ORIGINAL ARTICLE

Herpes zoster in kidney transplant recipients: protective effect of anti-cytomegalovirus prophylaxis and natural killer cell count. A single-center cohort study

Mario Fernández-Ruiz[1], Julia Origüen[1], David Lora[2], Francisco López-Medrano[1], Esther González[3], Natalia Polanco[3], Rafael San Juan[1], Tamara Ruiz-Merlo[1], Patricia Parra[1], Amado Andrés[3] & José María Aguado[1]

1 Unit of Infectious Diseases, Hospital Universitario "12 de Octubre", Instituto de Investigación Hospital "12 de Octubre" (i+12), School of Medicine, Universidad Complutense, Madrid, Spain
2 Unit of Clinical Research, Instituto de Investigación Hospital "12 de Octubre" (i+12), Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
3 Department of Nephrology, Hospital Universitario "12 de Octubre", Instituto de Investigación Hospital "12 de Octubre" (i+12), Madrid, Spain

Correspondence
Mario Fernández-Ruiz MD, Unit of Infectious Diseases, Hospital Universitario "12 de Octubre", Centro de Actividades Ambulatorias, 2ª planta, bloque D. Avda. de Córdoba, s/n. Postal code 28041, Madrid, Spain.
Tel: +34 913908000;
Fax: +34 914695775;
e-mail: mario_fdezruiz@yahoo.es

SUMMARY

Despite its impact on quality of life and potential for complications, specific risk and protective factors for herpes zoster (HZ) after kidney transplantation (KT) remain to be clarified. We included 444 patients undergoing KT between November 2008 and March 2013. Peripheral blood lymphocyte subpopulations were measured at baseline and months 1 and 6. The risk factors for early (first post-transplant year) and late HZ (years 1–5) were separately assessed. We observed 35 episodes of post-transplant HZ after a median follow-up of 48.3 months (incidence rate: 0.057 per 1000 transplant-days). Median interval from transplantation was 18.3 months. Six patients (17.1%) developed disseminated infection. Postherpetic neuralgia occurred in 10 cases (28.6%). The receipt of anti-cytomegalovirus (CMV) prophylaxis with (val)ganciclovir decreased the risk of early HZ [adjusted hazard ratio (aHR): 0.08; 95% CI: 0.01–1.13; *P*-value = 0.062], whereas the natural killer (NK) cell at month 6 was protective for the occurrence of late HZ [aHR (per 10-cells/μl increase): 0.94; 95% CI: 0.88–1.00; *P*-value = 0.054]. In conclusion, two easily ascertainable factors (whether the patient is receiving anti-CMV prophylaxis and the NK cell count at month 6) might be potentially useful to tailor preventive strategies according to individual susceptibility to post-transplant HZ.

Transplant International 2018; 31: 187–197

Key words
anti-cytomegalovirus prophylaxis, herpes zoster, immunological monitoring, incidence, kidney transplantation, risk factors

Received: 7 May 2017; Revision requested: 20 June 2017; Accepted: 18 September 2017

Introduction

The reactivation of varicella-zoster virus (VZV) latent infection from cranial nerve or dorsal root ganglia in form of herpes zoster (HZ) represents a notable source of decrease in health-related quality of life in the general population, particularly when followed by postherpetic neuralgia (PHN) [1]. Patients undergoing solid organ transplantation (SOT) face an increased risk of HZ due to the deleterious effect of post-transplant immunosuppression and are more prone to develop potentially life-threatening forms of disease [2–4].

doi:10.1111/tri.13076

Preventive strategies for post-transplant HZ have been limited, as the use of the live attenuated virus vaccine derived from the Oka VZV strain is contraindicated in immunocompromised hosts [5]. The advent of a new subunit vaccine containing the VZV glycoprotein E and the $AS01_B$ adjuvant system (HZ/su) has opened promising perspectives in the prevention of this condition [6]. No safety or efficacy data are available so far for the SOT population, although clinical trials in kidney transplant (KT) recipients are ongoing (ClinicalTrials.gov identifier: NCT02058589).

Previous studies focused on HZ among KT recipients were performed more than 15 years ago (limiting its applicability to contemporary immunosuppression practices) [2], jointly considered different SOT populations [3,7] or suffer from short post-transplant follow-up [4,8] or lack of data granularity [3]. Most importantly, none of them investigated any surrogate of the VZV-specific host's immune response. By gaining more insight into the factors modulating the susceptibility to this complication, targeted preventive approaches might be focused on those recipients most expected to benefit from certain measures. Thus, we aimed at investigating the incidence and risk factors for HZ in a large single-center cohort of KT recipients with long-term follow-up by taking advantage from the immune monitoring program to which these patients were subjected.

Materials and methods

Study population and design

Consecutive adult patients (≥18 years) undergoing KT at our center between November 2008 and March 2013 were included in an immunological monitoring program, as detailed elsewhere [9,10]. Patients with primary immunodeficiency syndrome, human immunodeficiency virus (HIV) infection, simultaneous pancreas-kidney transplantation, or primary graft nonfunction were excluded, as were those who died or developed graft loss requiring graft removal within the first week. Participants were enrolled at the time of transplantation and followed up until March 2016 (unless death or graft loss occurred earlier). They underwent an immune status assessment at scheduled times (baseline [within 12 h before transplantation] and post-transplant months 1 and 6 [±2 weeks]) that included the enumeration of peripheral blood lymphocyte subpopulations (PBLSs), as detailed below and elsewhere [10]. The research was performed in accordance with the ethical standards defined by the Helsinki Declaration and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. The local Ethics Committee approved the study protocol, and written informed consent was obtained from all participants.

