristics did not significantly differ between patients with exanthema who received or did not receive a skin biopsy (Table 1). Median duration of follow-up of the patients with virus PCR-analyses from skin was 516 days (interquartile range 176–1,449).

Viral genome was detected in 23 (41.8%) of the 55 biopsies (Table 2). PCR analyses were positive in 6 of 48 cases (12.5%) for CMV, 6 of 51 cases for EBV (11.8%), 3 of 30 cases for HHV6 (10%), 0 of 14 cases for HHV8, 0 of

Table 1. Patient and transplant characteristics of all patients with exanthema, with skin rash without virus PCR of skin, with skin rash and virus PCR of skin, and with positive virus DNA, CMV, EBV, HHV6, or Parvovirus B19 PCR in skin

	Exanthema, no virus PCR performed	Exanthema, virus PCR performed	Virus PCR negative	Virus PCR positive	CMV PCR positive	EBV PCR positive	HHV6 PCR positive	Parvovirus B19 PCR positive
<i>N</i>	41	55	32		6	6	3	12
Age, years (IQR); <i>p</i>	57 (53–64)	52 (45–62); 0.386	56 (46–56)	48 (36–58)	53 (42–66)	45 (33–54)	61 (58–61)	37 (30–46)
Gender, male, <i>n</i> (%); <i>p</i>	22 (53.7)	34 (61.8); 0.531	21 (65.6)	13 (56.5)	2 (33.3)	4 (66.7)	2 (66.7)	8 (66.7)
Karnofsky >80%, <i>n</i> (%); <i>p</i>	29 (70.1)	39 (70.9); 1.0	24 (75)	15 (65.2)	3 (50)	5 (83.3)	3 (100)	6 (50)
HCT-CI, <i>n</i> (%)								
0	7 (17.1)	18 (32.7)	9 (28.1)	9 (39.1)	3 (50)	2 (33.3)	1 (33.3)	6 (50)
1–2	17 (41.5)	15 (27.3)	10 (31.3)	5 (21.7)	1 (16.7)	1 (16.7)	1 (33.3)	2 (16.7)
>2	17 (41.5)	22 (40.0)	13 (40.6)	9 (39.1)	2 (33.3)	3 (50)	1 (33.3)	4 (33.3)
<i>p</i>		0.168						
Disease, <i>n</i> (%)								
AML/MDS/MPN	17 (41.5)	31 (56.4)	19 (59.4)	12 (52.2)	4 (83.7)	2 (33)	2 (66.7)	5 (41.7)
NHL	10 (24.4)	14 (25.5)	12 (37.5)	2 (8.7)	0	1 (16.7)	0	1 (8.3)
MM	11 (26.8)	5 (9.1)	0	5 (21.7)	1 (16.7)	2 (33.3)	1 (33.3)	2 (16.7)
ALL	2 (4.9)	5 (9.1)	1 (3.1)	4 (17.4)	0	1 (16.7)	0	4 (33.3)
AA	1 (2.4)	0	0	0	0	0	0	0
<i>p</i>		0.1						
HLA, <i>n</i> (%)								
MRD	8 (19.5)	15 (27.3)	11 (34.4)	4 (17.4)	1 (16.7)	1 (16.7)	1 (33.3)	1 (8.3)
MUD	33 (80.5)	33 (60)	19 (59.4)	14 (60.9)	5 (83.3)	4 (66.7)	1 (33.3)	7 (58.3)
MMUD		6 (10.9)	2 (6.3)	4 (17.4)	0	1 (16.7)	0	4 (33.3)
Haplo		1 (1.8)	0	1 (4.3)	0	0	1 (33.3)	0
<i>p</i>		0.471						
CMV, <i>n</i> (%)								
Neg in neg	11 (26.8)	9 (16.4)	6 (18.8)	3 (13)	2 (33.3)	4 (66.7)	1 (33.3)	1 (8.3)
Others	30 (80.5)	46 (83.6)	24 (81.2)		4 (66.7)	2 (33.3)	2 (66.7)	11 (91.7)
<i>p</i>		0.31						
Conditioning, <i>n</i> (%)								
MA	12 (29.3)	15 (27.3)	8 (25)	7 (30.4)	2 (33.3)	4 (66.7)	1 (33.3)	5 (41.7)
RIC/NMA	29 (70.7)	40 (72.7)	24 (75)	14 (60.9)	4 (83.3)	2 (33.3)	2 (66.7)	7 (58.3)
<i>p</i>		1						
GvHD prophylaxis, <i>n</i> (%)								
CsA + MTX	15 (36.6)	13 (23.6)	7 (21.9)	16 (69.6)	1 (16.7)	2 (33.3)	0	6 (50)
CsA + MMF	25 (61)	41 (74.5)	25 (78.1)	6 (26.1)	5 (83.3)	4 (66.6)	2 (66.7)	6 (50)
Other	1 (2.4)	1 (1.8)	0	1 (4.3)	0	0	1 (33.3)	0
<i>p</i>		<0.352						

AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; AVT, antiviral treatment; ASCT, allogeneic stem cell transplantation; CMV, cytomegalovirus; CsA, cyclosporine A; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; HCT-CI, hematopoietic stem cell transplantation comorbidity index; HHV6, herpes virus 6; HLA, human leukocyte antigen; IQR, interquartile range; IST, immunosuppressive treatment; Karnofsky, Karnofsky performance status; MA, myeloablative conditioning; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MM, multiple myeloma; MMUD mismatched unrelated donor; MNA, nonmyeloablative conditioning; MPN, myeloproliferative neoplasm; MUD, matched related donor; MRD, matched related donor; MTX, methotrexate; NHL, non-Hodgkin lymphoma; *p*, *p* value for comparison between “exanthema, no virus PCR performed” vs. “exanthema, virus PCR performed”; Parvovirus B19, parvovirus B19, PCR, polymerase chain reaction; PS, performance status; RIC, reduced intensity conditioning; SCT, allogeneic stem cell transplantation; TBI, total body irradiation.

Table 2. Virus PCR analyses of skin biopsies in exanthema after SCT

PCR	All viruses	CMV	EBV	HHV6	HHV8	HSV	VZV	ParvoB19
Analyzed, <i>n</i>	55	48	51	30	14	34	34	45
Positive, <i>n</i> (%)	23 (41.8)	6 (12.5)	6 (11.8)	3 (10)	0	0	1 (2.9)	12 (26.7)
Negative, <i>n</i> (%)	32 (58.2)	42 (87.5)	45 (88.2)	27 (90)	14 (100)	34 (100)	33 (97.1)	33 (73.3)

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV6, herpes virus 6; HHV8, human herpes virus 8; HSV, herpes simplex virus; *n*, number; ParvoB19, parvovirus B19; PCR, polymerase chain reaction; VZV, varicella zoster virus.

Table 3. Associations between result of virus PCR of skin and histopathology of skin rash biopsies

	Virus PCR (<i>n</i> = 55)		CMV PCR (<i>n</i> = 48)		EBV PCR (<i>n</i> = 51)		HHV6 PCR (<i>n</i> = 30)		ParvoB19 PCR (<i>n</i> = 45)	
	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
Histo virus, <i>n</i>	6	4	1	8	1	8	2	4	3	5
Histo other, <i>n</i>	17	28	5	34	5	37	1	23	9	28
GvHD	11	19	3	24	2	26	0	16	6	18
Drug toxicity	2	3	0	3	1	4	0	2	1	3
Normal skin	1	1	1	1	0	2	0	1	0	1
Not defined	3	5	1	6	2	5	0	4	2	5
<i>p</i>	0.29		0.477		0.268		0.061		0.443	

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV6, herpes virus 6; *n*, number; *p*, *p* value; ParvoB19, parvovirus B19; PCR, polymerase chain reaction.

34 cases for HSV, 1 of 34 cases for VZV (2.9%), and 12 of 45 cases for ParvoB19 (26.7%). Two patients had positive PCR for both EBV and ParvoB19, and 1 patient had positive PCR for CMV, EBV, VZV, and ParvoB19. Due to the small number of positive tests for VZV, HSV, and HHV8, these viruses were not included in further analyses.

