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Association of Host Factors With Antibody Response to Seasonal Influenza Vaccination in Allogeneic Hematopoietic Stem Cell Transplant Patients

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Background. Influenza vaccination efficacy is reduced after hematopoietic stem cell transplantation (HSCT) and patient factors determining vaccination outcomes are still poorly understood.

Methods. We investigated the antibody response to seasonal influenza vaccination in 135 HSCT patients and 69 healthy volunteers (HVs) in a prospective observational multicenter cohort study. We identified patient factors associated with hemagglutination inhibition titers against A/California/2009/H1N1, A/Texas/2012/H3N2, and B/Massachusetts/2012 by multivariable regression on the observed titer levels and on seroconversion/seroprotection categories for comparison.

Results. Both regression approaches yielded consistent results but regression on titers estimated associations with higher precision. HSCT patients required 2 vaccine doses to achieve average responses comparable to a single dose in HVs. Prevaccination titers were positively associated with time after transplantation, confirming that HSCT patients can elicit potent antibody responses. However, an unrelated donor, absolute lymphocyte counts below the normal range, and treatment with calcineurin inhibitors lowered the odds of responding.

Conclusions. HSCT patients show a highly heterogeneous vaccine response but, overall, patients benefited from the booster shot and can acquire seroprotective antibodies over the years after transplantation. Several common patient factors lower the odds of responding, urging identification of additional preventive strategies in the poorly responding groups.

Clinical Trials Registration. NCT03467074.

Keywords. influenza; vaccination; hematopoietic stem cell transplantation; immunosuppression; graft-versus-host disease; hemagglutination inhibition titer; seroconversion; categorical regression; sequential model.

Community-acquired viruses, such as influenza, pose a high risk for hematopoietic stem cell transplantation (HSCT) patients, with reported case fatalities of up to 20%–30% for seasonal and pandemic influenza [1]. Vaccination is the primary intervention against influenza, but vaccine effectiveness is lower in HSCT patients than in healthy individuals [2]. To develop better vaccination strategies for HSCT patients and to identify patients at high risk for morbidity and fatal outcome, it is important to understand which host factors influence vaccination outcomes.

Several studies investigated the influenza vaccine-induced antibody response in HSCT patients (reviewed in [3, 4]). Most studies agree that immunosuppressive treatment determines vaccination success [5–8] along with the time after transplantation (transplantation-to-vaccination interval), especially within the first year post-HSCT [8–11]. However, there are mixed results on the effect of chronic graft-versus-host disease (cGVHD) [5, 6, 8, 11–14] and little is known on the effect of donor relationship and mismatch [15]. In addition, host genetic factors have been proposed to influence the vaccine response [16–18]. Genetic factors may be especially important in immunocompromised populations where compensating mechanisms are potentially impaired [19] but, so far, genetic factors have not been investigated in HSCT patients. One of the main strategies to improve vaccine effectiveness

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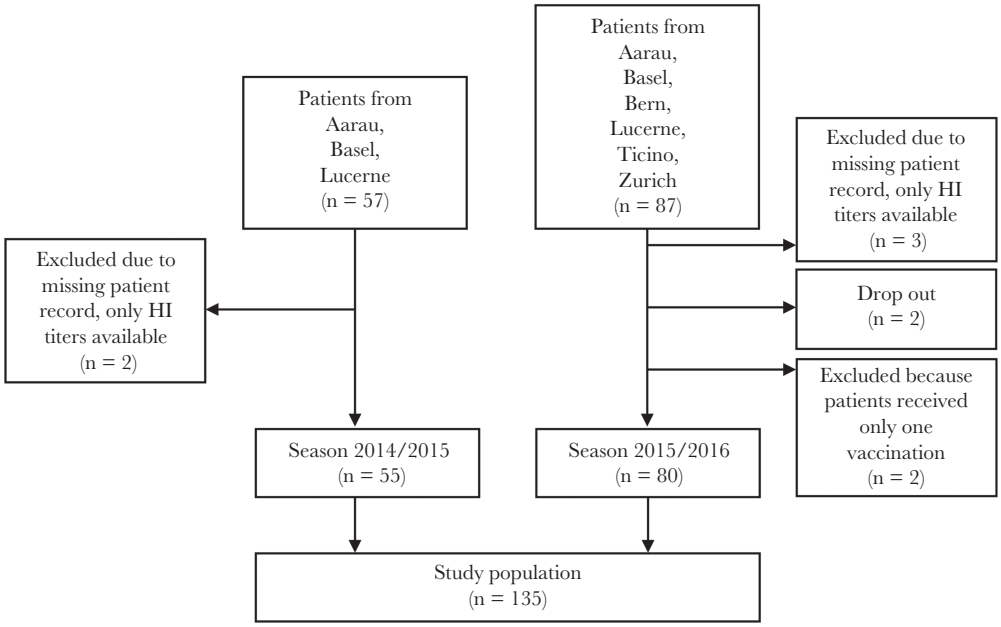

Figure 1. Overview of patient recruitment. All patients participated in only 1 of the seasons.

is administering a second dose [19], but there are also conflicting data on the benefit of a booster shot for HSCT patients [5, 15, 20, 21].

Understanding the vaccine response in HSCT patients is particularly challenging. Controlled studies are unethical or unfeasible, and the number of patients in observational studies is usually low, which hampers statistical power and can introduce bias in the estimated effects [22]. Moreover, HSCT patients are highly heterogeneous, for example in medication and comorbidities, and many host factors depend on each other, which further complicates the comparison of published studies. To disentangle host factor associations, adjustment for relevant factors is crucial, such as in a multivariable regression analysis. In addition, statistical analysis is commonly performed on dichotomized outcomes, for example seroconversion and seroprotection, instead of the full data, which can further decrease statistical power [23].

