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Predictive factors for vaccine failure to guide vaccination in allogeneic hematopoietic stem cell transplant recipients

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Vaccination after hematopoietic stem cell transplantation (HSCT) is essential to protect high-risk patients against potentially lethal infections. Though multiple studies have evaluated vaccine specific responses, no comprehensive analysis of a complete vaccination schedule post-HSCT has been performed and little is known about predictors for vaccine failure. In this context, allogeneic HSCT (alloHSCT) patients were included and vaccinated starting one year post-transplantation. Antibody responses were measured by Multiplex Immuno Assay for pneumococcal (PCV13), meningococcal C, diphtheria, pertussis, tetanus and *Haemophilus influenzae* type b one month after the last vaccination and correlated to clinical and immunological parameters. Vaccine failure was defined as antibody response above vaccine-specific cut-off values for less than four out of six vaccines. Ninety-six patients were included of which 27.1% was found to have vaccine failure. Only 40.6% of all patients responded adequately to all six vaccines. In multivariate analysis, viral reactivation post-HSCT (OR 6.53; $P = 0.03$), B-cells <135 per mm^3 (OR 7.24; $P = 0.00$) and NK-cells <170 per mm^3 (OR 11.06; $P = 0.00$) were identified as predictors for vaccine failure for vaccination at one year post-alloHSCT. Measurement of antibody responses and an individualized approach for revaccination guided by clinical status and immune reconstitution of B-cells and NK-cells may improve vaccine responses.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a promising and often the only curative option for patients with hematologic malignancies [1]. Post-HSCT, previous immunity is often lost and the patient's immune system is severely impaired due to pre-HSCT conditioning, post-HSCT immune suppression and time needed for immune reconstitution [1]. Therefore, infectious complications are a major cause of death post-HSCT. Guidelines recommend preventive strategies such as antibiotics, antiviral and antifungal prophylaxis and vaccination. Required vaccines are clearly described in guidelines [2–5], but data on vaccine efficacy, antibody responses and parameters to predict these responses are scarce [2–6]. Insight in vaccine responses and identification of predictors for vaccine failure may guide an individualized approach in timing of vaccination and improve vaccine responses.

Vaccination within the first year post-HSCT may result in lower responses due to ongoing immune reconstitution and might thereby not sufficiently diminish the risk of infection [7]. Though delayed vaccination will theoretically result in better and longer lasting responses [7], this leaves patients for a longer period unprotected and at higher risk for infections. Therefore, insight in vaccine responses and predictors for vaccine failure is of great importance. Besides clinical parameters, such as graft-versus-host disease [8], immunological markers for immune reconstitution may also be predictive for vaccine failure. However, integrated

studies on clinical and immunological parameters are currently lacking.

Within this context, we assessed responses to a complete vaccination schedule in allogeneic HSCT (alloHSCT) recipients between December 2012 and March 2018 and studied clinical and immunological influencing vaccine failure. Vaccine response was measured by antibody responses and implemented as standard care. The stem cell transplantation program was accredited by the Joint Accreditation Committee of European Society for Blood and Marrow Transplantation (EBMT) and International Society for Cellular Therapy (ISCT) (JACIE) and linked to a biobanking study protocol.

METHODS

Study design and population

We performed an observational cohort study among HSCT patients eligible for vaccination. Patients aged ≥ 18 in the HSCT-program of the Hematology department from the University Medical Center Utrecht (UMCU), The Netherlands were included (protocol number METC 11–063) and informed consent was obtained. Conform to local protocol, vaccination was initiated one year post-HSCT in all post-HSCT patients. In patients who were clinically unstable, had severe GVHD (grade $\geq \text{II}$ and daily use of ≥ 20 mg prednisone) or had any active infection, vaccination was postponed until either recovery or dose reduction of prednisone <10 mg daily use. Patients were vaccinated according to a standard vaccination schedule for

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diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b (Hib), poliomyelitis and hepatitis B at twelve months, thirteen months and fourteen months post-HSCT. A conjugate pneumococcal vaccine was administered at twelve, thirteen and fourteen months post-HSCT and meningococcal C vaccine at twelve months post-HSCT (polysaccharide meningococcal vaccine C or quadrivalent meningococcal vaccine ACWY from May 2017). Blood was drawn for determination of antibody titers at fifteen months post-HSCT for pneumococcal, meningococcal C, diphtheria, tetanus, pertussis and Hib. If vaccination was postponed, blood sample collection was equally postponed, ensuring antibody response measurement at one month after the last vaccination. This moment was defined as end of follow-up.