Comparison of Results from Skin Virus PCR, Skin Histopathology, and Immunohistochemistry

In an analysis including all studied viruses, no association between PCR result and histopathological diagnosis was seen ($p = 0.29$). Out of the 23 patients with detection of viral DNA in skin, 6 patients had histopathological diagnosis of viral exanthema, whereas 17 patients had different diagnoses (11 aGvHD, 2 drug-induced skin alterations, 3 equivocal skin alterations, 1 normal skin). Four of the 32 patients with negative virus PCR from skin had histopathological diagnosis of virus exanthema. Separate analyses for the CMV, EBV, HHV6, and ParvoB19 subgroups did not reveal an association between PCR-based detection of viral DNA and histopathological diagnosis of exanthema (Table 3).

All cases with negative PCR were also immunohistochemistry negative for CMV (7/7), EBV (10/10), and Par-

voB19 (6/6). Two of 3 cases with positive skin CMV PCR were immunohistochemistry positive. No EBV or ParvoB19 PCR-positive case was available for immunohistochemistry.

Comparison of Results from Skin Virus PCR and Blood Virus PCR

We identified 45, 46, 10, and 13 patients with concomitant PCR analysis from skin and blood for CMV, EBV, HHV6, and ParvoB19. PCR results from skin and blood were associated with CMV ($p = 0.016$), EBV ($p < 0.001$), and HHV6 ($p = 0.067$). For Parvo19, PCR from blood was negative in every case, and no association between skin and blood PCR was noticed (Table 4).

Association between Results of PCR from Skin, Exanthema Characteristics, and Treatment

No significant differences regarding exanthema characteristics were identified when patients with negative virus PCR were compared to patients with positive PCR for any single or particular viruses (Table 5). IST was started in 26 of 32 patients without positive virus PCR versus 5 of 12 patients with positive ParvoB19-PCR ($p = 0.023$). Antiviral treatment (AVT) was started in 5 of 32 patients

Table 4. Associations between results of virus PCR in skin and virus PCR in blood

	Skin virus PCR (<i>n</i> = 55)		Skin CMV PCR (<i>n</i> = 45)		Skin EBV PCR (<i>n</i> = 46)		Skin HHV6 PCR (<i>n</i> = 10)		Skin ParvoB19 PCR (<i>n</i> = 13)	
	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
Blood virus PCR positive	18	11	4	6	6	7	2	1	0	0
Blood virus PCR negative	5	21	2	33	0	33	0	7	3	10
<i>p</i>	<0.001		0.016		<0.001		0.067		n.a.	

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV6, herpes virus 6; *n*, number; *p*, *p* value; ParvoB19, parvovirus B19; PCR, polymerase chain reaction.

Table 5. Characteristics of the exanthema and GvHD staging/grading at time of skin rash biopsy; treatment and diagnosis

	Virus PCR negative	Virus PCR positive	CMV PCR positive	EBV PCR positive	HHV6 PCR positive	ParvoB19 PCR positive
<i>N</i>	32	23	6	6	3	12
Time after ASCT, median (min–max), days; <i>p</i>	23 (15–44)	37 (20–100); 0.886	47.5 (13–143); 0.807	41 (19–70); 0.807	130 (n.a.); n.a.	32 (14–80); 0.54
Time from exanthema to biopsy, median (min–max), days; <i>p</i>	3 (1–15)	2 (1–12); 0.36	3 (0–12); 0.624	6 (1–23); 0.682	4 (n.a.); 0.178	2 (1–12); 0.373
GvHD skin stage at biopsy, <i>n</i> (%)						
≤2	20 (62.5)	20 (87)	6 (100)	6 (100)	3 (100)	9 (75)
>2	12 (37.5)	3 (13)	0	0	0	3 (25)
<i>p</i>		0.066	0.149	0.149	0.536	0.5
GvHD overall grade at biopsy, <i>n</i> (%)						
≤II	31 (96.9)	22 (95.7)	6 (100)	6 (100)	3 (100)	11 (91.7)
>II	1 (3.1)	1 (4.3)	0	0	0	1 (8.3)
<i>p</i>		1	1	1	1	0.476
Maximal GvHD overall grade, <i>n</i> (%)						
No GvHD or ≤grade II	26 (81.2)	18 (78.3)	5 (83.3)	5 (83.3)	3 (100)	9 (75)
>grade II	6 (18.8)	5 (15.6)	1 (16.7)	1 (16.7)	0	3 (25)
<i>p</i>		1	1	1	1	0.687
Further symptoms ^a present, <i>n</i> (%); <i>p</i>	12 (37.5)	7 (30.4); 0.775	1 (16.7); 0.643	3 (50%); 0.663	1 (33.3); 1.0	5 (41.7); 1.0
IST increased or newly introduced, <i>n</i> (%); <i>p</i>	26 (81.3)	12 (52.2); 0.037	3 (50); 0.131	3 (50); 0.131	2 (66.8); 0.499	5 (41.7); 0.023
AVT increased or newly introduced, <i>n</i> (%); <i>p</i>	5 (15.6)	13 (56.5); 0.003	5 (83.3); 0.003	6 (100); <0.001	3 (100); 0.009	3 (25); 0.663
Response of exanthema to treatment ^b , <i>n</i> (%); <i>p</i>	26 (81.3)	20 (87); 0.72	5 (83.3); 1.0	5 (83.3); 1.0	3 (100); 1.0	7 (58.3); 0.653

