Received: 16 August 2023 | Revised: 22 November 2023 | Accepted: 27 November 2023

DOI: 10.1111/ejh.14147

ORIGINAL ARTICLE

European Journal of Haematology

WILEY

Risk factors for hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation in a letermovir-exposed CMV-free population receiving PTCy

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Abstract

Hemorrhagic cystitis (HC) is a highly impacting complication in allogeneic hematopoietic stem cell transplantation (HSCT), occurring in 12%–37% of patients. The impact of transplant- and patient-specific variables has been described, with a possible role for JCV and BKV, which may be cooperating with cytomegalovirus (CMV). Here, we analyze 134 letermovir-exposed, CMV-free patients, treated with the same cyclophosphamide-based graft-versus-host disease (GVHD) prophylaxis, describing risk factors for HC. The overall incidence of HC was 23%. Patients with HLA mismatched transplant, higher comorbidity score, and receiving three alkylating agents with TBF (thiotepa, busulfan, and fludarabine) conditioning regimen had a higher risk of HC in multivariate analysis (OR: 4.48, 6.32, and 1.32, respectively). A HC-score including male gender, TBF conditioning, and HLA-mismatch stratifies the risk of HC in the first 100 days after HSCT. The role of BKV and JCV was not highly impacting in those patients, suggesting a possible synergistic effect between CMV and JCV in causing HC. HC can be interpreted as the combination of patient-related factors, chemotherapy-related toxicities—especially due to alkylating agents—and immunological elements.

KEYWORDS
allogeneic hematopoietic stem cell transplantation, CMV, hemorrhagic cystitis, letermovir

Novelty statements

What is the new aspect of your work?

This manuscript explores for the first time the incidence and risk factors for hemorrhagic cystitis in a homogeneous population of patients receiving allogeneic hematopoietic stem cell

transplantation under letermovir CMV-prophylaxis, all with no CMV reactivation, given the same conditioning and GVHD prophylaxis.

What is the central finding of your work?

We found that TBF conditioning and HLA-mismatch provide augmented risk of HC in this population, while the role for JCV and BKV seems reduced when compared to the current literature.

What is (or could be) the specific clinical relevance of your work?

We propose a score able to stratify patients according to risk of HC. This could help- for example- in tailoring prophilactic strategies when administering PTCy.

1 | INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) can be a curative cell therapy approach for patients with many types of hematological cancer. HSCT is normally preceded by a conditioning chemotherapy regimen, which can be myeloablative (MAC) or reduced-intensity (RIC). The main toxicities are mainly due to regimen-related toxicities, including infectious risk, specific organ toxicities, and bleeding risk, together with the onset of acute or chronic graft-versus-host disease (a/c-GVHD). All those factors may contribute to Non-Relapse Mortality (NRM).

Among viral infections, cytomegalovirus (CMV) is one of the most impacting pathogens. Patients receiving GVHD prophylaxis with post-transplant cyclophosphamide (PTCy) show an incidence of CMV infection of 35%–76% of cases, with a full CMV-disease in up to 17% of cases.[1] In patients receiving an HSCT, CMV disease shows high mortality—up to 70%[2]—and tends to present more frequently with CMV pneumonitis, followed by gastrointestinal CMV disease, despite some cases of hemorrhagic cystitis (HC) due to CMV has also been reported in immunocompromised patients.[3–5]

Overall, an incidence of HC between 12.2% and 36.9% is generally reported in the setting of HSCT. Most commonly, HC occurs within the first month after HSCT. Studies on HC are generally retrospective and heterogeneous. With those limitations, some risk factors have been identified, such as pediatric age, haploidentical or mismatched unrelated donors, and myeloablative regimens.[6–8]

The role of reactivation of latent viruses like JCV and BKV has been widely associated with HC in the setting of HSCT. According to ECIL guidelines, BKV-related HC occurs in 8%–25% of pediatric and 7%–54% of adult patients receiving an HSCT. In an Indian report of 2022, Lionel and colleagues report BKV-related HC in 8% of patients, mainly favored by aGVHD and active disease.[9]