Data collection and markers for response

Data on baseline characteristics were collected. At each hospital visit, infections and acute GVHD (aGVHD) were scored. All data were collected as part of the JACIE-associated obligation to report on patient outcome [9]. Specific infections (bacterial, fungal, BK-virus and respiratory viruses) and viral reactivations (Epstein-Barr virus (EBV) and cytomegalovirus (CMV)) and use of immunosuppressive medication were evaluated for the follow-up period. Data on immunological parameters were collected. If these were not yet determined for standard care, they were determined on biobank samples if available.

Definitions

Infections and reactivations. Infection was defined as clinical symptoms with a positive polymerase chain reaction (PCR) test. Routine screening for EBV and CMV reactivation was performed by PCR-testing. Cut-off values used for viral reactivation and pre-emptive treatment were for CMV > 250 IU/ml and EBV > 5000 IU/ml plasma (=1000 c/ml).

Use of immunosuppressive medication. Systematic usage of the following immunosuppressive drugs was taken into account: mycophenolate mofetil (MMF), steroids, calcineurin inhibitors (CNI), mTOR inhibitors, immunomodulatory drugs (IMiD's), tyrosine kinase inhibitors (TKI's), TNF α -inhibitors and B-cell depleting agents (Rituximab). To determine the immunosuppressive effect of specific immunosuppressants, dosage and duration of immunosuppressive effects were taken into account [10]. For each immunosuppressant the immunosuppressive period was defined from start till stop date plus duration of lasting immunosuppression after cessation. For steroids the equivalent of prednisone 10 milligrams per day for one month was considered relevant with an immunosuppressive effect lasting one month. The immunosuppressive effect of MMF, CNI/mTOR, TKI's, IMiD's and TNF α -inhibitors was defined as one month after cessation whereas the immunosuppressive effect of Rituximab was defined as six months after cessation [10]. Furthermore, any immunosuppressive medication started within 2 weeks after starting vaccination was considered to be relevant.

Immunological parameters at moment of vaccination. Immunological parameters as absolute cell counts of T-cells, CD4-cells, CD8-cells, B-cells and NK-cells were measured in blood samples at the moment of first vaccination from a range of 6 months before until 3 months after the first vaccination. Cell counts were measured by flowcytometry and hematopoietic cells were identified by CD45. Then, subsets were identified as following: T-cells CD3 (plus respectively CD4 and CD8 for CD4- and CD8-cells), B-cells CD19 and NK-cells CD16 and CD56. Immunological parameters were evaluated as predictors for vaccine failure and subsequently cell count variables were dichotomized for further analysis.

Assessment of vaccine responses

To estimate vaccine immunogenicity, antibody responses to administered vaccine antigens were measured. IgG antibodies against *Bordetella pertussis* (Ptx, FHA and Prn), diphtheria, tetanus, Hib, *Neisseria meningitidis* type C and 13 *Streptococcus pneumoniae* serotypes (PS) were measured in two dilutions using an in-house developed, validated and standardized fluorescent bead-based Multiplex Immuno Assays (Luminex xMAP technology). For the DTaP multiplex assay, samples were diluted in phosphate-buffered saline (PBS) containing 0.1% Tween-20 and 3% bovine serum albumin [11]. Samples for the Hib and meningococcal C assay were diluted in Surmodics containing 10% FCS [12]. Pneumococcal assay samples were diluted in Surmodics containing 10% FCS with 15 μ g/ml cell wall polysaccharide Multi (Statens Serum Institute, Copenhagen, Denmark) to reduce non-specific reactions [13]. In all assays a reference, controls and

blanks were included on each plate. All analyses were performed with a Bio-Plex 200 combined with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).

Antibody levels were considered protective according to vaccine-specific cut-off values. An adequate response was defined as an antibody level above this value. The following cut-off values were used: PS \geq 0.35 μ g/ml; [14] meningococcal C and Hib \geq 1.0 μ g/ml; [15, 16] diphtheria and tetanus \geq 0.1 International Units/ml (IU/ml) [17] and pertussis (Ptx) \geq 20 IU/ml [18]. For pneumococcal vaccination, responses for 13 PS were measured. Adequate response to the pneumococcal vaccine was defined as \geq 7 out of 13 PS titers \geq 0.35 μ g/ml [19].

The primary outcome was overall vaccine failure, defined as an adequate response for less than four out of six vaccines. Patients with vaccine failure were classified as non-responders; those with an adequate response to four out of six vaccines or more as responders. An optimal vaccine response was defined as an adequate response to all six out of six vaccines (pneumococcal, meningococcal C, Hib, diphtheria, tetanus and pertussis). Exploratory analyses were performed to determine the cut-off for non-responders versus responders.