Pretransplant, perioperative, and post-transplant variables were prospectively recorded by means of a standardized case report form. Additional information on post-transplant HZ episodes was specifically gathered by retrospective chart review. To ensure adequate capture of those mild cases of HZ that could have been treated in an outpatient setting, electronic medical records of primary care physicians were also screened through the Madrid Electronic Health Record (HORUS) system, which integrates comprehensive patient information from the entire regional healthcare system. The *primary study outcome* was the occurrence of post-transplant HZ. We also assessed the occurrence of complicated post-transplant HZ and PHN as *secondary outcomes*.

Definitions

Post-transplant HZ was clinically diagnosed on the basis of the appearance of a characteristic pruritic papulovesicular rash with a dermatomal distribution, preceded or not by prodromal pain. Virological (i.e., PCR or cell culture) or immunohistochemical confirmation was not required. Disseminated HZ was defined by the presence of typical lesions involving ≥2 noncontiguous dermatomes or varicella-like syndrome (disseminated vesicular rash with lesions in various stages of evolution). PHN was defined as pain arising or persisting in the areas originally affected by HZ for at least 3 months after the onset of the rash. Complicated HZ was defined by any of the following events: HZ ophthalmicus (conjunctivitis, keratitis, uveitis, or ocular cranial nerve palsy), central nervous system involvement (meningitis, encephalitis, myelitis, or stroke attributable to necrotizing arteritis), or any other visceral involvement with virological or immunohistochemical documentation.

Immunosuppression and prophylaxis regimens

Immunosuppression and prophylaxis regimens used have been previously described [9,10] and are detailed as Appendix S1. Regarding anti-cytomegalovirus (CMV) prevention strategies, antiviral prophylaxis with intravenous ganciclovir (GCV) (5 mg/Kg daily) followed by oral valganciclovir (val-GCV) (900 mg daily, with dose adjusted for renal function) was scheduled for 6 months in the presence of donor/recipient serological mismatch (D+/R−). Seropositive patients (R+) receiving induction therapy with

polyclonal antithymocyte globulin (ATG) were scheduled to receive CMV antiviral prophylaxis for 3 months [11,12]. The initiation of pre-emptive therapy in intermediate-risk patients (R+ not receiving ATG) with documented CMV infection was suggested to the attending physicians, although not systematically performed. Acyclovir prophylaxis was not administered for patients not at risk for CMV infection (D−/R−). No patient received the live attenuated VZV vaccine either prior to or after transplantation.

Enumeration of PBLSs

Whole blood samples were collected into Vacutainer tubes containing EDTA as anticoagulant and analyzed within 18 h. Whole blood (50 μl) was stained with 10 μl of BD Multitest six-color TBNK reagent in TruCount tubes for 15 min. Red blood cells were lyzed using fluorescence-activated cell sorting lysing solution. Determination of lymphocyte subsets ($CD3^+$, $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, and $CD3^-$ $CD56^+$ $CD16^+$ natural killer [NK] cells) was performed with a standard FACSCanto II flow cytometer using a single-platform technology and the following combination of fluorochrome-labeled monoclonal antibodies: FITC-labeled CD3 (clone SK7), APC-labeled CD4 (clone SK3), PE-labeled CD8 (clone SK1), PerCP-labeled CD45 (clone 2D1 [HLe-1]), PE-labeled CD16 (clone B73.1), PE-labeled CD56 (clone NCAM 16.2), and APC-labeled CD19 (clone SJ25C1) [13]. Data were analyzed by the FACSCanto clinical software (BD Biosciences, San Jose, CA, USA). It should be noted that this protocol does not allow the separate quantification of the different NK cell subpopulations on the basis of the relative expression of surface markers CD16 and CD56 ($CD56^{dim}$ $CD16^+$ and $CD56^{bright}$ $CD16^+$ subsets) and that the $CD56^{bright}$ $CD16^-$ NK cells were not captured, although this subset only represents ≈5% of the circulating NK cells and lacks antibody-dependent cellular cytotoxicity [14].

Statistical analysis

Quantitative data were shown as the mean ± standard deviation (SD) or the median with interquartile ranges (IQR), whereas qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the chi-squared test. Student's *t*-test, Mann–Whitney *U*-test or Wilcoxon signed-rank test were applied for continuous variables, as appropriate. The optimal cutoff value (i.e., that with the highest value for the combined sensitivity and specificity) of selected PBLSs to predict post-transplant HZ was identified by the Youden's index or *J* statistic (*J* = sensitivity + specificity − 1) [15] in the receiver operating characteristics (ROC) curve estimated for time-to-event outcomes. In addition, resampling (bootstrapping) and cross-validation methods were used to assess the variability of the proposed cutoff and to correct for the over-optimism of the risk estimates, in an attempt to minimize the inherent bias induced by model building and estimation of effects within the same data set [16]. We applied the "minimum *P*-value method" (in which the cutoff is selected such that the *P*-value for the comparison of observations below and above such a threshold is a minimum) to confirm the validity of the chosen value. The minimum *P*-value was corrected for overestimation by means of the so-called shrinkage factor. Cross-validation was then carried out among 800 bootstrap samples of equal size generated by sampling with replacement. In each of these bootstrap samples, the optimal cutoff value was also obtained.

The incidence of post-transplant HZ was plotted by Kaplan–Meier curves and differences between groups compared with the log-rank test. Time-to-first-event Cox regression models were used to identify independent risk factors for the occurrence of post-transplant HZ (primary study outcome), with death and graft loss considered as competing risk events. To adequately assess the effect of anti-CMV prophylaxis (that is only administered for 3–6 months), and considering that the overall amount of immunosuppression usually decreases over time, we constructed two separate models to predict the occurrence of HZ during the first 12 months, and thereafter until year 5 after transplantation (early and late HZ, respectively). Only the first episode of HZ recorded for an individual patient within each of these periods was included in the analysis. Certain variables were modeled as time-dependant covariates and forced into the models regardless of its univariate significance. Associations were expressed as HRs with 95% confidence intervals (CIs). All the significance tests were two-tailed. Statistical analysis was performed using SPSS v. 20.0 (IBM Corp., Armonk, NY, USA) and R software (R Foundation for Statistical Computing, Vienna, Austria), whereas graphics were generated with PRISM v. 6.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Baseline characteristics and incidence of post-transplant HZ

We included 444 patients whose demographics and baseline characteristics are shown in Table 1. The

Table 1. Clinical characteristics of the study cohort (n = 444).