Both CMV and JCV can be latently hosted in renal cells. Viruses can not only generate pathologic effects on their direct target but can also favor other viruses to exercise their pathogenetic potential. When affecting the same patient, two viruses can pass through a viral interference mechanism, with one virus competing to suppress the replication of another, or through a synergy. A "virologic" synergy applies when viruses enhance the replication of other viruses, while viral noninterference—or "clinical" synergy—applies when viruses have different tissue tropisms on the same individual.[10] Viruses produce damage in different tissue and clinical relevance need to be framed case by case. The effects of CMV have been widely described in the setting of HSCT, resulting in one of the most widely impacting opportunistic infections in immunocompromised patients. Some data suggest the possibility that JCV may be transactivated by CMV with a virologic synergy.[11]

In a recent paper on 173 HSCT receiving various conditioning regiments and MTX-based GVHD prophylaxis, CMV infection was found to be the only independent variable significantly associated with HC in both univariate and multivariate analyses, while acute GVHD was a risk factor for CMV reactivation. In that study, 46% patients experienced CMV reactivation, while the incidence of HC was 30.6%. In that report, the incidence of HC among patients with no CMV reactivation was 9.5%, compared to 55.7% in patients with CMV infection ($p < .001$).[12]

Recently, the CMV-terminase complex inhibitor letermovir has been approved and the administration as prophylaxis in all CMV IgG-positive HSCT recipients has become available in Italy since September 2018.[13] In the Phase-3 trial, patients receiving letermovir as a prophylaxis had a CMV reactivation of 37%, compared to 60% of patients receiving a placebo.[14] Real-life experiences confirm the experimental data, with a low incidence of CMV infections and very low rates of CMV disease.[15]

2 | AIMS

Our study aimed to identify major risk factors for HC in a CMV-free/letermovir-exposed population receiving PTCy, focusing on the possible residual role of JCV and BKV. Together with this, we aimed to draw an identikit of the high-risk patient and to quantify this risk; other points of interest were to explore the impact of the HC event on NRM and OS and the role of regime-related toxicity of busulfan.

3 | METHODS

3.1 | Patients

All consecutive patients who received allogeneic HSCT between September 2018 and November 2022 were considered for the study. Patients with positive IgG serology for CMV received letermovir

240 mg daily dose, as per the European Medicines Agency (EMA) indications. For being included in the study, patients had to have received prophylaxis with letermovir, and GVHD prophylaxis with PTCy, mycophenolate mofetil, and cyclosporin-A. Patients receiving additional administration of anti-thymocyte globulins (ATG) were also included. The analysis was subsequently focused on patients with no CMV reactivation occurring after HSCT.

Baseline demographic and disease-related characteristics were collected, as well as transplant-related variables. Conditioning regimens are described in Supporting Information.

The comorbidity burden was quantified through the HCT-CI score as described by Sorror and colleagues.[16] HLA-matched transplants, both siblings and matched unrelated donors, were analyzed together and compared with HLA mismatched unrelated and haploidentical-related transplants. Conditioning regimen TBF, containing a double alkylating strategy (see Supporting Information), was compared versus all other strategies.

3.2 | HC and virology

Adverse events were accounted for when registered between days 0 and 100 from the transplant. HC and other adverse effects were graded according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0; when analyzing data, HC graded 0–1 was compared to HC of Grade 2 or more, in conformity to recent reports.[12,17] Data on the time of onset, duration, and treatment of HC were collected as well.

All patients were tested for the presence of JCV and BKV in urine at baseline before transplant hospitalization and repeated in case of urinary symptoms or HC; patients with a positive viruria were compared to the others. Viral nucleic acid extractions from urine samples were performed using automatic commercially extractor QIA Symphony (Qiagen GmbH, Hilden, Germany), and Real-time PCR amplifications were carried out with commercially available kits for JC and BK (Clonit Srl, Milano, Italy).

3.3 | Outcomes and statistics

The prognostic impact of categorical and continuous variables on HC was calculated with a logistic regression test, in univariate analysis.