Here, we performed a multivariable regression analysis directly on the observed antibody titers to investigate the association of patient factors with vaccination outcomes in allogeneic HSCT patients, including interferon-λ (IFN-λ) genotypes that were reported to be associated with vaccine response [16, 17, 19]. We assessed the antibody response against 3 influenza types (H1N1, H2N3, and B), which enabled us to study strain-specific differences, and, more importantly, investigate strain-independent host factors associated with vaccine response. Our results obtained by titer regression are consistent with results obtained by the commonly used binary logistic regression on seroconversion/seroprotection categories but our approach yields higher precision in the estimated effects.

METHODS

Ethics and Regulatory Requirements

The study was conducted following the Declaration of Helsinki, approved by the local ethics committee (EKNZ ID, 2014-141) and registered at ClinicalTrials.gov (ID, NCT03467074). All participants signed informed consent.

Study Design, Participants, and Data Collection

We recruited allogeneic HSCT patients at 6 hematological centers in Switzerland from October 2014 to January 2015 and October 2015 to January 2016 (Figure 1). Only adult patients (aged ≥18 years) with time post-HSCT ≥ 1 year and without known vaccine intolerance were eligible for participation. Patients received 2 doses of nonadjuvanted seasonal influenza vaccine (Table 1). The booster shot was given 30 days after the

Table 1. Vaccine Composition

	Season 2014–2015	Season 2015–2016
Manufacturer	Agrippal, Novartis, Switzerland	Fluarix Tetra, GSK, UK
Influenza strains	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2) B/Massachusetts/2/2012 (Yamagata lineage)	A/California/7/2009 (H1N1) A/Switzerland/9715293/2013 (H3N2) B/Phuket/3073/2013 (Yamagata lineage)[a] B/Brisbane/60/2008 (Victoria lineage)[b]

Participants received a nonadjuvanted seasonal influenza vaccine by intramuscular injection comprising inactivated subunit viruses with 15 µg hemagglutinin (HA) antigen per strain according to influenza season.

[a]Hemagglutination inhibition (HI) titers were not measured against this strain.

[b]HI titers against this strain were excluded in the final analysis as the HI assay showed too low immunogenicity (see Supplementary Material).

first, following the standard of care after HSCT. Serum samples and peripheral blood mononuclear cells were collected before the first vaccination (day 0) and afterward (days 7, 30, 60, and 180) and stored in aliquots at −80°C.

The study team documented patients' medication and cGVHD grade at study inclusion according to National Institutes of Health consensus criteria [24]. Patients were asked to document side effects on day 7 and day 37 in a questionnaire. Absolute lymphocyte counts were available from routine laboratory tests from the same day or the same week of the first vaccination, except for 1 patient in season 2014/2015 and 7 patients in season 2015/2016 for which counts were measured at a later/earlier time. The genotype of the transplanted stem cells (the donor's genotype) was determined from blood samples using TaqMan quantitative real-time polymerase chain reaction (PCR) assays. Patients were advised to consult a physician in case of influenza-like illness, and tested for influenza infection by PCR.

In addition, we collected serum samples from healthy volunteers (HVs) for comparison (n = 25 in 2014/2015 and n = 44 in 2015/2016, n = 69 in total). In contrast to HSCT patients, HVs received only 1 dose of the seasonal influenza vaccine.

Genotyping

We investigated SNPs that were reported to be associated with vaccine and antiviral immune response [16, 17, 19, 25–29]. All single-nucleotide polymorphisms (SNPs) were genotyped using TaqMan Real-Time PCR assay kits from Applied Biosystems (Supplementary Table 1) as previously described [30]. The genomic DNA of the donor was isolated from EDTA blood as described by the manufacturer (QIAamp DNA Blood Mini Kit; Qiagen). Samples and positive controls were run in duplicates.

Hemagglutination Inhibition Assay

Antibody levels were determined as hemagglutination inhibition (HI) titers [31] using a previously published protocol [32]. In both seasons, we measured HI titers against all vaccine strains shown in Table 1 except for B/Phuket/3073/2013.

Table 2. Patient Characteristics

Characteristic	Season 2014–2015	Season 2015–2016	All
Total	55	80	135
Age, y			
Median, IQR, range	55, 44–64, 22–72	54, 47–63, 24–74	54, 46–64, 22–74
≥ 65 y	13 (24)	18 (22)	31 (23)
Sex			
Female	26 (47)	32 (40)	58 (43)
Male	29 (53)	48 (60)	77 (57)
Underlying disease			
Acute myeloid leukemia	21 (38)	29 (36)	50 (37)
Acute lymphoblastic leukemia	10 (18)	12 (15)	22 (16)
Multiple myeloma	6 (11)	8 (10)	14 (10)
Chronic myeloid leukemia	6 (11)	6 (8)	12 (9)
Chronic lymphocytic leukemia	6 (11)	0	6 (4)
Myelodysplastic syndromes	3 (5)	8 (10)	11 (8)
Non-Hodgkin lymphoma	1 (2)	6 (8)	7 (5)
Myeloproliferative neoplasms	1 (2)	5 (6)	6 (4)
Other	1 (2)	6 (8)	7 (5)
Time after transplantation, y			
Median, IQR, range	4, 2–8, 1–25	4, 2–7, 1–22	4, 2–7, 1–25
1–2 y	19 (35)	33 (41)	52 (39)
3–5 y	15 (27)	21 (26)	36 (27)
>5 y	21 (38)	26 (33)	47 (35)
Absolute lymphocyte count, 10^9 cells/L			
Median, IQR, range	1.5, 1.0–2.4, 0.3–7.5	1.7, 1.2–2.3, 0.5–5.5	1.7, 1.1–2.3, 0.3–7.5
Disease state			
Remission	51 (93)	40 (50)	91 (67)
Recurrence	4 (7)	6 (8)	10 (7)
Unknown	0	34 (43)	34 (25)
Transplant source			
Peripheral blood stem cells	49 (89)	74 (92)	123 (91)
Bone marrow	6 (11)	6 (8)	12 (9)
Donor source			
Matched donor	45 (82)	60 (75)	105 (78)

