Association of host factors with antibody response to seasonal influenza vaccination in allogeneic hematopoietic stem cell transplant (HSCT) patients

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Abstract: 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 yield consistent results but regression on titers estimated associations with higher precision. HSCT patients required two vaccine doses to achieve average responses comparable to a single dose in HVs. Pre-vaccination 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 lower 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 to identify additional preventive strategies in the poorly responding groups.

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1 Short summary

Patients after hematopoietic stem cell transplantation (HSCT) show a highly heterogeneous antibody response to seasonal influenza vaccination that can be partially explained by easily accessible clinical data such as the type of immunosuppressive treatment, absolute lymphocyte count, and donor relationship.
Abstract

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 yield consistent results but regression on titers estimated associations with higher precision. HSCT patients required two vaccine doses to achieve average responses comparable to a single dose in HVs. Pre-vaccination 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 lower 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 to identify additional preventive strategies in the poorly responding groups.

ClinicalTrials.gov Identifier: NCT03467074

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

Community-acquired viruses, such as influenza, pose a high risk for 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], [6], [7], [8], along with the time after transplantation (transplantation-to-vaccination interval), especially within the first year post HSCT [9], [10], [11], [8]. However, there are mixed results on the effect of chronic graft-versus-host disease (cGVHD) [12], [11], [13], [5], [6], [14], [8], 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], [17], [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 is administering a second dose, but there is 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, e.g., 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. Besides, statistical analysis is commonly performed on dichotomized outcomes, e.g., 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 IFN-λ genotypes that were reported to be associated with vaccine response [16], [17], [19]. We assessed the antibody response against three influenza types (H1N1, H2N3, 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 six hematological centers in Switzerland from October 2014–January 2015 and October 2015–January 2016 (Figure 1). Only adult patients (≥18 years) with time post HSCT ≥1 year and without known vaccine intolerance were eligible for participation. Patients received two doses of non-adjuvanted seasonal influenza vaccine (Table 1). The booster shot was given 30 days after the first, following the standard of care after HSCT. Serum samples and peripheral blood mononuclear cells were collected before the first vaccination (d0) and afterward (d7, d30, d60, d180) and stored in aliquots at −80°C.

The study team documented patients’ medication and cGVHD grade at study inclusion according to NIH consensus criteria [24]. Patients were asked to document side effects on d7 and d37 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 one patient in season 2014/15 and seven patients in season 2015/16 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 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 2014/15 and n=44 in 2015/16, n=69 in total). In contrast to HSCT patients, HVs received only one 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], [26], [27], [28], [29]. All SNPs were genotyped using TaqMan Real-Time PCR assay kits by 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 by Qiagen, #51106). 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.

Investigated endpoints and data analysis

Primary endpoints were investigating the association of patient factors with (i) vaccine-induced antibody response (relative HI titer increase) and seroconversion (HI titer fold change ≥ 4), and (ii) pre-vaccination antibody levels, i.e., HI titer level and seroprotection (HI titer ≥ 40) on d0. Secondary endpoints were (i) comparing vaccine responses between HSCT patients and HVs, and (ii) investigating the association of local side effects with vaccine response in HSCT patients.

For analysing 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], [34], [35]. We compared the estimated effects from titer regression on HI titers with the commonly used binary regression on seroconversion/seroprotection. We included the patientsâ€™ 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 (d0) titer and time point when analysing response. In total, we considered 24 variables when analysing response on d30 and d60 (810 titers in total) and 22 variables when analysing pre-vaccination titers on d0 (405 titers). For all details on regression models, included variables, missing data imputation, sensitivity analyses, and cross-reactivity between measured strains see Supplementary Information.
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 d7 and 41/118 (35%) on d37. The most frequent event was pain, followed by swelling, redness and warm skin (Table 3). There were eight influenza infections in the first season and one 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 one titer level) on d7, d30, d60, and d180 compared to d0 over both seasons and all influenza types (Supplementary Information). HVs showed a stronger response on d7 and d30, while responses were comparable on d60 and d180 (Figure 2c), even though HSCT patients received an additional vaccine dose on d30. The difference was largest on d7, 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 yrs (IQR 32–49 yrs) compared to 54 yrs (IQR 46–64 yrs) — and influenza vaccine efficacy in adults decreases with increasing age [36]. Differences in pre-vaccination titers might additionally complicate the comparison between patients and HVs (Figure 2a and 2b).
**HSCT patients benefited from booster shot**