Variables with an impact in univariate analysis were analyzed together in multivariate analysis. with the same technique. A *p*-value lower than .05 was considered significant. NRM and Relapse incidence were assessed with the Cumulative Incidence method, while Overall Survival (OS) was assessed with the Kaplan–Meier curves. The impact of a categorical variable on survival outcomes was assessed with the Cox regression method. Similarly, risk factors for time-to-HC (counted as time from transplant to HC, also called HC-free survival), were explored with Cox regressions. The impact of the HC-score (see later) on the development of HC was calculated with the cumulative incidence tool, considering time-to-HC as time from transplant to the onset of grade 2–5 HC. For cumulative incidence, the statistical power was determined with Gray's test. Statistical analysis was conducted with the NCSS 2020 Statistical Software (2020). NCSS, LLC, Kaysville, UT, USA. All patients provided informed consent for the anonymized use of data. This study was conducted in conformity with the Helsinki Declaration and approved by the local ethical committee (Prot.n.0016991/21).

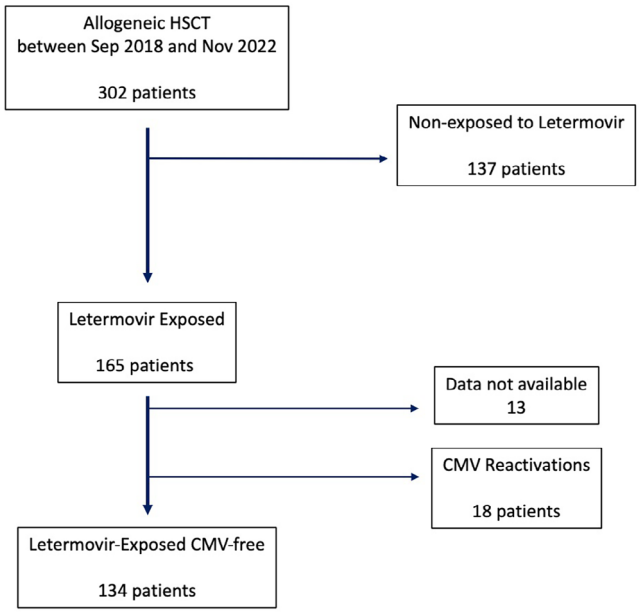

FIGURE 1 Patients disposition. The diagram shows how patients were screened and included in the study. All patients receiving allogeneic HSCT between September 2018—when letermovir was available—and November 2022 were screened. HSCT, hematopoietic stem cell transplantation.

4 | RESULTS

4.1 | Patients

Between September 2018 and November 2022, 302 patients received an allogeneic HSCT; 137 patients did not receive letermovir, in accordance with their CMV serologic status, and were therefore excluded. Eighteen letermovir-exposed patients experienced CMV reactivation after transplant: despite being ineligible for the study, their data were collected and only provided for a non-determining comparison, as they were not considered for the key aim of the study. Thirteen letermovir-exposed patients had not enough available data on their medical records and were also excluded, resulting in 134 patients finally being eligible for the study (Figure 1). Data from patients with CMV reactivations were all 134 patients had received PTCy-based GVHD prophylaxis, with 14 patients receiving additional ATG.

Patients were affected by acute myeloid leukemia in 53 (40%) cases, chronic myeloproliferative diseases in 38 (28%), acute lymphoid

TABLE 1 Characteristics of population, overall and analyzed by incidence of hemorrhagic cystitis, and univariate association in logistic regression.