Variable	
Age of recipient, years [mean ± SD]	55.0 ± 14.9
Gender (male) [*n* (%)]	277 (62.4)
Pretransplant chronic comorbidities [*n* (%)]	
Smoker (current or previous)	161 (38.1)
Hypertension	357 (80.4)
Diabetes mellitus	117 (26.4)
Coronary heart disease	37 (8.3)
Chronic obstructive pulmonary disease	52 (11.7)
Peripheral arterial disease	35 (7.9)
Chronic liver disease	21 (4.7)
Pretransplant immunosuppressive therapy [*n* (%)]	53 (11.9)
Previous solid organ transplantation [*n* (%)]	91 (20.5)
Underlying end-stage renal disease [*n* (%)]	
Glomerulonephritis	96 (21.6)
Diabetic nephropathy	80 (18.0)
Policystosis	57 (12.8)
Nephroangiosclerosis	56 (12.6)
Chronic interstitial nephropathy	39 (8.8)
Reflux nephropathy	16 (3.6)
Congenital nephropathy	15 (3.4)
Unknown	38 (8.6)
Other	47 (10.5)
Pretransplant HCV seropositivity [*n* (%)]	34 (7.7)
Pretransplant VZV seropositivity [*n* (%)]*	420 (95.5)
Pretransplant CMV serostatus [*n* (%)]	
D+/R+	291 (65.5)
D−/R+	59 (13.3)
D unknown/R+	58 (13.1)
D+/R−	30 (6.8)
D−/R−	6 (1.4)
Pretransplant renal replacement therapy [*n* (%)]	418 (94.1)
Hemodialysis	362 (81.5)
Continuous ambulatory peritoneal dialysis	56 (12.6)
Dialysis vintage, days [median (IQR)]	648 (314–1311)
Age of donor, years [mean ± SD]	53.4 ± 16.6
Type of donor [*n* (%)]	
DBD donor	285 (64.2)
DCD donor	145 (32.7)
Living donor	14 (3.2)
Cold ischemia time, hours [mean ± SD]	16.8 ± 6.5
Induction therapy [*n* (%)]	
None	75 (16.9)
ATG	216 (48.6)
Basiliximab	153 (34.5)
Alefacept	1 (0.2)
Primary immunosuppression scheme [*n* (%)]	
Tacrolimus, mycophenolate mofetil, and steroids	393 (88.5)
Tacrolimus, azathioprine, and steroids	47 (10.6)
Tacrolimus, everolimus, and steroids	2 (0.5)
CMV antiviral prophylaxis [*n* (%)]	214 (48.2)
Scheduled for 3 months	185 (41.7)
Scheduled for 6 months	29 (6.5)
Post-transplant complications [*n* (%)]	
Reintervention within the first month	51 (11.5)
Renal artery stenosis	76 (17.1)
De novo post-transplant diabetes mellitus	63 (14.2)
≥1 episode of acute graft rejection	111 (25.0)
2 episodes	16 (3.6)

ATG: antithymocyte globulin; CMV: cytomegalovirus; D: donor; DBD: donation after brain death; DCD: donation after circulatory death; HCV: hepatitis C virus; IQR: interquartile range; KT: kidney transplant; R: recipient; SD: standard deviation; VZV: varicella-zoster virus.

*Data available for 440 patients.

median follow-up was 48.3 months (IQR: 22.5–66.4). One- and five-year survival rates were 91.0% and 84.0%, respectively, and 56 patients (12.6%) died at a median interval of 427.0 days after transplantation. Death-censored graft survival at one and five years was 96.0% and 85.0%.

Thirty-five patients were diagnosed with 35 episodes of post-transplant HZ, accounting for a cumulative incidence at the end of follow-up of 7.9% (95% CI: 5.4–10.4%) and an incidence rate of 0.057 per 1,000 transplant-days (95% CI: 0.041–0.079). The median interval between transplantation and diagnosis was 18.3 months (95% CI: 6.1–34.2). Most cases (68.6% [24/35]) occurred beyond the first post-transplant year, although the incidence rates for both periods were roughly similar (0.066 and 0.048 cases per 1000

transplant-days, for early and late HZ, respectively; *P*-value = 0.417).

Clinical features of post-transplant HZ

The clinical characteristics of the episodes of post-transplant HZ are detailed in Table 2. Most of them involved a single dermatome and had a thoracic distribution, although almost one-fifth of patients developed a disseminated (i.e., multimetameric or varicella-like) infection. The clinical characteristics of these six episodes of disseminated HZ are detailed in Table S1. Patients with disseminated HZ were more likely to have been previously given steroid boluses as antirejection therapy, although the difference did not attain statistical significance (50.0% [3/6] vs. 20.7% [6/23]; *P*-value = 0.162). They also exhibited a lower $CD8^+$ T-cell count in the preceding monitoring point as compared with those with uncomplicated HZ (0.165 vs. 0.426×10^3 cells/μl, respectively; *P*-value = 0.022). The majority of episodes of post-transplant HZ were managed in an outpatient setting, and oral famciclovir was the most commonly administered antiviral drug.

The clinical course was complicated with PHN in approximately one-third of cases. Patients with PHN trended to be older (64.9 ± 13.2 vs. 57.5 ± 15.3 years; *P*-value = 0.189) and more likely to have received induction therapy with ATG (60.0% [6/10] vs. 32.0% [8/17]; *P*-value = 0.151) compared to those remaining free of this complication. There were no differences with regard to the type of antiviral agent used. One single patient—a 71-year-old male who received his first KT 42 months before—had a complicated infection in form of HZ ophthalmicus with keratitis and loss of vision. Of note, he had been previously diagnosed with CMV syndrome and visceral leishmaniasis. There were no recurrences of HZ during the follow-up.