HSCT patients showed a stronger vaccine response on d60 (OR = 3.35, 95% CI = [2.77, 4.05], \( P < 10^{-12} \)) compared to d30 (OR = 2.78 [2.30, 3.36], \( P < 10^{-12} \)) (Figure 2c). Consistent with these estimates, slightly more patients seroconverted on d60 than d30 across all seasons in both seasons (Supplementary Table 4). Although HSCT patients showed a weaker response on d7 and d30 compared to HVs, both groups showed a similar response on d60 and d180, 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 one titer level on d60 compared to d30, adjusting for relevant patient and experimental factors. Patients had higher odds for an increased HI titer on d60 compared to d30 with an estimated OR = 1.26 (95% CI = [1.03, 1.54], \( P = 0.022 \)). In comparison, HVs showed non-significantly lower odds for HI titer increase on d60 than d30 (OR = 0.75 [0.55, 1.03], \( P = 0.080 \)). Nevertheless, the estimated odds ratios were significantly different from each other (Welch’s 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, i.e., as a positive or negative shift in antibody concentration (see Supplementary Information). 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, 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 pre-vaccination (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 [0.24, 0.53], \( P = 2 \cdot 10^{-7} \)) and tacrolimus (OR = 0.48 [0.34, 0.66], \( P = 7 \cdot 10^{-6} \)). For mycophenolate mofetil
(MMF)/mycophenolate sodium, we observed a significant negative effect on baseline titers (OR = 0.49 [0.30, 0.78], \( P = 0.003 \)), while for prednisone, we observed a (not significant) positive effect (OR = 1.54 [0.98, 2.42], \( P = 0.060 \)). We could not investigate the effect of rituximab, because only three 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 one year ago. However, the time after transplantation was the most important predictor for baseline titers (Figure 4a). Importantly, the average effect on HI titer increase of four years post HSCT (OR = 3.84 [2.47, 5.99], Figure 4a) was comparable to the average effect of vaccination on d60 (OR = 3.35 [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 250-7500 cells per L 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 L showed a stronger response but not significantly different baseline titers (Figure 4c). All other estimated effects 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 [0.30, 0.65] per cGVHD grade, \( P = 4 \cdot 10^{-5} \)). Thus, patients with a class I mismatch showed a stronger vaccine response than fully matched patients (OR = 3.50 [2.05, 5.98], \( P = 4 \cdot 10^{-5} \)), 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 [0.99, 2.04], \( P = 0.059 \)), suggesting that this association highly depends on the patient’s cGVHD state. We did not detect a significant interaction between cGVHD and class II mismatch (OR = 0.78 [0.56, 1.09], \( P = 0.139 \), \( n = 5/14 \) without cGVHD). Instead, we found a strong negative association between class II mismatch and baseline titers (OR = 0.45 [0.25, 0.79], \( P = 0.006 \)). 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 [1.43, 1.86], \( P = 7.7 \cdot 10^{-13} \) with mismatch interaction, OR
= 1.53 [1.34, 1.75], \( P = 1.6 \cdot 10^{-10} \) 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).

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% = [0.44, 0.71], \( P = 1.7 \cdot 10^{-6} \) when adjusted for mismatch, OR = 0.62 [0.50, 0.78], \( P = 4.0 \cdot 10^{-5} \) when unadjusted).

Patients with relapse showed a significantly weaker vaccine response than patients in complete remission (OR = 0.68 [0.47, 0.99], \( P = 0.043 \)). 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 twelve patients (9%) received bone marrow stem cells).