	ALL *n*	Non - HC, *n* (%)	HC *n* (%)	Univariate *p* Value	Univariate odds ratio
	134	103 (77)	31 (23)		
Gender					
Female	69	58 (84)	11 (16)		
Male	65	45 (69)	20 (31)	**0.044**	2.34 (1.01–5.38)
Age					
Median (years)				**0.082**	1.03 (0.99–1.07)
<60 years	83	66 (79)	17 (21)		
≥60 years	51	37 (72)	14 (28)	0.35	1.46 (0.65-3-31)
HCT-CI					
Median				**0.013**	1.26 (1.04–1.53)
0–2	58	49 (84)	9 (16)		
>2	76	54 (71)	22 (29)	0.071	2.21 (0.9–5.2)
Disease					
ALL/AML/MDS	77	59 (76)	18 (24)		
MPN	38	27 (71)	11 (29)	0.518	1.33 (0.55–3.21)
Lymphoma	12	11 (92)	1 (8)	0.261	0.29 (0.03–2.46)
SAA	7	6 (86)	1 (14)	0.587	0.54 (0.06–4.84)
State at transplant					
First/second CR	48	35 (73)	13 (27)		
Active disease	47	15 (74)	12 (26)	0.912	0.95 (0.38–2.97)
Missing	34				
HLA					
Matched	95	80 (84)	15 (16)		
Mismatched	39	23 (59)	16 (41)	**0.002**	3.71 (1.59–8.62)
Conditioning					
Others	39	35 (90)	4 (10)		
TBF	95	68 (72)	27 (28)	**0.030**	3.47 (1.12–10.71)
Intensity					
RIC	41	32 (78)	9 (22)		
MAC	93	71 (76)	22 (24)	**0**.831	1.10 (0.45–2.65)
GVHD prophylaxis					
PTCY CSA MMF	120	94 (78)	26 (22)		
PTCY CSA MMF + ATG	14	9 (64)	5 (36)	0.245	2.00 (0.61–6.51)

Note: Values with $p < 0.05$ are highlighted in bold.

Abbreviations: ALL, acute lynmphoblastic anemia; AML, acute myeloid leukemia; ATG, anti thymocyte globulin; CR, complete remission; CSA, cyclosporin; GVHD, graft-versus-host disease; MAC, reduced intensity conditioning; MDS, myelodysplastic syndromes; MMF, mycofenolate mofetil; MPN, myeloproliferative neoplasia; PTCY, post transplant cyclophosphamide; RIC, reduced intensity conditioning; SAA, severe aplastic anemia; TBF, thiotepa-busulfan-fludarabine.

leukemia in 13 (10%), myelodysplastic syndromes in 12 (9%), lymphoproliferative diseases in 11 (8%), and aplastic anemia in 7 cases (5%). The donor was a matched sibling, a matched unrelated, or a mismatch unrelated/ haploidentical related in 27, 68, and 39 patients, respectively.

The median follow-up was 21 months (range 4–41). Overall, 31 (23%) patients experienced a clinically relevant episode of HC graded 2 or more, and 33 (25%) patients died for transplant-related or disease-related reasons (see later in the text).

Median engraftment occurred at Day 22 (range 15–99) and 24 (14–371) for PMN and platelets, respectively.

4.2 | Risk factors for HC

HC occurred in 31 (23%) patients, overall. The impact on HC of patients-related (age, gender, comorbidity score) and transplant-related (HLA matching, conditioning regimens, GVHD prophylaxis) variables

was explored (Table 1). Nine out of 31 (29%) patients with HC patients required continuous bladder irrigation, two (6%) patients required specific antiviral treatment, and one (3%) patient was treated with endoscopic diathermocoagulation. The median onset of HC was at Day 6 (range 1–84). Overall, 72% of HC occurred within Day 10 after transplant, 18% of cases occurred between Days 11 and 20, and only 10% of cases occurred after Day 21. CTCAE grading of HC was 1, 2, and 3 in 13%, 70%, and 17% of cases, respectively. The median duration of HC was 10 days (range 1–30 days) both for patients who received only supportive therapy and 10 days (range 3–66 days) for patients treated with bladder irrigation or other "active" interventions.

In 65 male patients, 31% experienced HC, compared to 16% of events in the female population ($p = .0044$, OR: 2.34, 95% CI: 1.01–5.38). A trend for higher risk of HC was observed for elderly patients ($p = .082$), but a clear cut-off was not identified. Similarly, we have observed a higher incidence of HC in patients with higher HCT-CI ($p = .013$), without identifying a clear cut-off value. Patients receiving HLA-mismatched (mismatched unrelated or haploidentical-related transplants) developed HC in 41%, with an OR of 3.71 ($p = 0.002$, 95% CI: 1.59–8.62) when compared to matched related or unrelated transplants, which had an HC in 16% of cases. Furthermore, patients receiving TBF conditioning had a higher risk of HC when compared to all other regimens (28% vs 10%, $p = .030$, OR: 3.47, 95% CI: 1.12–10.71).