AVT, antiviral treatment; ASCT, allogeneic stem cell transplantation; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GVHD, acute graft-versus-host disease; HHV6, herpes virus 6; IST, immunosuppressive treatment; *n*, number; *p*, *p* value for comparison against group “virus PCR negative”; ParvoB19, parvovirus B19; PCR, polymerase chain reaction. ^a Further symptoms: fever, fatigue, hepatosplenomegaly or lymph node enlargement. ^b Response of exanthema to treatment defined as alive and no secondary IST increase/ initiation.

with negative virus PCR; all of these 5 patients had systemic herpesvirus reactivation. In comparison, 5 of 6 patients with positive CMV-PCR ($p = 0.003$), 6 of 6 patients with positive EBV-PCR ($p < 0.001$), and 3 of 3 patients with positive HHV6-PCR ($p = 0.009$) were started on AVT.

Association of Results from Skin Virus PCR with NRM

NRM did not differ between patients with negative versus positive skin PCR for either CMV ($p = 0.2$), EBV ($p = 0.259$), or HHV6 ($p = 0.208$). In contrast, patients with ParvoB19 PCR-positive exanthemas had a lower NRM (NRM-free survival not reached) than patients with

Fig. 2. Transplant-related mortality for virus PCR-negative vs. -positive skin rash for ParvoB19 (negative, $n = 24$, vs. positive, $n = 12$; $p = 0.041$). ParvoB19, parvovirus B19; PCR, polymerase chain reaction.

negative PCR for any of the tested viruses (median NRM-free survival 16.9 months; $p = 0.041$; Fig. 2). The 12 patients with positive PCR for ParvoB19 tended to have lower maximal extent of exanthema than the 36 with negative PCRs (stage 1–2: 66.7 vs. 41.4%, $p = 0.289$) and had fewer initiations of IST (no IST: 58.3 vs. 16.7%, $p = 0.015$).

We performed a multivariate analysis including ParvoB19 skin PCR results, grade of aGvHD, initiation of IST, donor/recipient CMV serostatus, HLA-match, conditioning intensity, and HCT-CI. Detection of ParvoB19 DNA and GvHD grade III–IV were significant predictors of TRM in this analysis (Table 6). In contrast, initiation of IST, CMV serostatus, HLA-match, HCT-CI, and conditioning intensity did not remain significantly associated with NRM.

Regarding the causes of death, 6 (50%) of the 12 patients with positive ParvoB19 died; 4 died due to relapse of the malignant disease (67%) and 2 due to NRM (33%). Of the 24 patients with negative PCRs for ParvoB19 and other viruses, 18 (75%) died. Of these, 2 died due to relapse of the malignant disease (11%), but 16 died due to NRM (89%).

Discussion

To our best of our knowledge, this is the first study evaluating the incidence and the clinical relevance of positive PCR for herpesviruses and ParvoB19 in skin rash

Table 6. Multivariate analysis for NRM risk factors

Variable	Categories	HR (95% CI)	<i>p</i> value
ParvoB19 DNA detection	No vs. yes	0.12 (0.02–0.7)	0.019
GvHD grade	I–II vs. III–IV	5.11 (1.5–17)	0.008
Initiation of IST	No vs. yes	1.20 (0.3–4.7)	0.845
HCT-CI	0 vs. ≥ 1	0.48 (0.2–1.5)	0.209
CMV serostatus	Neg in neg vs. other	4.50 (0.9–22)	0.067
HLA	10/10 ident vs. other	1.76 (0.3–9.8)	0.516
Conditioning intensity	MA vs. other	0.06 (0.1–1)	0.058