Statistical methods and analytical approach

Antibody responses to the complete vaccination schedule were evaluated on patient level. Additionally, vaccine specific responses were studied. Associations between clinical and immunological parameters and the antibody response were studied to determine predictive factors for adequate response. Exploratory analyses were performed to determine cut-off values for the immunological parameters by ROC curves. To assess the influence of timing of the measurement of immunological parameters, repetitive analyses were performed excluding outliers. Finally, the definition of a responder was explored by sensitivity analyses adapting this outcome measure (for example: responder defined as response to five out of six instead of four out of six vaccines).

All data were analysed with SPSS Statistics for Windows, version 26.0. Categorical variables were analysed with χ^2 test or Fisher's exact test. Continuous variables were analysed with Student's *t* test with Levene's test for equality of variances. All analyses used 2-tailed significance level of $P < 0.05$. Univariate analysis was completed for each individual variable where odds ratios (OR), confidence intervals (CI) and *P* values were calculated. Multivariate logistic regression modeling was performed with $P < 0.10$ for the primary outcome as criterion for inclusion in this modeling. The model was stepwise reduced through exclusion of variables with backward selection based on the probability of Wald statistics and the correct model was based on the Nagelkerke R square. The reliability of the multivariate logistic regression model was determined by the Hosmer-Lemeshow goodness-of-fit statistics. The area under the receiver operator characteristic curve (AUC) was calculated to indicate the model's discriminative ability.

RESULTS

Baseline characteristics

Study population. In December 2019, ninety-six patients who had completed their follow-up were enrolled. These patients had received their HSCT between December 2012 and March 2018. Baseline characteristics are described in Table 1. Median age at HSCT was 50 years old (range 18–73), 61.5% were male. Median time from HSCT till vaccination was 376 days (range: 261–1399; 25th percentile (Q1): 366; 75th percentile (Q3): 395). Sixty-seven patients (69.8%) were conditioned following a myeloablative regimen. Twenty-one patients (21.9%) developed aGVHD grade \geq II. Seventy-five patients (78.1%) had either an infection or a viral reactivation between HSCT and end of follow-up. Median number of infections and reactivations during follow-up was 2, and twelve patients (12.5%) had more than 4 infections or reactivations. Thirty-two patients (33.3%) used relevant immunosuppressants. No patients used intravenous immunoglobulins. Cell counts were measured at median 5 days before first vaccination (range –149 till +69; Q1: –15; Q3: 0 (= day of vaccination)).

Protocol adherence and missing values. Vaccination was postponed in seven patients (7.3%) due to protocol adherence. Five patients had both severe GVHD and active infection. One patient had severe GVHD and one patient was clinically unstable:

Table 1. Baseline characteristics of study patients.

Variable	Patients (n = 96)
Time between HSCT and first vaccination, median days (Q1–Q3)	376 (366–395)
Age at HSCT, mean years ± SD (range)	50 ± 14 (18–73)
Male sex	59 (61.5)
Hematological diagnosis	
AML	41 (42.7)
MM	6 (6.3)
MF	10 (10.4)
Lymphoma	11 (11.5)
Others	28 (29.2)
Donor type	
FD matched	26 (27.1)
FD mismatched	1 (1.0)
MUD matched	49 (51.0)
MUD mismatched	9 (9.4)
CBU	11 (11.5)
Cell source	
BM	6 (6.3)
PB	80 (83.3)
CBU	10 (10.4)
Myeloablative conditioning	67 (69.8)
Anti-thymocyte globulin (ATG)	75 (78.1)
Total body irradiation (TBI) (2–4 Gray)	35 (36.5)
T-cell depletion	52 (54.2)
aGVHD grade ≥ II	21 (21.9)
aGVHD grade ≥ II within 3 months prior to vaccination	4 (4.2)
Infections during follow-up	54 (56.3)
Bacterial	24 (25.0)
Fungal	12 (12.5)
BK-virus	24 (25.0)
Respiratory viruses	20 (20.8)
Viral reactivations during follow-up	60 (62.5)
Epstein-Barr virus (EBV)	32 (33.3)
Cytomegalovirus (CMV)	40 (41.7)
Infections or reactivations within 3 months prior to vaccination	3 (3.1)
Total number of infections and reactivations during follow-up, median (Q1–Q3)	2 (1–3)
Total number of infections and reactivations during follow-up > 4	12 (12.5)
Usage of relevant immunosuppressive drugs	32 (33.3)
Steroids	14 (14.6)
Calcineurin inhibitors (CNI) or mTOR inhibitors	19 (19.8)
Immunomodulatory imide drugs (IMiD's) or Tyrosine kinase inhibitors (TKI's)	7 (7.3)
B-cell depleting agents (Rituximab)	2 (2.1)
Cell counts at first vaccination, median cells per mm ³ (Q1–Q3)	
T-cells	633 (292–1251)
CD4-cells	205 (114–315)
CD8-cells	371 (127–705)
B-cells	254 (94–457)
NK-cells	250 (130–360)