Table 2. Continued

Characteristic	Season 2014–2015	Season 2015–2016	All
Matched unrelated donor	19 (35)	26 (33)	45 (33)
Mismatched donor	10 (18)	20 (25)	30 (22)
Mismatched unrelated donor	8 (15)	12 (15)	20 (15)
HLA class I mismatch	5 (9)	6 (8)	11 (8)
HLA-A, -B, -C	3, 0, 2	4, 1, 1	7, 1, 3
HLA class II mismatch	4 (7)	10 (13)	14 (10)
HLA-DP, -DQ, -DR	2, 2, 0	5, 1, 4	7, 3, 4
HLA-haploidentical donor	1 (2)	0	1 (1)
Not available mismatch type	0	4 (5)	4 (3)
Immunosuppressive treatment[a]			
None	25 (45)	45 (56)	70 (52)
Tacrolimus	16 (29)	14 (18)	30 (22)
Prednisone	13 (24)	16 (20)	29 (22)
Mycophenolate[b]	9 (16)	11 (14)	20 (15)
Cyclosporine A[c]	5 (9)	13 (16)	18 (13)
Rituximab[d]	3 (5)	0	3 (2)
Chronic GVHD			
None	20 (36)	40 (50)	60 (44)
Mild, grade 1	12 (22)	16 (20)	28 (21)
Moderate, grade 2	12 (22)	6 (8)	18 (13)
Severe, grade 3	11 (20)	14 (18)	25 (19)
Not available	0	4 (5)	4 (3)
IFNL3/4 genotype			
rs8099917, GT/GG	23 (42)	27 (34)	50 (37)
rs12979860, CT/TT	35 (64)	40 (50)	75 (56)
IFNLR1 genotype			
rs10903035, AG/GG	25 (46)	44 (55)	69 (51)
Influenza infection[e]			
Influenza A	5 (9)	1 (1)	6 (4)
Influenza B	3 (5)	0	3 (2)

Data are No. (%) except where indicated. Columns refer to 2 consecutive influenza seasons. For frequencies of all determined genotypes, see Supplementary Table 2.

Abbreviations: GVHD, graft-versus-host disease; HLA, human leukocyte antigen; IQR, interquartile range; PCR, polymerase chain reaction.

[a]Before vaccination (documented at the time of inclusion).

[b]Mycophenolate mofetil (CellCept) or mycophenolate sodium (Myfortic).

[c]Sandimmun Neoral.

[d]MabThera within the previous 6 months

[e]PCR-confirmed influenza infection during flu season; for time of detection by PCR, see Supplementary Figure 3.

Investigated Endpoints and Data Analysis

Primary endpoints were investigating the association of patient factors with (1) vaccine-induced antibody response (relative HI titer increase) and seroconversion (HI titer fold change ≥ 4), and (2) prevaccination antibody levels, that is HI titer level and seroprotection (HI titer ≥ 40) on day 0. Secondary endpoints were (1) comparing vaccine responses between HSCT patients and HVs, and (2) investigating the association of local side effects with vaccine response in HSCT patients.

For analyzing HI titers, we used a generalized linear regression model for ordinal data where response categories are reached successively, known as the sequential model or stopping-ratio model (referred to as titer regression in this paper) [33–35]. We compared the estimated effects from titer regression on HI titers with the commonly used binary regression on seroconversion/seroprotection. We included the patients' characteristics summarized in Table 2 in our multivariable regression analysis, except for the underlying disease. We corrected for influenza season, influenza strain, inclusion center, and experimental batches in all analyses and additionally for baseline (day 0) titer and time point when analyzing response. In total, we considered 24 variables when analyzing response on day 30 and day 60 (810 titers in total) and 22 variables when analyzing prevaccination titers on day 0 (405 titers). For all details on regression models, included variables, missing data imputation, sensitivity analyses, and cross-reactivity between measured strains see Supplementary Material.

Data and Code Availability

Data and results are available on GitLab with R scripts for reproducibility (https://gitlab.com/csb.ethz/hsct-study).

RESULTS

Patient Characteristics

HSCT patient characteristics are summarized in Table 2. Occurrence of cGVHD was similarly distributed among patients with an unrelated/related donor, and among fully matched/mismatched patients (Supplementary Table 3). Intake of immunosuppressive treatment increased with cGVHD grade (Supplementary Figure 1). No serious adverse events were reported. In total, 46/118 (39%) patients reported any local side effect on day 7 and 41/118 (35%) on day 37. The most frequent event was pain, followed by swelling, redness, and warm skin (Table 3). There were 8 influenza infections in the first season and 1 in the second (see Supplementary Figure 3 for time of detection by PCR). Seroprotection and seroconversion rates are summarized in Supplementary Table 4.