The addition of ATG to standard triple PTCy-based GVHD prophylaxis did not influence the occurrence of HC (36% in ATG recipients versus 22% in patients, $p = .238$). When focusing on the most represented diseases, 25% of patients with acute myeloid leukemia and 30% of patients with chronic myeloproliferative diseases experienced HC. A lower incidence of HC seemed to affect patients with acute or chronic lymphoproliferative diseases (8% in acute lymphoblastic leukemia, 9% in lymphoma).

In multivariate analysis, we have tested together variables with a statistical p-value lower than .05. We found that increasing HCT-CI (OR: 1.32), HLA mismatch (OR: 4.48), and TBF conditioning (OR: 6.32) were independent risk factors for HC. Male gender kept a trend in predicting HC, while positive JCV lost its significance (Table 2).

With regards to HCT-CI, this variable was associated with HC as a continuous variable ranging from 0 to 8: to find a cut-off value capable to predict the onset of HC with sufficient specificity and sensibility, we have performed a ROC analysis, without finding an optimal cut-off that satisfied the criteria.

So, we moved to identify an identikit of high-risk patients: we have built a specific score (HC-score) assigning 1 point for each categorical variable with multivariate p-value lower than .1, namely male gender, HLA mismatch, and TBF conditioning. Consequently, patients were grouped into four groups (0–1–2–3) according to the HC score. In patients with an HC-score of 0, 1, 2, or 3, the incidence of HC was 0%, 10%, 30%, and 50% 30 days after transplant and 0%, 12%, 32%, and 58% 90 days after transplant ($p < .001$) (Figure 2A).

In all but 16 patients, BKV and JCV have been searched in urines. A positive viruria for BKV and JCV was found in 19/118 (16%) and in 40/118 (34%) patients, respectively, with 14/118 (12%) patients being positive for both viruses. A positive finding of JCV was associated with macroscopic HC (OR: 2.52, 95% CI: 1.07–5.91, $p = .039$), while no association with BKV was found. In the 18 patients with CMV reactivating during letermovir prophylaxis, the overall incidence of HC was 33%.

In our study population, we have found a cumulative incidence of 50% of HC among BKV or JCV-positive patients, compared to 27% in BKV-negative and 14% in JCV-negative patients (data not shown). Besides, we have found an overall incidence of HC in 18% of patients receiving PTCy-based prophylaxis not exposed to letermovir, without significant differences when compared to patients receiving letermovir (data not shown). Overall, when patients with or without letermovir are compared together, risk factors for survival without HC were BKV (OR: 2.29, $p = .005$) and JCV (OR: 2.70, $p < .001$), but not letermovir exposure (OR: 0.72, $p = .228$).

TABLE 2 Multivariate logistic regression: variables with statistical significance in univariate analysis are tested together for independence in predicting HC.

		Multivariate p value	Odds ratio (95% CI)
HCT-CI		**0.013**	1.32 (1.05–1.64)
Gender	Male	0.097	2.26 (0.86–5.96)
HLA	Mismatch	**0.003**	4.48 (1.64–12.17)
Conditioning	TBF	**0.012**	6.32 (1.48–26.89)
JCV	Detected	0.181	1.93 (0.73–5.12)

Note: Values with $p < 0.05$ are highlighted in bold.
Abbreviations: HC, hemorrhagic cystitis; TBF, thiotepa, busulfan, and fludarabine.

4.3 | HC and outcomes

Patients with HC had worse NRM and OS. The cumulative incidence of NRM at 90, 180, and 360 days after transplant was 5%, 10%, and 10% for patients with no HC compared to 3%, 12%, and 25% in patients who experienced HC ($p = .038$). Similarly, OS 90, 180, and 360 days after transplant was 95%, 87%, and 84% in non-HC patients versus 89%, 76%, and 54% in patients with HC ($p < .001$) (Figure 2B,C). No relevant difference in terms of relapses was observed among groups ($p = .126$).