Female patients showed slightly higher odds for vaccine-induced HI titer increase, although not significantly higher (OR = 1.30 [0.96, 1.77], \( P = 0.092 \)), and increasing age was negatively associated with baseline titers (OR = 0.81 [0.71, 0.93] for a 10-year difference in age, \( P = 0.002 \)).

We observed a positive association of the \( \text{IFNL3} \) rs8099917 minor allele (GT/GG) with response (OR = 1.54 [1.13, 2.09], \( P = 0.006 \)) and baseline titers (OR = 1.55 [1.00, 2.40], \( P = 0.050 \)). The other investigated genotypes showed no significant associations (Figure 3c). However, since all patients carrying the rs8099917 minor allele also carried the \( \text{IFNL3} \) rs12979860 minor allele (CT/CC) (Supplementary Figure 2), the positive association could also be due to an interaction effect between these two genotypes.

There was a significant negative association between PCR-confirmed influenza infection (influenza A or B) with both response (OR = 0.50 [0.34, 0.72], \( P = 3 \cdot 10^{-4} \)) and baseline titers (OR = 0.55 [0.32, 0.94], \( P = 0.030 \)), confirming that low antibody levels increase the risk for infection.
Patients reporting at least one local side effect on d7 had a stronger titer increase on d30 compared to patients without side effects (OR = 1.72 [1.26, 2.35], $P = 7 \cdot 10^{-4}$), and analogously, patients with any side effect on d37 showed stronger response on d60 (OR = 1.66 [1.23, 2.25], $P = 0.001$). 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], [20], [11], [21]. A randomized trial in 65 HSCT patients vaccinated with a non-adjuvanted quadrivalent influenza vaccine did not detect a significant effect [21]. However, the median time after transplantation was only one year (IQR 0.3–2 yrs) in this trial, while complete B-cell reconstitution can take up to two years [37]. Our patient population had a median time of four years (IQR 2–7 yrs), 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 [41], [42], [36], but have not yet been reported for HSCT patients. For cGVHD, previous studies reported either no significant effect on vaccine response [12], [13], [15], [5], [8], or a negative effect [11], [14], [6] but these studies are not directly comparable: A study that determined active GVHD (acute GVHD of grade $\geq 2$ 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 [11], [14], [6], 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 $\leq 6$ months post HSCT [11], [10], [20], [13], whereas another study with a time-post-HSCT distribution like ours observed no significant effect [8]. Our results also suggest that class I vs class II mismatched patients are differently affected by cGVHD and might respond to vaccination differently. However, classification of mismatches into only two 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.

**Conclusion**

On average, HSCT patients benefited from the booster shot and showed comparable responses after two vaccine doses to HVs after one. The strong association of time after transplantation with pre-vaccination 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 (unkown 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, e.g., with time after transplantation or medical history, might need to be considered in future studies. Since 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.
Author contributions
Study design and conceptualization: A.E., J.S., J.H. Funding acquisition: A.E., J.S. Experiments: M.S., D.V., L.K. Data management and data curation: D.V., Y.H., A.M.S.M., J.L. Methodology and formal analysis: J.L., H.M.K. Code implementation: J.L. Administration and resources: A.E., J.S., J.H., N.C., S.R.G., U.S., T.P., G.S., M.W. Supervision: J.S., A.E., H.M.K. Writing the first draft: J.L. Reviewing and editing: all.

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Conflict of interests
Potential conflicts of interest. N.C. has been a consultant to Celgene and Novartis, received honoraria for lectures and presentations from AbbVie, Celgene, Gilead, Novartis, OrPha Swiss, Pfizer, Sandoz, Sanofi-Aventis, and Shire, received support for attending meetings from AbbVie, Amgen, Celgene, and Gilead, and is a member of advisory boards for AbbVie, Alexion, Amgen, BMS, Celgene, Gilead, Incyte, Janssen-Cilag, Novartis, OrPha Swiss, Pfizer, Roche, Sandoz, Sanofi-Aventis, Shire, and Takeda.

The statistical methodology and parts of the results of this study were presented at the 31st Conference of the Austro-Swiss Region of the International Biometric Society in Lausanne, Switzerland (9 to 12 Sept 2019).
References

[1] Kunisaki K, Janoff E. Influenza in immunosuppressed populations: a review of infection frequency, morbidity, mortality, and vaccine responses. Lancet Infect Dis. 2009;9(8):493–504.