HSCT Patients Show High Variability in Antibody Titers

HSCT patients showed a more diverse vaccine response for all measured influenza strains and over the whole study period compared to HVs (Figure 2A and 2B). However, HVs showed larger differences in HI titers between strains and seasons. To compare the average effect of vaccination between HSCT patients and HVs, we estimated the odds ratios for showing higher titers (increase by at least 1 titer level) on days 7, 30, 60, and 180 compared to day 0 over both seasons and all influenza types (Supplementary Material). HVs showed a stronger response on days 7 and 30, while responses were comparable on days 60 and 180 (Figure 2C), even though HSCT patients received an additional vaccine dose on day 30. The difference was largest on day 7, suggesting that many HVs responded with a rapid antibody production by memory B cells. Thus, the slower and weaker antibody response in patients might be partially explained by fewer memory recall responses. Moreover, HVs were younger than HSCT patients—with a median age of 37 years (interquartile range [IQR], 32–49 years) compared to 54 years (IQR, 46–64 years)—and influenza vaccine efficacy in adults decreases with increasing age [36]. Differences in prevaccination titers might additionally complicate the comparison between patients and HVs (Figure 2A and 2B).

Table 3. Number of Hematopoietic Stem Cell Transplantation Patients Reporting Local Side Effects

Side Effect	No. of Patients (%) on Day 7	No. of Patients (%) on Day 37
Pain	38 (32)	32 (27)
Swelling	26 (22)	16 (14)
Warm skin	21 (18)	14 (12)
Redness	20 (17)	16 (14)
Restricted arm movement	11 (9)	12 (10)
Itching	9 (8)	11 (9)
Any	46 (39)	41 (35)

Questionnaires were available from n = 118 patients.

HSCT Patients Benefited From Booster Shot

HSCT patients showed a stronger vaccine response on day 60 (odds ratio [OR] = 3.35; 95% confidence interval [CI], 2.77–4.05; $P < 10^{-12}$) compared to day 30 (OR = 2.78; 95% CI, 2.30–3.36; $P < 10^{-12}$; Figure 2C). Consistent with these estimates, slightly more patients seroconverted on day 60 than day 30 across all strains in both seasons (Supplementary Table 4). Although HSCT patients showed a weaker response on days 7 and 30 compared to HVs, both groups showed a similar response on days 60 and 180, suggesting that the booster shot had a compensating effect (Figure 2C).

We quantified the effect of the booster shot by estimating the odds ratio for showing an increase in titer by at least 1 titer level on day 60 compared to day 30, adjusting for relevant patient and experimental factors. Patients had higher odds for an increased HI titer on day 60 compared to day 30 with an estimated OR = 1.26 (95% CI, 1.03–1.54; $P = .022$). In comparison, HVs showed nonsignificantly lower odds for HI titer increase on day 60 than day 30 (OR = 0.75; 95% CI, .55–1.03; $P = .080$). Nevertheless, the estimated odds ratios were significantly different from each other (Welch test on log-transformed OR, $P < 10^{-3}$; Figure 2D), suggesting that the booster shot helped patients to mount a stronger antibody response.

Regression on Titers Yields Higher Precision in Estimated Effects

An illustrative comparison between the binary regression on seroconversion (or seroprotection) categories and the regression model on HI titer levels is shown in Figure 3A. It has been previously suggested that regression on titers increases statistical power compared to regression on dichotomized outcomes [23]. The effects inferred by both approaches can be interpreted similarly, that is as a positive or negative shift in antibody concentration (Supplementary Material). Binary and titer regression yielded qualitatively similar results in all our analyses, but regression on titers inferred effects with higher precision due to the higher resolution in patient's underlying antibody level (Figure 3C and Supplementary Figures 4–11).

Association of Host Factors with Vaccine Response and Baseline Titers in HSCT Patients

We identified the most important host factors determining prevaccination (baseline) titers (Supplementary Figure 7) and relative HI titer increase (Figure 3B) in terms of their contribution to the explained residual deviance.

Immunosuppressive Treatment

Patients receiving calcineurin inhibitors showed lower odds for vaccine-induced HI titer increase, specifically cyclosporine A (OR = 0.36; 95% CI, .24–.53; $P = 2 \times 10^{-7}$) and tacrolimus (OR = 0.48; 95% CI, .34–.66; $P = 7 \times 10^{-6}$). For mycophenolate mofetil (MMF)/mycophenolate sodium, we observed a significant negative effect on baseline titers (OR = 0.49; 95% CI, .30–.78; $P = .003$), while for prednisone, we observed a