The major causes of death were equally distributed between NRM and relapse (48% vs. 52%, $p = .386$). Active disease (42%), infections (18%), and GVHD (15%) were the leading causes of death, overall.

In multivariate analysis, the only independent risk factor for NRM was HLA-mismatch ($p = .001$, OR: 5.97, 95% CI: 1.95–18.29) with a trend for higher HCT-CI ($p = .060$), while independent risk factors for OS were elevated HCT-CI ($p = .026$, OR: 1.2, 95% CI: 1.02–1.42), HLA mismatch ($p = .019$, OR: 2.52, 95% CI: 1.16–5.50), and HC ($p = .019$, OR: 2.69; 95% CI: 1.17–6.20).

4.4 | Sub-analysis in patients receiving TBF

Ninety-five patients had received TBF conditioning regimen, with one, two, or three daily doses of 3.2 mg/kg busulfan in 18 (19%), 57 (60%),

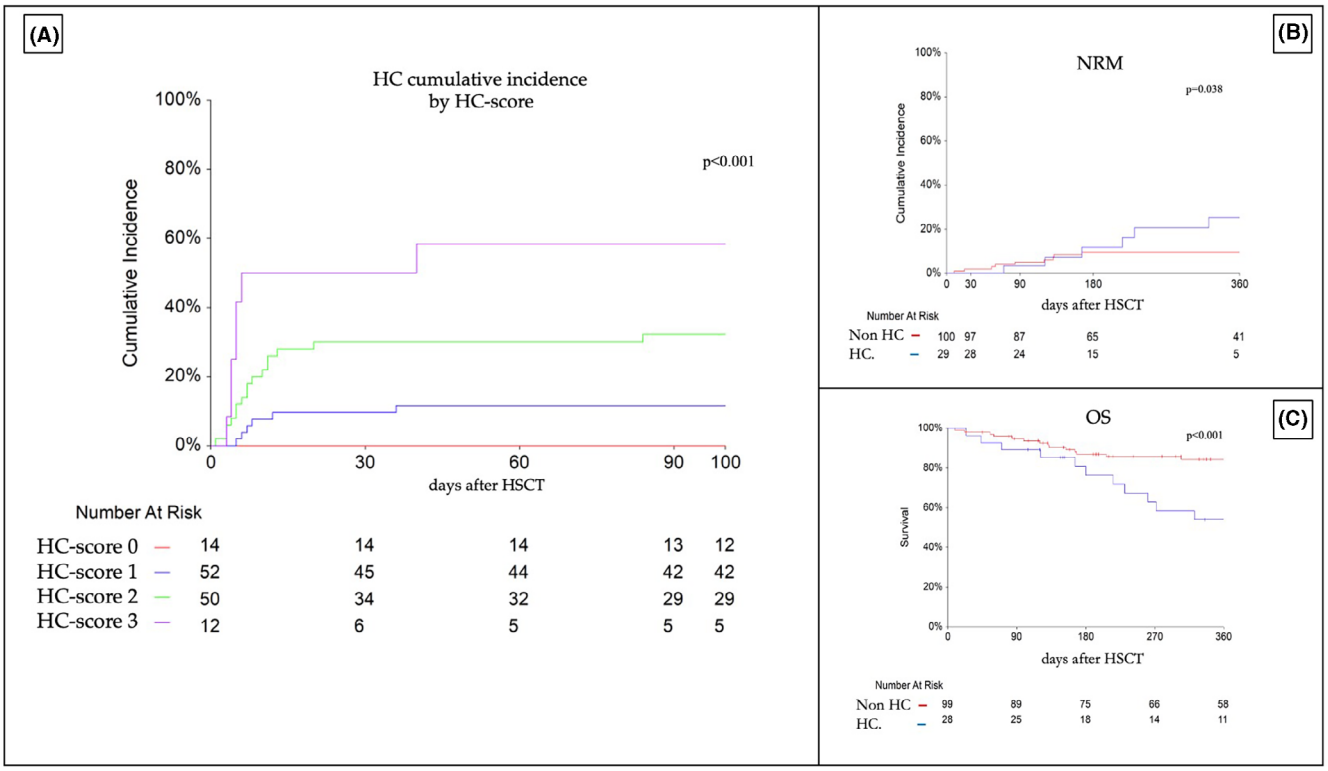

FIGURE 2 Risk factors and impact of HC on outcomes. (A) Incidence of HC by the HC score, assigning 1 point to each of the following: male gender, TBF conditioning, HLA mismatch. The majority of cases onset in the first 15 days after HSCT. (B) Non-relapse mortality in the first year after transplant according to HC (blue: no incidence of HC, red: HC occurred). (C) Overall survival in the first year after transplant according to HC (blue: no incidence of HC, red: HC occurred). HC, hemorrhagic cystitis; HSCT, hematopoietic stem cell transplantation; NRM, non-relapse mortality; OS, overall survival; TBF, thiotepa, busulfan, and fludarabine.

and 20 (21%) patients, respectively. Sixty patients (63%) had received their daily dose in one single administration, while 35 (37%) patients had received their daily dose in four fractioned doses of 0.8 mg/kg every 6 h. When analyzing the impact of cumulative busulfan dose, having received a total dose of 6.4 mg/kg or 9.6 mg/kg did not augment the risk of HC ($p = .5464$ and $p = .825$, respectively). Similarly, the fractionated busulfan schedule did not change the risk of developing HC when compared to the single-dose schedule ($p = .980$).

5 | DISCUSSION

HC is a highly impacting adverse effect after HSCT. Despite the pathogenesis of HC is thought to be multifactorial, a growing focus on risk factors may identify some variables like age, type of chemotherapy exposure, or HLA matching.