Table 2. Characteristics of the episodes of post-transplant HZ (*n* = 35).

Variable	
Dermatomal distribution [*n* (%)]	
Thoracic	12 (34.3)
Disseminated	6 (17.1)
Facial	4 (11.4)
Abdominal	4 (11.4)
Lumbosacral	3 (8.6)
Cervical	1 (2.9)
Unknown	5 (14.3)
Pretransplant history of HZ [*n* (%)]	1 (2.0)
Requirement for hospital admission [*n* (%)]	8 (22.9)
Initial treatment [*n* (%)]	
IV acyclovir	8 (22.9)
Oral acyclovir	8 (22.9)
Famciclovir	9 (25.7)
Brivudin	3 (8.6)
Valacyclovir	2 (5.7)
Unknown	5 (14.3)
Development of PHN [*n* (%)]	10 (28.6)
Complicated forms [*n* (%)]	1 (2.0)
HZ ophthalmicus	1 (2.0)

HZ: herpes zoster; IV: intravenous; PHN: postherpetic neuralgia.

Risk factors for early post-transplant HZ

The results of the uni- and multivariate models of predicting factors for the occurrence of HZ during the first 12 months (early HZ) are depicted in Table 3. Patients suffering from HZ within this period were older and less likely to have received induction therapy with ATG and CMV antiviral prophylaxis compared to the remaining recipients, whereas no differences were found in PBLS counts at baseline (data not shown) or month 1. There were no significant differences between both groups in the 12-month cumulative incidence of acute graft rejection, and no significant impact was observed either when this variable was included as a time-dependant covariate in the model with death and graft loss treated as competing risk events. On the other hand, the receipt of CMV antiviral prophylaxis exerted a near significant protective effect (adjusted HR [aHR]: 0.08; 95% CI: 0.01–1.13; *P*-value = 0.062). In accordance with this finding, early HZ-free survival was significantly lower among those patients that were not given prophylaxis (12-month survival rate: 94.0% vs. 100.0%; log-rank test *P*-value = 0.005) (Fig. 1).

Risk factors for late post-transplant HZ

We next assessed the risk factors for the development of HZ between years 1 and 5 after transplantation (late HZ). As shown in Table 4, neither the receipt of CMV antiviral prophylaxis nor the occurrence of acute rejection had a significant impact on the incidence of HZ during this period. This lack of association persisted when rejection was analyzed as a time-dependant covariate (data not shown). When comparing PBLSs at months 6, patients with late post-transplant HZ had a significantly lower count of NK cells than those without. The area under the ROC curve for predicting this complication was 0.669 (95% CI: 0.537–0.802) (Fig. S1).

Table 3. Cox regression models for the occurrence of post-transplant HZ throughout the first 12 months after transplantation (i.e., early HZ), with death and graft loss considered as competing risk events.

				Univariate analysis			Multivariate analysis		
	No early HZ (n = 433)	Early HZ (n = 11)	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Age of recipient, years [mean ± SD]	54.8 ± 14.9	63.0 ± 10.8	0.071	1.04	1.00–1.08	0.028	-	-	-
Gender (male) [n (%)]	269 (62.1)	8 (72.7)	0.547						
Pretransplant diabetes mellitus [n (%)]	114 (26.3)	3 (27.3)	1.000						
Pretransplant immunosuppressive therapy [n (%)]	52 (12.0)	1 (9.1)	1.000						
Previous solid organ transplantation [n (%)]	89 (20.6)	2 (18.2)	1.000						
Pretransplant VZV seropositivity [n (%)]*	406 (94.6)	10 (90.9)	0.464						
CMV serostatus D+/R− [n (%)]	30 (6.9)	0 (0.0)	1.000						
Age of donor, years [mean ± SD]	53.2 ± 16.6	60.6 ± 14.7	0.147						
DCD donor [n (%)]	143 (33.0)	2 (18.2)	0.516						
Induction therapy with ATG [n (%)]	214 (49.4)	2 (18.2)	0.041	0.23	0.04–1.06	0.060	-	-	-
CMV antiviral prophylaxis [n (%)]†	218 (50.7)	1 (9.1)	0.006	0.09	0.01–0.77	0.027	0.08	0.01–1.13	0.062
PBLSs at month 1, $\times 10^3$ cells/μl [median (IQR)]									
$CD3^+$ T-cell count	0.543 (0.216–1.125)	0.705 (0.170–1.030)	0.857						
$CD4^+$ T-cell count	0.270 (0.073–0.722)	0.342 (0.098–0.548)	0.956						
$CD8^+$ T-cell count	0.218 (0.086–0.370)	0.147 (0.055–0.354)	0.633						
B-cell count	0.138 (0.069–0.244)	0.075 (0.049–0.175)	0.138						
NK cell count	0.050 (0.022–0.140)	0.047 (0.016–0.091)	0.764						
Acute rejection during the first 12 months [n (%)]†	92 (21.2)	4 (36.4)	0.263	1.84	0.50–6.74	0.322	-	-	-

ATG: antithymocyte globulin; CMV: cytomegalovirus; D: donor; DCD: donation after circulatory death; HR: hazard ratio; IQR: interquartile range; KT: kidney transplant; PBLSs: peripheral blood lymphocyte subpopulations; R: recipient; SD: standard deviation.

*Data available for 440 patients.

†Time-dependant covariate.