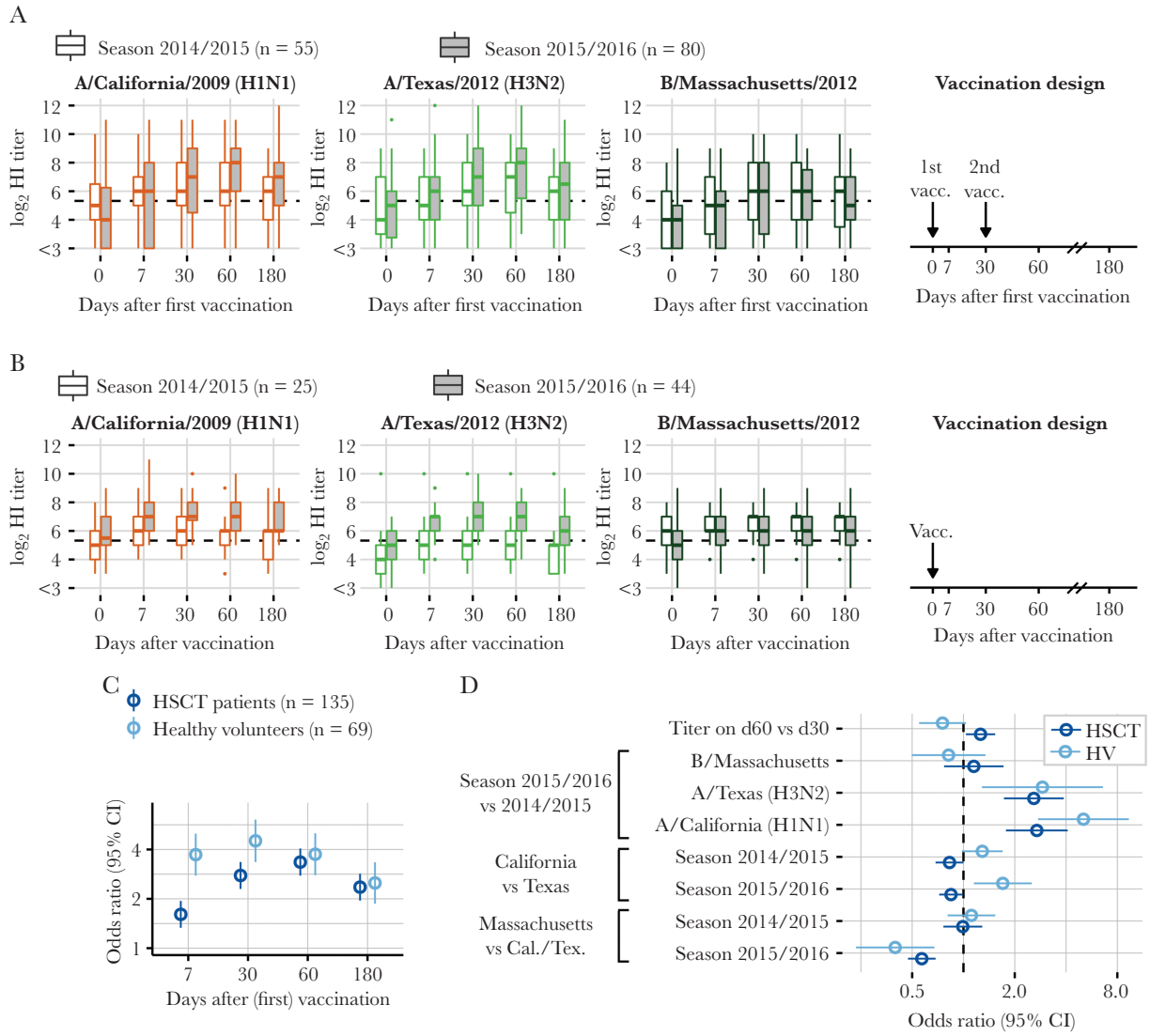

Figure 2. Antibody titers in hematopoietic stem cell transplant (HSCT) patients and healthy volunteers (HVs). *A* and *B*, Hemagglutination inhibition (HI) titers against 3 different influenza strains (*A*) in HSCT patients and (*B*) in HVs from 2 consecutive flu seasons. *C*, Average effect of vaccination on HI titer increase (relative to influenza strain- and season-specific baseline levels) in HSCT patients and HVs. Effects are expressed as the odds ratio for an increase in at least 1 titer level on days 7, 30, 60, and 180 compared to day 0. *D*, Estimated differences in vaccine response between time points, season, and influenza strains. Effects are expressed as the odds ratio for an HI titer increase by at least 1 level compared to the reference, specifically, day 60 vs day 30, season 2015/2016 vs 2014/2015 (by strain), A/California titers vs A/Texas titers, and B/Massachusetts titers vs others (by season).

(not significant) positive effect (OR = 1.54; 95% CI, .98–2.42; P = .060). We could not investigate the effect of rituximab because only 3 patients received rituximab along with other immunosuppressants (Supplementary Figure 1).

Time After Transplantation

The time after transplantation was not significantly associated with vaccine response, probably because we included only patients who received hematopoietic stem cells at least 1 year before enrollment in the study. However, the time after transplantation was the most important predictor for baseline titers (Figure 4A). Importantly, the average effect on HI titer increase of 4 years post-HSCT (OR = 3.84; 95% CI, 2.47–5.99; Figure 4A) was comparable to the average effect of vaccination on day 60 (OR = 3.35; 95% CI, 2.77–4.05; Figure 2C). Hence, HSCT patients can acquire seroprotective antibody levels over the years, probably by repeated vaccinations.

Absolute Lymphocyte Count

Our patient population showed a large variability in absolute lymphocyte count ranging from approximately 250 to 7500 cells per µL blood (Figure 4B). We observed a strong association between lymphocyte count and vaccine response (Figure 4C), similar to a previous study [15]. Patients with lymphocyte counts above 1000 cells per µL showed a stronger response but not significantly different baseline titers (Figure 4C). All other estimated effects