CI, confidence interval; CMV, cytomegalovirus; GvHD, graft-versus-host disease; HCT-CI, hematopoietic stem cell transplantation comorbidity index; HLA, human leukocyte antigen; HR, hazard ratio; IST, immunosuppressive therapy; MA, myeloablative; PCR, polymerase chain reaction; ParvoB19, parvovirus B19.

after ASCT. Detection of CMV, EBV, or HHV6 in skin rash was not associated with exanthema presentation, clinical course, or TRM but was strongly associated with systemic reactivation. In contrast, detection of ParvoB19 in skin was not associated with systemic reactivation but was associated with low NRM in uni- and multivariate survival analyses.

Published data on the frequency of the detection of DNA from herpesviruses and ParvoB19 virus in skin rashes until 100 days after ASCT are sparse. Hentrich et al. [33] detected HHV6 in 6 cases and concomitant ParvoB19 in 2 cases of skin rash after ASCT but did not report the frequency of HHV6 and ParvoB19-positive cases. Sawada et al. [36] analyzed 5 biopsies of skin or mucosa with high clinical suspicion for herpes virus infection with a multiplex PCR against HSV1/2, VZV, CMV, EBV, and HHV6. They detected EBV in 2, VZV in 2, and HSV in 1 sample. In the present study, detection of viral DNA in skin rash after ASCT was common with 42% positive biopsies. Regarding particular viruses, DNA of CMV, EBV, and HHV6 could be detected in about 12% of skin biopsies, respectively. In line with the regular acyclovir prophylaxis in the studied cohort, VZV was identified in only 1 rash, and HSV PCR was negative in all samples. PCR for HHV8 was negative in all cases. Serological prevalence of HHV8 in Europe is low and reactivation after ASCT is rare [10]. ParvoB19 was detected in 27% of the

exanthemas. Outside of the ASCT setting, ParvoB19 DNA was identified in skin affected by erythema but also in healthy individuals as the virus has the capacity to persist in skin [31, 37]. Thus, clinical relevance of detection of ParvoB19 DNA in skin biopsies outside of ASCT setting is under debate.

The value of histopathology for diagnosis of acute skin GvHD is limited. This is shown by multiple analyses which did not find an association between histological and definite clinical diagnosis of exanthema [27, 30, 38]. In the present study, diagnoses by histopathology were not associated with the result of the virus PCRs from the skin. Only in 6 out of 17 cases with positive virus PCR, a potential viral etiology of exanthema was reported by histopathology. This might be due to, for example, parallel occurrence of virus infection and GvHD but also due to the limited value of skin virus PCR analyses to identify the pathogenic cause of the rash. Serial biopsies could help to depict the sequence of virus and aGvHD occurrence.

In PCR negative cases, the results of CMV, EBV, and ParvoB19 immunohistochemistry were in concordance with the PCR results. Two of the 3 CMV PCR-positive cases showed positive intraepithelial staining by immunohistochemistry indicating local infection. However, the number of cases is too small to draw conclusions about the association of IHC with PCR. No EBV- and ParvoB19-positive case was available for immunohistochemistry.

The comparison of PCR results concomitantly analyzed from skin and blood revealed a strong association for the analyzed Herpesviridae. All cases with positive skin PCR for EBV and HHV6 and 67% with positive skin PCR for CMV had a positive blood PCR. This can be due to blood contamination of the biopsy by concomitant viremia even if skin was repetitively rinsed with saline before PCR in our analysis. Thus, detection of herpesvirus DNA in exanthematous skin might reflect viremia in some cases and could make skin-specific analysis dispensable. In concordance with that, Hentrich et al. [33] detected HHV6 DNA in the blood of 4 out of 6 patients with HHV6 in skin rash. Virus-specific immunohistochemistry could help to clarify the relevance of blood contamination. In contrast to herpes viruses, ParvoB19 was not detected in blood even if skin biopsy was positive both in our and in a PCR analysis of multiple viruses in repetitive blood samples after ASCT by Inazawa et al. [39].