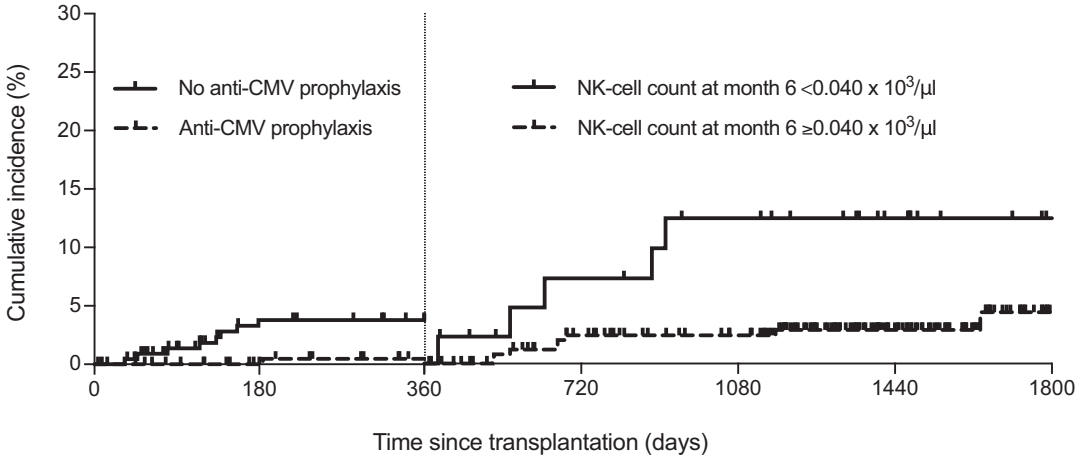

Figure 1 Cumulative incidence curves for post-transplant late HZ according the receipt of CMV antiviral prophylaxis (follow-up truncated at 12 months [i.e., early HZ]) (log-rank test *P*-value = 0.005) and NK cell count at month 6 after transplantation (follow-up truncated at 5 years [i.e., late HZ]) (log-rank test *P*-value = 0.015). CMV: cytomegalovirus; NK: natural killer; HZ: herpes zoster.

Such an estimate was roughly similar when the ROC curve was plotted for time-to-event outcomes (0.665; 95% CI: 0.521–0.809). The kinetics of NK cell counts demonstrates a notable decrease at month 1 from baseline that only partially recovered at month 6 (Fig. S2). Recipients suffering from late HZ were also more likely to have been diagnosed with CMV disease within the first 6 months after transplantation. Only the NK cell count remained (aHR [per 10-cells/µl increase]: 0.94; 95% CI: 0.88–1.00; *P*-value = 0.054) in the final multivariate model in which the use of CMV antiviral prophylaxis was forced despite its lack of univariate significance.

To further refine the analysis of the predictive accuracy of this parameter, we explored different thresholds (including the median and first and third quartiles) and selected a cutoff value of 0.040×10^3 NK cells/µl as that with the highest Youden's index (sensitivity: 42.9%; 95% CI: 17.7–71.1; specificity: 76.0%; 95% CI: 70.7–80.9) (Table S2). Such cutoff offered the best positive predictive value (11.4%; 95% CI: 5.7–21.5) without significantly compromising negative predictive value (96.5%; 95% CI: 94.9–97.6). In addition, this threshold was also selected as the optimal according to the "minimum *P*-value method" (unadjusted HR: 3.26; 95% CI: 1.11–9.55; *P*-value = 0.031), even after correction for over-optimism (shrinkage factor: 0.78). Cross-validation confirmed that the NK cell count of 0.040×10^3 cells/µl was the optimal cutoff value in 270 of 800 bootstrap samples (33.7%), being the highest rate among the various alternative points explored (Table S2). Overall, 14.3% (58/404) of patients with evaluable NK cell measurement at month 6 were below this cutoff. As expected, late HZ-free survival was significantly lower among patients with NK cell counts below this threshold at month 6 compared to those above (5-year survival rate: 88.0% vs. 96.0%, respectively; log-rank test *P*-value = 0.015) (Fig. 1). The deleterious impact of having a NK cell count $<0.040 \times 10^3$ cells/µl at month 6 was not confirmed in the multivariate Cox regression model with death and graft loss as competing risk events (aHR: 2.51; 95% CI: 0.77–8.19; *P*-value = 0.126).

We additionally analyzed the predictive value of the NK cell count at month 6 by stratifying the incidence of late HZ according to ascending categories of this variable (<0.040, 0.040–0.120, 0.120–0.250, and $>0.250 \times 10^3$ cells/µl). There was a clear gradient in the cumulative incidence at year 5 across these categories (11.4% [5/44], 4.7% [5/106], 3.8% [4/106], and 0.0% [0/46], respectively; *P*-value = 0.074), although the low number of events in each interval precludes the demonstration of significant differences in survival curves (log-rank test *P*-value = 0.053). However, when the central categories were collapsed into a single one, differences in late HZ-free survival were also evident (5-year survival rates: 88.0%, 95%, and 100.0% for <0.040, 0.040–0.250, and $>0.250 \times 10^3$ cells/µl, respectively; log-rank test *P*-value = 0.023) (Fig. S3).

Discussion

Post-transplant HZ constitutes a non-negligible complication for KT recipients, as demonstrated by the cumulative incidence in the present cohort (7.9%). Other authors have reported similar figures [2,4], although follow-up periods were not homogeneous across studies.

Table 4. Cox regression models for the occurrence of post-transplant HZ between years 1 and 5 after transplantation (i.e., late HZ), with death and graft loss considered as competing risk events.