Latent CMV, as well as JCV and BKV, can be harbored in renal cells. This has led to investigate the relationship between those viruses. Lionel et al. reported a univariate correlation between CMV reactivation and BKV-related HC, which was not independent of aGVHD.[9] As aGVHD is often related to other predisposing factors for HC, such as additional immunosuppression and mismatch donor, this loss of statistical power may not be fully relevant. Interestingly, in an in-vitro experience on human fibroblasts—which are normally nonpermissive for replication of JCV alone—the coinfection with CMV led to considerable JCV DNA replication. Conversely, the inhibition of CMV replication with ganciclovir produced a concomitant inhibition of JCV replication.[18]

According to Zhang and colleagues, ATG did not impact HC. In that report, HLA mismatch predicted HC, but not in multivariate analysis. In that report, for all receiving MTX-based prophylaxis, the incidence of HC in CMV-negative patients was 9.5%. In our experience, the incidence of HC in CMV-negative patients was 23%. It is notable that our population received uniformly PTCy-based prophylaxis. As cyclophosphamide is considered a risk factor for HC, this difference appears justified by the different GVHD prophylaxis strategies. In a previous description, we have reported that cyclophosphamide-based GVHD prophylaxis, higher age, male gender, HLA mismatch, and myeloablative conditioning may raise the risk for HC in univariate analysis, despite age alone resulting independent.[17] In the same paper, we report a role of JCV and BKV infection in the occurrence of HC, as well as a potential role for prostatic hypertrophy in the male population, while no role for comorbidities was found.

When deciding to observe the presentation of HC in a letermovir-exposed CMV-free population, we aimed also to explore if a favoring role of CMV on other viruses, such as BKV and JCV, can be clinically observed. When no replication CMV occurs, as in our population, there is no independent pathogenic role for JCV or BKV in generating

HC. Thus, it is possible to hypothesize that those highly endemic viruses may be favored by CMV as a co-pathogenic agent. This hypothesis is enforced by some experimental or epidemiological findings, often coming from the experience on solid organ transplants.[19,20] In our report, we found that the role of viruses appears as non-leading risk factor for HC when CMV is absent, and the incidence of HC seemed up to 50% in non-letermovir exposed patients with at least one replicating virus between BKC and JCV. A second hypothesis is that letermovir may produce an inhibitory effect also on BKV and JCV, but this has not been proven. To make this scenario less probable, it should also be noticed that we have observed in our population a similar- and not reduced-incidence of JCV/BKV viruria compared to other studies.