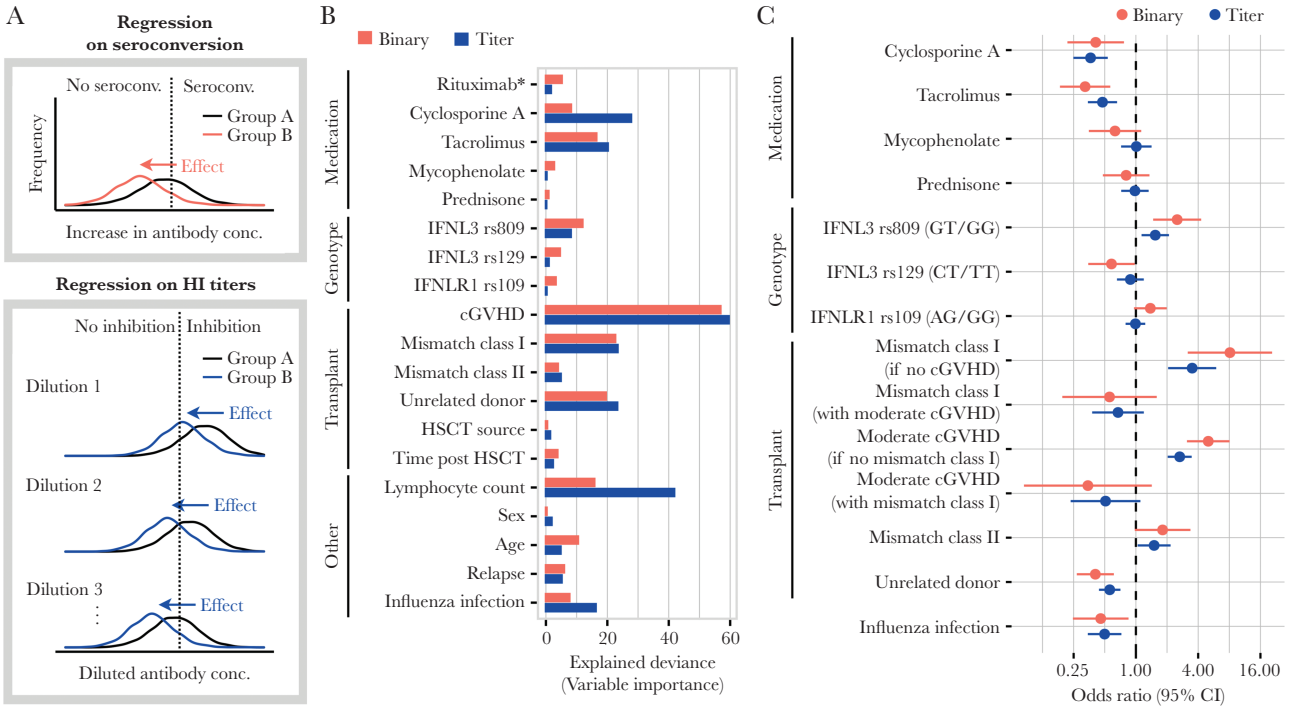

Figure 3. Host factors associated with vaccine response in HSCT patients. *A*, Illustrative explanation of the compared models, the commonly used binary regression on seroconversion vs titer regression on HI titer levels. An effect inferred from binary logistic regression can be interpreted as the odds ratio between groups A and B for showing seroconversion. In contrast, an effect inferred from titer regression used in this study can be interpreted as the odds ratio for showing HI when HI has also been observed in all preceding dilution steps (ie, increase in HI titer by at least 1 level). Alternatively, effects can also be interpreted as a shift in antibody concentration between the compared subpopulations. Because all serum samples are diluted equally, this shift is the same at all dilution steps. For details on the regression models, see Supplementary Material. Note that we corrected for baseline titers when analyzing postvaccination titers because we want to infer effects on the relative HI titer increase, which means that we compared patients with the same baseline titer levels. In general, in the multivariable setting, an inferred effect gives the odds ratio between groups A and B for showing a higher HI titer/seroconversion/seroprotection when all the other variables are held constant. *B*, Variable importance in terms of contribution to the explained residual deviance of all investigated host factors. Results can only be compared within each model but not across models. *Only 3 patients received rituximab. *C*, Estimated effects for important patient factors. All inferred effects are shown in Supplementary Figure 5. Abbreviations: cGVHD, chronic graft-versus-host disease; CI, confidence interval; HI, hemagglutination inhibition; HSCT, hematopoietic stem cell transplantation.

were not affected when we did not correct for lymphocyte count (Supplementary Figure 5), showing that the other host-factor associations were statistically independent of lymphocyte count.

HLA Mismatch

We found a positive association between vaccine response and mismatch in HLA class I, but in conjunction with a strong negative interaction effect with cGVHD (OR = 0.44; 95% CI, .30–.65 per cGVHD grade; $P = 4 \times 10^{-5}$). Thus, patients with a class I mismatch showed a stronger vaccine response than fully matched patients (OR = 3.50; 95% CI, 2.05–5.98; $P = 4 \times 10^{-6}$), but only if they did not suffer from cGVHD (Figure 3C). The effect was weaker and not significant when we removed the interaction effect (OR = 1.44; 95% CI, .99–2.04; $P = .059$), suggesting that this association depends highly on the patient's cGVHD state. We did not detect a significant interaction between cGVHD and class II mismatch (OR = 0.78; 95% CI, .56–1.09; $P = .139$; n = 5/14 without cGVHD). Instead, we found a strong negative association between class II mismatch and baseline titers (OR = 0.45; 95% CI, .25–0.79; $P = .006$). However, this effect was unstable in our sensitivity analysis (Supplementary Figure 10), suggesting that patients showed high heterogeneity in antibody titers that was not explained by our model. All these associations were only marginally affected when we did not correct for an unrelated donor (Supplementary Figures 5 and 9).

Chronic GVHD

In contrast to the mismatch effect, the positive association with cGHVD was also significant without the interaction effect (OR = 1.63; 95% CI, 1.43–1.86; $P = 7.7 \times 10^{-13}$ with mismatch interaction; OR = 1.53; 95% CI, 1.34–1.75; $P = 1.6 \times 10^{-10}$ without) and very stable (Supplementary Figures 4–6). In our study, cGVHD might also be confounded with repeated vaccinations (patients' vaccination history was unknown), although patients with cGVHD did not show significantly higher baseline titers than patients without cGVHD (Supplementary Figure 9).