[2] Beck C, McKenzie B, Hashim A, Harris R, University of Nottingham Influenza and the ImmunoCompromised (UNIIC) Study Group, a, Nguyen-Van-Tam J. Influenza vaccination for immunocompromised patients: systematic review and meta-analysis by etiology. J Infect Dis. 2012;206(8):1250–1259.

[3] Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant. 2008;42(10):637–641.

[4] Caldera F, Mercer M, Samson SI, Pitt JM, Hayney MS. Influenza vaccination in immunocompromised populations: Strategies to improve immunogenicity. Vaccine. 2021;.

[5] Gueller S, Allwinn R, Mousset S, Martin H, Wieters I, Herrmann E, et al. Enhanced immune response after a second dose of an AS03-adjuvanted H1N1 influenza A vaccine in patients after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2011;17(10):1546–1550.

[6] Roll D, Ammer J, Holler B, Salzberger B, Schweiger B, Jilg W, et al. Vaccination against pandemic H1N1 (2009) in patients after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. Infection. 2012;40(2):153–161.
[7] Nazi I, Kelton JG, Larché M, Snider DP, Heddle NM, Crowther MA, et al. The effect of rituximab on vaccine responses in patients with immune thrombocytopenia. Blood. 2013;122(11):1946–1953.

[8] Fukatsu Y, Nagata Y, Adachi M, Yagyu T, Ono T. Serum IgM levels independently predict immune response to influenza vaccine in long-term survivors vaccinated at> 1 year after undergoing allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2017;105(5):638–645.

[9] Engelhard D, Nagler A, Hardan I, Morag A, Aker M, Baciu H, et al. Antibody response to a two-dose regimen of influenza vaccine in allogeneic T cell-depleted and autologous BMT recipients. Bone Marrow Transplant. 1993;11:1–1.

[10] Avetisyan G, Aschan J, Hassan M, Ljungman P. Evaluation of immune responses to seasonal influenza vaccination in healthy volunteers and in patients after stem cell transplantation. Transplantation. 2008;86(2):257–263.

[11] Mohty B, Bel M, Vukicevic M, Nagy M, Levrat E, Meier S, et al. Graft-versus-host disease is the major determinant of humoral responses to the AS03-adjuvanted influenza A/09/H1N1 vaccine in allogeneic hematopoietic stem cell transplant recipients. Haematologica. 2011;96(6):896–904.

[12] Pauksen K, Linde A, Hammarström V, Sjölin J, Carneskog J, Jonsson G, et al. Granulocyte-macrophage colony-stimulating factor as immunomodulating factor together with influenza vaccination in stem cell transplant patients. Clin Infect Dis. 2000;30(2):342–348.

[13] Issa NC, Marty FM, Gagne LS, Koo S, Verrill KA, Alyea EP, et al. Seroprotective titers against 2009 H1N1 influenza A virus after vaccination in allogeneic hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2011;17(3):434–438.
[14] Dhédin N, Krivine A, Le Corre N, Mallet A, Lioure B, Bay JO, et al. Comparable humoral response after two doses of adjuvanted influenza A/H1N1pdm2009 vaccine or natural infection in allogeneic stem cell transplant recipients. Vaccine. 2014;32(5):585–591.

[15] Engelhard D, Zakay-Rones Z, Shapira MY, Resnick I, Averbuch D, Grisariu S, et al. The humoral immune response of hematopoietic stem cell transplantation recipients to AS03-adjuvanted A/California/7/2009 (H1N1) v-like virus vaccine during the 2009 pandemic. Vaccine. 2011;29(9):1777–1782.

[16] Haralambieva IH, Ovsyannikova IG, Kennedy RB, Vierkant RA, Pankratz VS, Jacobson RM, et al. Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. Vaccine. 2011;29(45):7883–7895.