HLA matching remained a determining risk factor for the development of HC. The impact of this variable may sustain different mechanisms. First, haploidentical transplants and unrelated mismatched transplants are at higher risk for developing GVHD. In this scenario, mucosae are more often impaired, and patients suffer more frequently from low peripheral blood counts and tend to receive augmented immunosuppression. All those elements confer an evident higher risk of HC. Second, and related to this point, immune surveillance may be weaker for mismatched transplants when compared to HLA-matched. This may also enforce the contribution of infections in the pathogenesis of HC. The role of alkylating-containing regimens, such as TBF combined with PTCy prophylaxis, was found to confer a higher risk of HC in our patients. Alkylating agents act by intercalating alkyl groups between the double helix of DNA, thus damaging replication processes and inducing cell death. Thiotepa and its metabolites N, N′,N″-triethylenephosphoramide (TEPA), N,N′-diethylene, N″-2-chloroethyl phosphoramide (monochloroTEPA), and thioTEPA—mercapturate, have been founded in urines of patients receiving intravenous thiotepa; the same was true also for busulfan and its metabolites 3-hydroxy sulfolane, tetrahydrothiophene 1-oxide, and sulfolane, and for cyclophosphamide and its metabolites: especially when used together, those substances may result in altered and damaged urothelium and favored occurrence of HC.[21–24]

HC resulted as a good alarm tool for identifying patients at high risk of NRM and overall mortality. With regards to NRM, HC loses its role in multivariate analysis, revealing that it may simply represent the epiphenomenon of coexisting transplant-related and patients-related risk factors for NRM: therefore only HLA-mismatch, and less clearly HCT-CI, resist in multivariate analysis as true predictors of NRM. The same data can be exported to the OS; however, in this scenario, HC remains an independent factor. In such instances, individuals with HC may face an elevated risk of mortality, both due to NRM, as previously discussed, and an increased susceptibility to relapse. This is attributed to lower platelet counts observed in relapsed patients, either due to the underlying disease or subsequent treatments. Consequently, on the whole, patients with HC exhibit a tendency toward poorer survival.

While offering valuable insights, we acknowledge the study's limitations. The retrospective, single-center design and limited patient cohort, combined with the highly specific characteristics of the study population, raise caution regarding the generalizability of the findings.

Overall, HC can be interpreted as the combination of patient-related factors and frailties, together with chemotherapy-related toxicities—especially due to alkylating agents—and immunological elements. Our single-center experience on a CMV-negative population exposed to letermovir seems to suggest that the pathogenetic role of BKV and JCV may be modest when CMV is inactive thanks to letermovir prophylaxis, with a leading role of HLA mismatch, male gender, and double alkylating TBF conditioning.

AUTHOR CONTRIBUTIONS

Conceptualization: Simona Sica and Eugenio Galli. *Methodology*: Simona Sica, Rosaria Santangelo, Elisabetta Metafuni, and Eugenio Galli. *Validation*: Patrizia Chiusolo and Federica Sorà. *Formal Analysis*: Eugenio Galli. *Resources*: Simona Sica. *Data curation*: Eugenio Galli, Andrea Mattozzi, and Elisabetta Metafuni. *Writing—original draft preparation*: Eugenio Galli. *Writing—review and editing*: Simona Sica, Federica Sorà, Maria Assunta Limongiello, Carlo Gandi, Sabrina Giammarco, Andrea Bacigalupo, and Patrizia Chiusolo.

ACKNOWLEDGMENTS

The authors acknowledge the support of "Centro di Ricerca sulle cellule staminali emopoietiche e le terapie cellulari 'Università Cattolica del Sacro Cuore, Roma'" research funding.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

For data availability, please contact the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Galli E, Metafuni E, Gandi C, et al. Risk factors for hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation in a letermovir-exposed CMV-free population receiving PTCy. *Eur J Haematol*. 2024;1-8. doi:10.1111/ejh.14147