Unrelated Donor

Having an unrelated donor was an important negative factor for vaccine response as previously reported [15]. This effect was statistically independent of HLA mismatch (OR = 0.55; 95% CI,

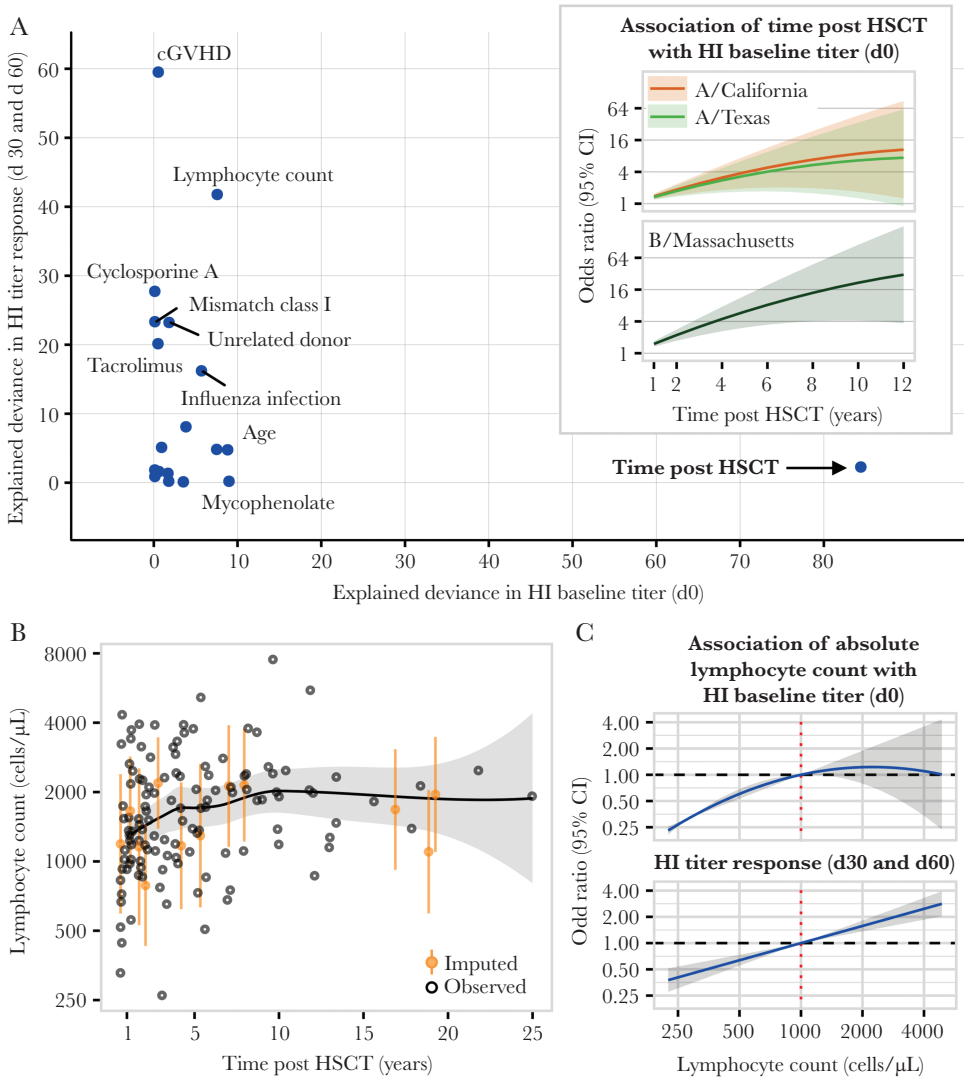

Figure 4. Association of the time after transplantation (time post-HSCT) and absolute lymphocyte count with vaccination outcomes. *A*, Comparison of variable importance (in terms of contribution to the explained residual deviance) for HI baseline titers (day 0) vs HI titer response (days 30 and 60). While multiple host factors determined vaccine response, baseline titers were mostly explained by the time after transplantation. The association of time post-HSCT was significantly stronger for HI titers against B/Massachusetts compared to the influenza A strains ($P = .001$). There was no significant difference between A/California (H1N1) and A/Texas (H3N2) ($P = .340$). *B*, Scatterplot showing lymphocyte counts by time after transplantation for the investigated HSCT patient population. For patients with missing values, data show the mean and standard deviation of imputed values. Data were fitted by a smoothing spline. *C*, Association of absolute lymphocyte count with response and baseline titers. Normal lymphocyte counts range from 1000 to 4800 cells per µL blood. Shaded area indicates 95% confidence intervals in each graph. Abbreviations: cGVHD, chronic graft-versus-host disease; CI, confidence interval; HI, hemagglutination inhibition; HSCT, hematopoietic stem cell transplant.

.44–0.71; $P = 1.7 \times 10^{-6}$ when adjusted for mismatch; OR = 0.62; 95% CI, .50–0.78; $P = 4.0 \times 10^{-5}$ when unadjusted).

Disease State and HSCT Source

Patients with relapse showed a significantly weaker vaccine response than patients in complete remission (OR = 0.68; 95% CI, .47–.99; $P = .043$). However, the effect was small and unstable (Supplementary Figure 4), indicating that the missing entries on the disease state in 25% of the patients hampered the analysis. We did not detect a significant association with transplant source (peripheral blood stem cells vs bone marrow; only 12 patients (9%) received bone marrow stem cells).

Sex and Age

Female patients showed slightly higher odds for vaccine-induced HI titer increase, although not significantly higher (OR = 1.30; 95% CI, .96–1.77; $P = .092$), and increasing age was negatively associated with baseline titers (OR = 0.81; 95% CI, .71–0.93 for a 10-year difference in age; $P = .002$).