[17] Kennedy RB, Ovsyannikova IG, Haralambieva IH, O’Byrne MM, Jacobson RM, Pankratz VS, et al. Multigenic control of measles vaccine immunity mediated by polymorphisms in measles receptor, innate pathway, and cytokine genes. Vaccine. 2012;30(12):2159–2167.

[18] Linnik JE, Egli A. Impact of host genetic polymorphisms on vaccine induced antibody response. Hum Vaccin Immunother. 2016;12(4):907–915.

[19] Egli A, Santer DM, O’Shea D, Barakat K, Syedbasha M, Vollmer M, et al. IL-28B is a key regulator of B- and T-cell vaccine responses against influenza. PLoS Pathog. 2014;10(12):e1004556.
[20] de Lavallade H, Garland P, Sekine T, Hoschler K, Marin D, Stringaris K, et al. Repeated vaccination is required to optimize seroprotection against H1N1 in the immunocompromised host. Haematologica. 2011;96(2):307–314.

[21] Karras NA, Weeres M, Sessions W, Xu X, DeFor T, Young JAH, et al. A randomized trial of one versus two doses of influenza vaccine after allogeneic transplantation. Biol Blood Marrow Transplant. 2013;19(1):109–116.

[22] Greenland S, Mansournia MA, Altman DG. Sparse data bias: a problem hiding in plain sight. BMJ. 2016;352:i1981.

[23] Capuano AW, Dawson JD, Gray GC. Maximizing power in seroepidemiological studies through the use of the proportional odds model. Influenza Other Respir Viruses. 2007;1(3):87–93.

[24] Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945–956.

[25] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O’Hare D, O’Huin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461(7265):798–801.

[26] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009;41(10):1105.
[27] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461(7262):399–401.

[28] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. Nat Genet. 2009;41(10):1100.

[29] Jiménez-Sousa M, Berenguer J, Rallon N, Guzmán-Fulgencio M, Lopez J, Soriano V, et al. IL 28 RA polymorphism is associated with early hepatitis C virus (HCV) treatment failure in human immunodeficiency virus-/HCV-coinfected patients. J Viral Hepat. 2013;20(5):358–366.

[30] Egli A, Levin A, Santer DM, Joyce M, O’Shea D, Thomas BS, et al. Immunomodulatory function of interleukin 28B during primary infection with cytomegalovirus. J Infect Dis. 2014;210(5):717–727.

[31] WHO. WHO manual on animal influenza diagnosis and surveillance. 2002;.

[32] Kaufmann L, Syedbasha M, Vogt D, Hollenstein Y, Hartmann J, Linnik JE, et al. An Optimized Hemagglutination Inhibition (HI) Assay to Quantify Influenza-specific Antibody Titers. J Vis Exp. 2017;(130):e55833.

[33] Tutz G. Sequential models in categorical regression. Comput Stat Data Anal. 1991;11(3):275–295.

[34] Agresti A. Categorical data analysis. vol. 482. John Wiley & Sons; 2003.
[35] Yee TW, et al. The VGAM package for categorical data analysis. J Stat Softw. 2010;32(10):1–34.

[36] Smetana J, Chlibek R, Shaw J, Splino M, Prymula R. Influenza vaccination in the elderly. Hum Vaccin Immunother. 2018;14(3):540–549.

[37] Williams KM, Gress RE. Immune Reconstitution and Implications for Immunotherapy Following Hematopoietic Stem Cell Transplantation. In: Allogeneic stem cell transplantation. Springer; 2010. p. 545–564.

[38] Allison AC, Eugui EM. Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. Transplantation. 2005;80(2S):S181–S190.

[39] Machado C, Cardoso M, Da Rocha I, Boas L, Dulley F, Pannuti C. The benefit of influenza vaccination after bone marrow transplantation. Bone Marrow Transplant. 2005;36(10):897–900.

[40] Nichols WG, Guthrie KA, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis. 2004;39(9):1300–1306.

[41] Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, Thibaut R, et al. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci USA. 2014;111(2):869–874.
[42] Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626.

[43] Kollman C, Spellman SR, Zhang MJ, Hassebroek A, Anasetti C, Antin JH, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016;127(2):260–267.