	No late HZ (n = 379)	Late HZ (n = 21)	P-value	Univariate analysis			Multivariate analysis		
				HR	95% CI	P-value	HR	95% CI	P-value
Age of recipient, years [mean ± SD]	54.0 ± 14.7	57.7 ± 15.8	0.265						
Gender (male) [n (%)]	237 (62.5)	10 (47.6)	0.171						
Pretransplant diabetes mellitus [n (%)]	92 (24.3)	6 (28.6)	0.656						
Pretransplant immunosuppressive therapy [n (%)]	47 (12.4)	3 (14.3)	0.736						
Previous solid organ transplantation [n (%)]	78 (20.6)	2 (9.5)	0.274						
Pretransplant VZV seropositivity [n (%)]*	352 (93.9)	21 (100.0)	0.624						
CMV serostatus D+/R− [n (%)]	28 (7.4)	1 (4.8)	1.000						
Age of donor, years [mean ± SD]	52.3 ± 16.6	57.9 ± 14.8	0.128						
DCD donor [n (%)]	130 (34.3)	7 (33.3)	0.928						
Induction therapy with ATG [n (%)]	185 (48.8)	12 (57.1)	0.457						
CMV antiviral prophylaxis [n (%)]	189 (50.3)	12 (57.1)	0.540	1.03	0.44–2.43	0.936	-	-	-
PBLSs at month 6, $\times 10^3$ cells/μl [median (IQR)]									
$CD3^+$ T-cell count	0.936 (0.516–1,435)	0.581 (0.403–1,159)	0.143						
$CD4^+$ T-cell count	0.398 (0.170–0.778)	0.241 (0.121–0.581)	0.158						
$CD8^+$ T-cell count	0.407 (0.249–0.654)	0.363 (0.235–0.569)	0.453						
B-cell count	0.070 (0.033–0.119)	0.065 (0.043–0.185)	0.479						
NK cell count	0.123 (0.062–0.205)	0.083 (0.029–0.130)	0.032	0.93†	0.87–0.99	0.026	0.94†	0.88–1.00	0.054
Acute rejection during the first 6 months [n (%)]	70 (18.5)	5 (23.8)	0.565						
New-onset diabetes after transplantation [n (%)]‡	56 (15.6)	3 (16.7)	1.000						
Severe bacterial infection during the first 6 months [n (%)]¶	95 (25.1)	7 (33.3)	0.398						
CMV disease during the first 6 months [n (%)]	47 (12.4)	6 (28.6)	0.045	3.57	1.51–8.44	0.004	-	-	-

ATG: antithymocyte globulin; CMV: cytomegalovirus; D: donor; DCD: donation after circulatory death; HR: hazard ratio; IQR: interquartile range; KT: kidney transplant; PBLSs: peripheral blood lymphocyte subpopulations; R: recipient; SD: standard deviation.

*Data available for 440 patients.

†HRs per 10-cells/μl increase in NK cell count at month 6.

‡Percentages were calculated after excluding patients with pretransplant diabetes mellitus.

¶Infection requiring hospital admission and parenteral antibiotic therapy.

Despite advances in immunosuppression and post-transplant care, the burden due to post-transplant HZ has remained largely unchanged over the last decades, as suggested by comparison of our incidence rate between 2008 and 2013 (0.057 episodes per 1000 transplant-days) to that observed in the US Veterans Administration between 1996 and 2007 (0.068 per 1000 transplant-days) [3]. Moreover, the rates of PHN found by us and others [2,4,8] more than triple that of the overall population [17].

The median interval to the occurrence of HZ in the present study (18.3 months) was similar to [4] or larger than those previously reported [8,18]. Although more than a third of the cases accumulated within the 12 months, the period at risk spanned thereafter through the following years. Such a case distribution suggests that the assessment of risk factors for post-transplant HZ should take into account the relative role of different conditions across time. By separately analyzing two different post-transplant timeframes (first year [early HZ] and from years 1 to 5 [late HZ]), we have identified some factors that appear to influence individual susceptibility for each period: the receipt of (val-)GCV and a low NK cell count at month 6, respectively. This study design seems reasonable as the effect of certain variables, such as the receipt of anti-CMV prophylaxis, may be evident only for limited post-transplant periods and would have otherwise remained hidden in a single comprehensive analysis spanning the 5-year period.

The *in vitro* activity of CGV against VZV has been shown to be similar to that of acyclovir [19,20]. It has been accordingly proposed that SOT recipients already receiving (val-)GCV to prevent CMV infection may be safely spared specific prophylaxis with acyclovir or it prodrugs against herpes simplex virus (HSV) or VZV [21]. However, clinical data supporting this recommendation is relatively scarce, in particular regarding HZ, and the results from various studies have been not entirely conclusive [3,4,7,8,22]. A recent subanalysis from the Swiss Transplant Cohort Study (STCS) found that SOT recipients on anti-CMV prophylaxis had a lower incidence of overall infection due to α-herpesviruses (mainly HSV), although this protective effect could not be specifically proven for HZ despite the large number of patients included [7]. Other authors have been unable to ascertain the impact of anti-CMV agents on the incidence of post-transplant HZ, either due to the universal use of (val-)GCV prophylaxis in the analyzed cohort [4] or to the lack of specific data [3]. In addition, some studies directly compared the effect of prophylaxis with anti-CMV and anti-HSV agents, reporting a nonsignificant trend toward a lower incidence of HZ among KT recipients under (val-)GCV as compared to those receiving acyclovir or its derivatives [2]. However, the administration of specific anti-HSV prophylaxis among CMV D−/R− patients in daily clinical practice is far from universal [7]. Of note, none of our patients that were not subjected to anti-CMV prophylaxis received acyclovir or any of its derivatives to prevent α-herpesvirus infection, regardless of their CMV D/R serostatus. This circumstance may have contributed to delineate more clearly the protective effect of (val-)GCV primarily given to prevent CMV infection. In fact, none of our patients developed HZ while on CMV antiviral prophylaxis, similarly to previous reports from heart transplant recipients [23]. Nevertheless, it should be stressed that we could only demonstrate a borderline significant protective effect of CMV antiviral prophylaxis on the risk of early HZ, likely reflecting insufficient statistical power due to the low number of events.

Acute graft rejection has been found to increase the risk of HZ in some [4] but not all [2,7] previous studies. Interestingly, after incorporating death and graft loss as competing risk events in the regression model, we found no apparent impact of such a complication on the occurrence of either early or late HZ. Likewise, the receipt of anti-CMV prophylaxis seems to outweigh the predictive value of recipient's age, which had been identified by other authors [2–4].