Donor's IFNL3 *Genotype*

We observed a positive association of the *IFNL3* rs8099917 minor allele (GT/GG) with response (OR = 1.54; 95% CI, 1.13–2.09; $P = .006$) and baseline titers (OR = 1.55; 95% CI, 1.00–2.40; $P = .050$). The other investigated genotypes showed

no significant associations (Figure 3C). However, because all patients carrying the rs8099917 minor allele also carried the *IFNL3* rs12979860 minor allele (CT/CC) (Supplementary Figure 2), the positive association could also be due to an interaction effect between these 2 genotypes.

Influenza Infection

There was a significant negative association between PCR-confirmed influenza infection (influenza A or B) with both response (OR = 0.50; 95% CI, .34–.72; $P = 3 \times 10^{-4}$) and baseline titers (OR = 0.55; 95% CI, .32–.94; $P = .030$), confirming that low antibody levels increase the risk for infection.

Local Side Effects

Patients reporting at least 1 local side effect on day 7 had a stronger titer increase on day 30 compared to patients without side effects (OR = 1.72; 95% CI, 1.26–2.35; $P = 7 \times 10^{-4}$), and analogously, patients with any side effect on day 37 showed stronger response on day 60 (OR = 1.66; 95% CI, 1.23–2.25; $P = .001$). Specifically, our data suggest that patients experiencing redness and pain might respond more strongly (Supplementary Figure 11).

DISCUSSION

Whether HSCT patients benefit from a booster shot is still under debate [5, 11, 20, 21]. A randomized trial in 65 HSCT patients vaccinated with a nonadjuvanted quadrivalent influenza vaccine did not detect a significant effect [21]. However, the median time after transplantation was only 1 year (IQR, 0.3–2 years) in this trial, while complete B-cell reconstitution can take up to 2 years [37]. Our patient population had a median time of 4 years (IQR, 2–7 years), suggesting that the booster effect depends also on patient factors.

Calcineurin inhibitors suppress T-cell activation and have been previously reported to reduce influenza vaccine responses in HSCT patients [5, 6, 8]. Mycophenolate inhibits T- and B-cell proliferation [38] and might have inhibited the production of long-lived B cells in our patient population, which are necessary for maintaining high antibody levels. A previous study in 82 HSCT patients observed less seroprotection among patients receiving MMF [13], but the effect was not significant, perhaps due to small sample size or dichotomization. Prednisone is a corticosteroid that suppresses inflammation, used to treat GVHD. Previous studies reported a protective role of steroids in influenza acquisition [39] and progression [40] and the authors hypothesized that steroids could help patients restore local immunity [39]. Additional studies with larger sample size are needed to confirm this positive effect.

Sex and age differences in vaccine responses are frequently observed in healthy adults [36, 41, 42], but have not yet been reported for HSCT patients. For cGVHD, previous studies reported either no significant effect on vaccine response [5, 8, 12, 13, 15], or a negative effect [6, 11, 14] but these studies are not directly comparable: a study that determined active GVHD (acute GVHD of grade ≥2 or chronic extensive GVHD) as the main negative determinant could not correct for immunosuppressive treatment [11]. In general, studies showing a negative effect investigated HSCT patients with a shorter transplantation to vaccination interval than in our patient population [6, 11, 14], while another study with a time post-HSCT distribution like ours observed no significant effect. Similarly, previous studies that detected a significant association of time after transplantation with vaccine response included patients with ≤6 months post-HSCT [10, 11, 13, 20], whereas another study with a time post-HSCT distribution like ours observed no significant effect [8]. Our results also suggest that class I versus class II mismatched patients are differently affected by cGVHD and might respond to vaccination differently. However, classification of mismatches into only 2 categories does not fully capture the biological diversity, and other donor factors, such as donor's age [43], might additionally influence patient's immune state.

Consistent with our results, a study in solid organ transplant patients reported that patients carrying the *IFNL3* rs8099917 minor allele were more likely to be seroconverted after influenza vaccination and had lower IFN-λ expression [19]. Recent studies showed that IFN-λ directly modulates B-cell proliferation [44] and has adjuvant effects in vaccinated mice [45], but the mechanistic role of IFN-λ in vaccine response is still unknown.

CONCLUSIONS

On average, HSCT patients benefited from the booster shot and showed comparable responses after 2 vaccine doses to HVs after 1 dose. The strong association of time after transplantation with prevaccination titers shows that HSCT patients can acquire durable antibody levels over the years. However, lymphocyte counts below the normal range, an unrelated donor, or calcineurin inhibitors lower the odds of responding. In addition, treatment with mycophenolate or a mismatch in HLA class II potentially reduce long-term antibody production. In conclusion, HSCT patients show a highly heterogeneous vaccine response that can be partially explained by easily accessible host factors. Although the current standard of care approach induces potent vaccine responses in some HSCT patients, poorly responding patient groups might benefit from additional, more targeted preventive strategies.

Regarding future studies, we note that factors related to the donor, such as mismatch and IFN-λ genotype, are potentially further modulating factors that have not been sufficiently investigated yet. Cytokine receptors may also be on recipient's cells and recipient's genotype (unknow in this study) might additionally influence the vaccine response. Moreover, the conflicting results on the effect of cGVHD suggest that interaction effects with other host factors, for example with time after

transplantation or medical history, might need to be considered in future studies. Because HSCT patients are rare, pooled studies are probably needed to reach adequate sample sizes to investigate such interactions. Finally, we demonstrated that performing regression directly on the observed HI titers increases information compared to the commonly used binary regression on seroconversion/seroprotection. The gain in precision of estimated effects is particularly large for small case numbers per treatment or other exposure categories, frequently the case for HSCT patients and other heterogeneous patient populations.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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