The present study did not reveal any association of the detection of herpes virus DNA in skin with the clinical presentation of aGvHD or TRM. Further data regarding the value of skin PCR analyses in this setting are lacking. Many studies analyzed the association between virus detection in

blood and aGvHD or NRM after ASCT. Regarding CMV, GvHD and its treatment with IST is a risk factor for CMV reactivation and disease [40]. On the other hand, CMV infection was linked to increased risk of GvHD in infected tissues [41], and positive pretransplant CMV serostatus of donor and or recipient as well as CMV reactivation after ASCT are associated with a 3–15% increased NRM [42–44]. In line with this, in our analysis, NRM was 55% in patients without and 63% in patients with herpesvirus reactivations in peripheral blood (data not shown). The associations between aGvHD and NRM with HHV6 are conflicting. Some studies identified a significant correlation of HHV6 detection with the development of aGvHD and NRM [33, 45] but others did not [46, 47]. Looking at ParvoB19, we found that patients with detection of ParvoB19 DNA in skin had a significantly reduced NRM compared to patients with negative virus PCR. This association remained significant in multivariate survival analysis indicating ParvoB19 detection as an independent prognostic factor. However, the analysis can neither elucidate the pathomechanistic role of ParvoB19 detection nor reliably exclude ParvoB19 DNA detection as a surrogate for other factors associated with low NRM. Irrespective of comparable overall GvHD grade, IST was significantly less often increased or newly introduced in patients with positive ParvoB19 skin PCR. It is not clear if PCR results influenced the initiation of IST as these analyses are hampered by the retrospective design of the study. The pathophysiological relevance of ParvoB19 in skin rash after ASCT is uncertain: virus induced cytopathic effects, the response of allogeneic T cells to the virus or its residues as well as tissue-specific damage by cross-reactive T cells could subsequently cause the skin rash [48]. On the other hand, and as discussed above, ParvoB19 DNA can also be detected in healthy skin and might be a bystander of skin disease. Additional immunohistochemical studies of PCR-positive cases demonstrating ParvoB19 in endothelial cells of the dermis are needed to pathogenically link ParvoB19 to skin rash mimicking or triggering GvHD. However, detection of ParvoB19 DNA in skin rash helped to identify a subgroup of patients who could benefit from withholding IST. This is in line with the report of 2 patients with self-limiting GvHD-like skin rash and detection of ParvoB19 in affected skin as well as in bone marrow reported by Muetherig et al. [21].

We reported a rate of 79% of patients with exanthema. Klager et al. [6] reported a rate of 62% (152 of 243 patients) of skin rashes of any causes, only 17.7% being due to GvHD after T-cell depleted ASCT. The incidence of GvHD in non-T-cell-depleted ASCT is 40–60%, and about 70% of these patients have skin involvement [49]. In most studies

focused on GvHD, skin rashes stage 1 and due to reasons other than GvHD are not reported. Taken together, this indicates a reasonable rate of skin rashes in our study.

During the time of the analysis, virus PCRs were not performed for all skin biopsies. This could have biased the analyses. Furthermore, the kits used are only validated for plasma and whole blood. We therefore decided to evaluate only qualitative test results to exclude methodological bias. Quantitative assessment of viral load by kits validated for skin would help to better identify the relevance of the virus DNA detection, as high viral load in, for example, plasma is associated with morbidity and mortality after ASCT [40].

A detailed clinical description of the exanthema would further help to characterize the skin rashes. Due to the retrospective design, we do not have these data available.

In conclusion, the present study demonstrated that herpes virus DNA can be detected in about 12% and ParvoB19 DNA in 27% of skin rash after ASCT. Virus DNA detection was not associated with histological diagnosis or with exanthema characteristics. The strong direct association between PCR results from blood and skin for herpes viruses might reflect viremia. In contrast, detection of ParvoB19 in skin rash after ASCT was not associated with viremia and is associated with fewer IST initiations and decreased NRM; thus, it identifies patients with

excellent clinical course. These data provide the rationale to study the relevance of ParvoB19 DNA detection in skin rash after ASCT in larger ASCT cohorts.

Statement of Ethics

The analysis was performed in accordance with the standards of the local independent review board and the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

T.W. designed study, analyzed data, wrote manuscript. A.S. and K.L. collected and analyzed data and wrote the manuscript. M.B. and A.R. performed, evaluated, and interpreted immunohistochemistry and contributed to manuscript writing. L.P.M. designed the study and wrote the manuscript.

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