Albeit still preliminary, a noteworthy finding of the present study is that low NK cell counts at month 6 appear to increase the risk for HZ over the following years. As for other herpesviruses, the competency of cell-mediated immunity is instrumental to prevent VZV from reactivating. There is mounting evidence on the protective role of VZV-specific T cells among different immunocompromised populations [24–26]. It has been also reported that percentage of VZV-specific $CD8^+$ effector memory T cells is significantly lower in KT recipients than in controls [27]. However, current methods for the enumeration of VZV-specific T cells—based on *in vitro* release of interferon-γ or other Th_1 cytokines upon antigen stimulation—are technically demanding and not standardized, and their implementation in clinical practice seems unfeasible in the medium term [26,28]. Although limited by the lack of an independent validation cohort, our study suggests that the NK cell count may be used as a convenient surrogate for the VZV-specific cell-mediated immunity. One of the hallmarks of NK cells is their ability to display potent cytolytic activity without

the need of previous clonal expansion and differentiation. Our group has recently shown that liver transplant recipients with low NK cell counts have an increased risk of opportunistic infection [29]. The relevance of NK cells in the host–pathogen interaction that leads to VZV dissemination or reactivation following primary infection is exemplified by the occurrence of severe forms of varicella among patients with NK cell deficiencies [30,31]. The increased risk of HZ associated with therapy with bortezomib in hematologic patients has been linked to the impact of this agent on NK and $CD8^+$ T-cell counts [32]. On the other hand, we cannot rule out that NK cell counts might to some extent mirror the enumeration of VZV-specific $CD4^+$ or $CD8^+$ T cells.

The lack of significant differences in NK cell counts at baseline or month 1 between patients with or without HZ supports the notion that anti-CMV prophylaxis acts a protective factor for this complication and that such effect appears strong enough to mute the impact of host's immune response. By assuming this hypothesis, it seems reasonable that the predictive value of the NK cell count could only emerge once prophylaxis has been discontinued. On the other hand, the dispersion observed in NK cell counts at month 6 was higher than at month 1 (Fig. S2), suggesting that the former measurement point would become more informative as the overall amount of immunosuppression gradually decreases (i.e., patients with persistently low NK cell counts remain more susceptible to HZ as compared to those that recover their baseline levels).

The present study has some limitations. Despite the large sample size, the relatively low number of episodes of HZ may have compromised the stability of multivariate models. Accordingly, associations only approached borderline statistical significance after multivariate adjustment. This caution is particularly necessary for interpreting the proposed cutoff value of the NK cell count at month 6, which should be taken as merely tentative. Although the study cohort was prospectively assembled, details on post-transplant HZ were retrospectively collected and we cannot rule out that some cases could have been missed. A more frequent monitoring of PBLSs would have been desirable to accurately delineate their post-transplant kinetics and potential impact on the development of HZ. Unfortunately, our immune monitoring schedule only spanned the first 6-month period after transplantation, thus lacking serial measurements of NK cell counts beyond that point and closer to the time of occurrence of HZ.

To conclude, HZ is still a relevant event during the course following KT both in terms of incidence and negative impact on quality of life. Two easily ascertainable factors (whether the patient is receiving or not anti-CMV antiviral prophylaxis and the NK cell count at post-transplant month 6) might be potentially useful to tailor preventive strategies according to individual susceptibility [21]. These would eventually include the use of anti-VZV prophylaxis with acyclovir or derivatives beyond the sixth month after transplantation, the tapering of immunosuppression, and the preferential administration—upon approval for this patient population—of the novel HZ/su vaccine for recipients with low NK cell counts.

Authorship

MFR, JO, DL, FLM, AA and JMA: participated in research design. MFR, JO, FLM, EG, NP and RSJ: participated in collecting clinical data. MFR and JO: participated in chart review. TRM and PP: participated in sampling and laboratory analyses. MFR, JO and DL: analyzed data. MFR: wrote the paper. FLM, RSJ, AA and JMA: critically revised and completed the final draft of the manuscript.

Funding

This study was supported by the Spanish Ministry of Economy and Competitiveness, Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias [FIS] 11/01538 and Proyecto Integrado de Excelencia [PIE] 13/00045). M.F.R. holds a clinical research contract "Juan Rodés" (JR14/00036) from the Spanish Ministry of Economy and Competitiveness, Instituto de Salud Carlos III.

Conflict of interest

All the authors declare they have no conflict of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1 Description of immunosuppression and prophylaxis regimens

Figure S1. Receiving operating characteristics curve for NK cell count at month 6 for predicting the occurrence of late post-transplant HZ.

Figure S2. Kinetics of NK cell counts during the first 6 months after transplantation.

Figure S3. Cumulative incidence curves for post-transplant late HZ according to ascending categories in

the NK cell count measured at month 6 (follow-up truncated at 5 years).

Table S1. Detailed clinical characteristics of the 6 episodes of disseminated post-transplant HZ.

Table S2. Diagnostic performance of different cut-off values of NK cell count at month 6 for predicting the occurrence of late post-transplant HZ.