[44] Syedbasha M, Bonfiglio F, Linnik J, Stuehler C, Wüthrich D, Egli A. Interferon-lambda enhances the differentiation of naive B-cells into plasmablasts via mTORC1 pathway. Cell Reports. 2020;33(1):108211.

[45] Ye L, Ohnemus A, Ong LC, Gad HH, Hartmann R, Lycke N, et al. Type I and type III interferons differ in their adjuvant activities for influenza vaccines. J Virol. 2019;93(23).
Figures

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

Figure 2: Antibody titers in hematopoietic stem cell transplant (HSCT) patients and healthy volunteers (HVs). (a) Hemagglutination inhibition (HI) titers against three different influenza strains in HSCT patients from two consecutive flu seasons. (b) As (a) but in HVs from the same 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 one titer level on d7/d30/d60/d180 compared to d0. (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 one level compared to the reference, specifically, d60 vs d30, season 2015/16 vs 2014/15 (by strain), A/California titers vs A/Texas titers and B/Massachusetts titers vs others (by season).

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 hemagglutination inhibition when hemagglutination inhibition has also been observed in all preceding dilution steps (i.e., increase in HI titer by at least one level). Alternatively, effects can also be interpreted as a shift in antibody concentration between the compared subpopulations. Since all serum samples are diluted equally, this shift is the same at all dilution steps. For details on the regression models, see Supplementary Information. Note that we corrected for baseline titers when analysing post-vaccination titers since we want to infer effects on the relative HI titer increase, which means that we compare 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. (c) Estimated effects for important patient factors. All inferred effects are shown in Supplementary Figure 5.
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 (d0) vs HI titer response (d30 & d60). 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 ($P = 0.001$). There was no significant difference between A/California (H1N1) and A/Texas (H3N2) ($P = 0.340$). (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 1000–4800 cells per L blood. Shaded area indicates 95% confidence intervals in all figures.
Tables

Table 1: Vaccine composition. Participants received a non-adjuvanted seasonal influenza vaccine by intramuscular injection comprising inactivated subunit viruses with 15 g HA antigen per strain (according to influenza season as specified below).

Table 2: Patient characteristics. Columns refer to two consecutive influenza seasons. For frequency of all determined genotypes, see Supplementary Table 2.

Table 3: Reported local side effects by HSCT patients (n=118). Questionnaires on side effects were not available for patients from Bern.
**Table 1. Vaccine composition.** Participants received a non-adjuvanted seasonal influenza vaccine by intramuscular injection comprising inactivated subunit viruses with 15 μg HA antigen per strain (according to influenza season as specified below).

| Manufacturer       | Season 2014/15                                                                 | Season 2015/16                                                                 |
|--------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
|                    | Agrippal, Novartis, Switzerland                                                 | Fluarix Tetra, GSK, UK                                                        |
| Influenza strains  | A/California/7/2009 (H1N1)                                                     | A/California/7/2009 (H1N1)                                                    |
|                    | A/Texas/50/2012 (H3N2)                                                        | A/Switzerland/9715293/2013 (H3N2)                                             |
|                    | B/Massachusetts/2/2012 (Yamagata lineage)                                      | B/Phuket/3073/2013 (Yamagata lineage)a                                       |
|                    |                                                                                | B/Brisbane/60/2008 (Victoria lineage)b                                        |

^aHI titers were not measured against this strain.

^bHI titers against this strain were excluded in the final analysis as the HI assay showed too low immunogenicity (see Supplementary Information).
Table 2. Patient characteristics. Columns refer to two consecutive influenza seasons. For frequencies of all determined genotypes, see Supplementary Table 2.