REFERENCES

1. Cohen JI. Clinical practice: herpes zoster. *N Engl J Med* 2013; **369**: 255.
2. Arness T, Pedersen R, Dierkhising R, Kremers W, Patel R. Varicella zoster virus-associated disease in adult kidney transplant recipients: incidence and risk-factor analysis. *Transpl Infect Dis* 2008; **10**: 260.
3. Pergam SA, Forsberg CW, Boeckh MJ, *et al.* Herpes zoster incidence in a multicenter cohort of solid organ transplant recipients. *Transpl Infect Dis* 2011; **13**: 15.
4. Pavlopoulou ID, Poulopoulou S, Melexopoulou C, Papazaharia I, Zavos G, Boletis IN. Incidence and risk factors of herpes zoster among adult renal transplant recipients receiving universal antiviral prophylaxis. *BMC Infect Dis* 2015; **15**: 285.
5. Harpaz R, Ortega-Sanchez IR, Seward JF. Advisory Committee on Immunization Practices Centers for Disease C, Prevention. Prevention of herpes zoster: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2008; **57**: 1; quiz CE2-4.
6. Lal H, Cunningham AL, Godeaux O, *et al.* Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. *N Engl J Med* 2015; **372**: 2087.
7. Martin-Gandul C, Stampf S, Hequet D, *et al.* Preventive strategies against cytomegalovirus and incidence of alpha-herpesvirus infections in olid-organ transplant recipients: a nationwide cohort study. *Am J Transplant* 2017; **17**: 1813.
8. Ko GB, Kim T, Kim SH, *et al.* Increased incidence of herpes zoster in the setting of cytomegalovirus preemptive therapy after kidney transplantation. *Transpl Infect Dis* 2013; **15**: 416.
9. Fernández-Ruiz M, López-Medrano F, Varela-Peña P, *et al.* Monitoring of immunoglobulin levels identifies kidney transplant recipients at high risk of infection. *Am J Transplant* 2012; **12**: 2763.
10. Fernández-Ruiz M, López-Medrano F, Allende LM, *et al.* Kinetics of peripheral blood lymphocyte subpopulations predicts the occurrence of opportunistic infection after kidney transplantation. *Transpl Int* 2014; **27**: 674.
11. de la Torre-Cisneros J, Farinas MC, Caston JJ, *et al.* GESITRA-SEIMC/REIPI recommendations for the management of cytomegalovirus infection in solid-organ transplant patients. *Enferm Infecc Microbiol Clin* 2011; **29**: 735.
12. Kotton CN, Kumar D, Caliendo AM, *et al.* Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation* 2013; **96**: 333.
13. BD Multitest™ CD3/CD16+CD56/CD45/CD19 (package insert). BD Biosciences, Becton, Dickinson and Company, 2016.
14. Poli A, Michel T, Theresine M, Andres E, Hentges F, Zimmer J. CD56^{bright} natural killer (NK) cells: an important NK cell subset. *Immunology* 2009; **126**: 458.
15. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950; **3**: 32.
16. Schumacher M, Holländer N, Sauerbrei W. Resampling and cross-validation techniques: a tool to reduce bias caused by model building? *Stat Med* 1997; **16**: 2813.
17. Friesen KJ, Chateau D, Falk J, Alessi-Severini S, Bugden S. Cost of shingles: population based burden of disease analysis of herpes zoster and postherpetic neuralgia. *BMC Infect Dis* 2017; **17**: 69.
18. Gourishankar S, McDermid JC, Jhangri GS, Preiksaitis JK. Herpes zoster infection following solid organ transplantation: incidence, risk factors and outcomes in the current immunosuppressive era. *Am J Transplant* 2004; **4**: 108.
19. Field AK, Davies ME, DeWitt C, *et al.* 9-([2-hydroxy-1-(hydroxymethyl)ethoxy] methyl)guanine: a selective inhibitor of herpes group virus replication. *Proc Natl Acad Sci USA* 1983; **80**: 4139.
20. Crumpacker CS. Ganciclovir. *N Engl J Med* 1996; **335**: 721.
21. Zuckerman RA, Limaye AP. Varicella zoster virus (VZV) and herpes simplex virus (HSV) in solid organ transplant patients. *Am J Transplant* 2013; **13** (Suppl 3): 55; quiz 66.
22. Slifkin M, Doron S, Snydman DR. Viral prophylaxis in organ transplant patients. *Drugs* 2004; **64**: 2763.
23. Koo S, Gagne LS, Lee P, *et al.* Incidence and risk factors for herpes zoster following heart transplantation. *Transpl Infect Dis* 2014; **16**: 17.
24. Watanabe D, Otani N, Suzuki S, *et al.* Evaluation of VZV-specific cell-mediated immunity in adults infected with HIV-1 by using a simple IFN-gamma release assay. *J Med Virol* 2013; **85**: 1313.
25. Distler E, Schnurer E, Wagner E, *et al.* Recovery of varicella-zoster virus-specific T cell immunity after T cell-depleted allogeneic transplantation requires symptomatic virus reactivation. *Biol Blood Marrow Transplant* 2008; **14**: 1417.
26. van der Heiden PL, de Boer R, van der Steen DM, *et al.* Identification of varicella-zoster virus-specific CD8 T cells in patients after T-cell-depleted allogeneic stem cell transplantation. *J Virol* 2009; **83**: 7361.
27. van Besouw NM, Verjans GM, Zuijderwijk JM, Litjens NH, Osterhaus AD, Weimar W. Systemic varicella zoster virus reactive effector memory T-cells impaired in the elderly and in kidney transplant recipients. *J Med Virol* 2012; **84**: 2018.
28. Salem Fourati I, Grenier AJ, Jolette E, Merindol N, Ovetchkine P, Soudeyns H. Development of an IFN-gamma ELISpot assay to assess varicella-zoster virus-specific cell-mediated immunity following umbilical cord blood transplantation. *J Vis Exp* 2014;(89). doi.org/10.3791/51643.
29. Fernández-Ruiz M, Silva JT, López-Medrano F, *et al.* Post-transplant monitoring of NK cell counts as a simple approach to predict the occurrence of opportunistic infection in liver transplant recipients. *Transpl Infect Dis* 2016; **18**: 552.
30. Orange JS. Natural killer cell deficiency. *J Allergy Clin Immunol* 2013; **132**: 515.
31. Ishida H, Imai K, Honma K, *et al.* GATA-2 anomaly and clinical phenotype of a sporadic case of lymphedema, dendritic cell, monocyte, B- and NK-cell (DCML) deficiency, and myelodysplasia. *Eur J Pediatr* 2012; **171**: 1273.
32. Uy GL, Goyal SD, Fisher NM, *et al.* Bortezomib administered pre-auto-SCT and as maintenance therapy post transplant for multiple myeloma: a single institution phase II study. *Bone Marrow Transplant* 2009; **43**: 793.