|                          | Season 2014/15 | Season 2015/16 | All            |
|--------------------------|---------------|---------------|----------------|
| Total                    | 55            | 80            | 135            |
| Age                      |               |               |                |
| Median, IQR, range (years) | 55, 44-64, 22-72 | 54, 47-63, 24-74 | 54, 46-64, 22-74 |
| ≥ 65 years               | 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 (AML) | 21 (38%)    | 29 (36%)      | 50 (37%)       |
| Acute lymphoblastic leukemia (ALL) | 10 (18%)  | 12 (15%)      | 22 (16%)       |
| Multiple myeloma (MM)    | 6 (11%)       | 8 (10%)       | 14 (10%)       |
| Chronic myeloid leukemia (CML) | 6 (11%)    | 6 (8%)        | 12 (9%)        |
| Chronic lymphocytic leukemia (CLL) | 6 (11%)  | 0             | 6 (4%)         |
| Myelodysplastic syndromes (MDS) | 3 (5%)      | 8 (10%)       | 11 (8%)        |
| Non-Hodgkin lymphoma (NHL) | 1 (2%)       | 6 (8%)        | 7 (5%)         |
| Myeloproliferative neoplasms (MPN) | 1 (2%)    | 5 (6%)        | 6 (4%)         |
| Other                    | 1 (2%)        | 6 (8%)        | 7 (5%)         |
| Time after transplantation |               |               |                |
| Median, IQR, range (years) | 4, 2-8, 1-25 | 4, 2-7, 1-22  | 4, 2-7, 1-25   |
| 1-2 years                | 19 (35%)      | 33 (41%)      | 52 (39%)       |
| 3-5 years                | 15 (27%)      | 21 (26%)      | 36 (27%)       |
| >5 years                 | 21 (38%)      | 26 (33%)      | 47 (35%)       |
| Absolute lymphocyte count |               |               |                |
| Median, IQR, range (10^9 cells/L) | 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%)      |
| 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 treatmenta |            |               |                |
| None                     | 25 (45%)      | 45 (56%)      | 70 (52%)       |
| Tacrolimus               | 16 (29%)      | 14 (18%)      | 30 (22%)       |
| Prednisone               | 13 (24%)      | 16 (20%)      | 29 (22%)       |
| Mycophenolateb           | 9 (16%)       | 11 (14%)      | 20 (15%)       |
| Cyclosporine Ac          | 5 (9%)        | 13 (16%)      | 18 (13%)       |
| Chronic GVHD | None | Mild (grade 1) | Moderate (grade 2) | Severe (grade 3) | Not available |
|--------------|------|---------------|-------------------|-----------------|--------------|
|              | 20 (36%) | 12 (22%) | 12 (22%) | 11 (20%) | 0 |
|              | 40 (50%) | 16 (20%) | 6 (8%) | 14 (18%) | 4 (5%) |
|              | 60 (44%) | 28 (21%) | 18 (13%) | 25 (19%) | 4 (3%) |
| IFNL3/4 genotype | | | | | |
| rs8099917 (GT/GG) | 23 (42%) | 27 (34%) | | 50 (37%) | |
| rs12979860 (CT/TT) | 35 (64%) | 40 (50%) | | 75 (56%) | |
| IFNLRI genotype | | | | | |
| rs10903035 (AG/GG) | 25 (46%) | 44 (55%) | | 69 (51%) | |
| Influenza infection | | | | | |
| Influenza A | 5 (9%) | 1 (1%) | | 6 (4%) | |
| Influenza B | 3 (5%) | 0 | | 3 (2%) | |

Abbreviations: IQR: interquartile range; GVHD: graft-versus-host disease; HLA: human leukocyte antigen.

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 six months
e PCR-confirmed influenza infection during flu season; for time of detection by PCR, see Supplementary Figure 3.
Table 3. Reported local side effects by HSCT patients (n=118). Questionnaires on side effects were not available for patients from Bern.

| Side effect               | Count on day 7 | Count 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%)        |
Excluded due to missing patient record, only HI titers available (n = 2)

Patients from Aarau, Basel, Bern, Lucerne (n = 57)

Season 2014/2015 (n = 55)

Patients from Aarau, Basel, Bern, Lucerne, Ticino, Zurich (n = 87)

Excluded due to missing patient record, only HI titers available (n = 3)

Drop-out (n = 2)

Excluded because patients received only one vaccination (n = 2)

Study population (n = 135)