Data are n (%) of patients unless otherwise indicated.

n number of patients, HSCT hematopoietic stem cell transplantation, Q1 25th percentile, Q3 75th percentile, SD standard deviation, AML acute myeloid leukemia, MM multiple myeloma, MF myelofibrosis, FD family donor, MUD matched unrelated donor, CBU cord blood unit, BM bone marrow, PB peripheral blood, aGVHD acute graft-versus-host disease.

vaccination was postponed due to long lasting use of a high dose TKI (Dasatinib 100–140 milligrams daily for 21 months). Data on immunological parameters were not complete: T-cells, CD4-cells, B-cells and NK-cells were missing for twenty-one patients (21.9%) and CD8-cells for twenty-two patients (22.9%).

Vaccine responses

Antibody responses were evaluated per vaccine: responses varied from 47.9% to 86.5%, which is poor compared to healthy populations [20, 21] (Fig. 1). Adequate response to pneumococcal vaccination was reached in 69.8% with considerable variation among serotypes: 47.9% response for PS6B; 85.4% for PS19F. Meningococcal C vaccination resulted in 58.3% response; Hib 67.7%; pertussis 62.5% and diphtheria 79.2%. Tetanus vaccination induced best responses: 86.5%. Calculating overall vaccine response, 72.9% were responders; 27.1% were non-responders, of whom six patients (6.3%) had no response to any of the vaccines. Only thirty-nine patients (40.6%) had an optimal vaccine response.

Clinical and immunological predictive factors for vaccine failure

Exploration of immunological parameters. CD4 + T-, CD8 + T-, B- and NK-cell counts were first evaluated as continuous parameters to determine their influence on vaccine failure (Fig. 2). Only variations in B-cell and NK-cell but not T-cell counts were significantly associated with response. Non-responders had lower B-cell or NK-cell counts as compared to responders; B-cells mean difference 180.23 (±64.68, $P=0.01$); NK-cells mean difference 120.49 (±42.88, $P=0.01$) (Fig. 2, Table 2). All immunological parameters were explored by constructing ROC-curves, resulting in dichotomized cut-off values for B-cells of 135 cells per mm³ and for NK-cells of 170 cells per mm³ (B-cells AUC 0.798 (95% CI 0.675–0.921); NK-cells AUC 0.757 (95% CI 0.639–0.876)).

Predictive factors for vaccine failure. In univariate analysis, acute GVHD grade ≥ II within three months prior to vaccination (OR 9.00; $P=0.06$), respiratory virus infection (OR 2.84; $P=0.04$), viral reactivation (OR 3.34; $P=0.02$), EBV reactivation (OR 2.68; $P=0.04$), number of infections or reactivations >4 (OR 3.20; $P=0.08$),

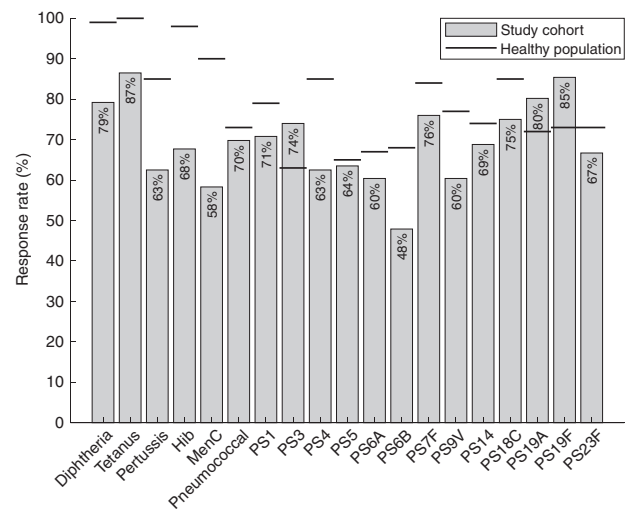


Fig. 1 Percentages of the study population with adequate antibody responses compared to the general healthy population. Healthy population responses: pneumococcal 73% (PS1 79%; PS3 63%; PS4 85%; PS5 65%; PS6A 67%; PS6B 68%; PS7F 84%; PS9V 77%; PS14 74%; PS18C 85%; PS19A 72%; PS19F 73%; PS23F 73%);[21] meningococcal C 90%; Hib 98%; diphtheria 99%, tetanus 100%; pertussis 85% [22]. Hib *Haemophilus influenzae* type b, MenC meningococcal C, PS pneumococcal serotype.

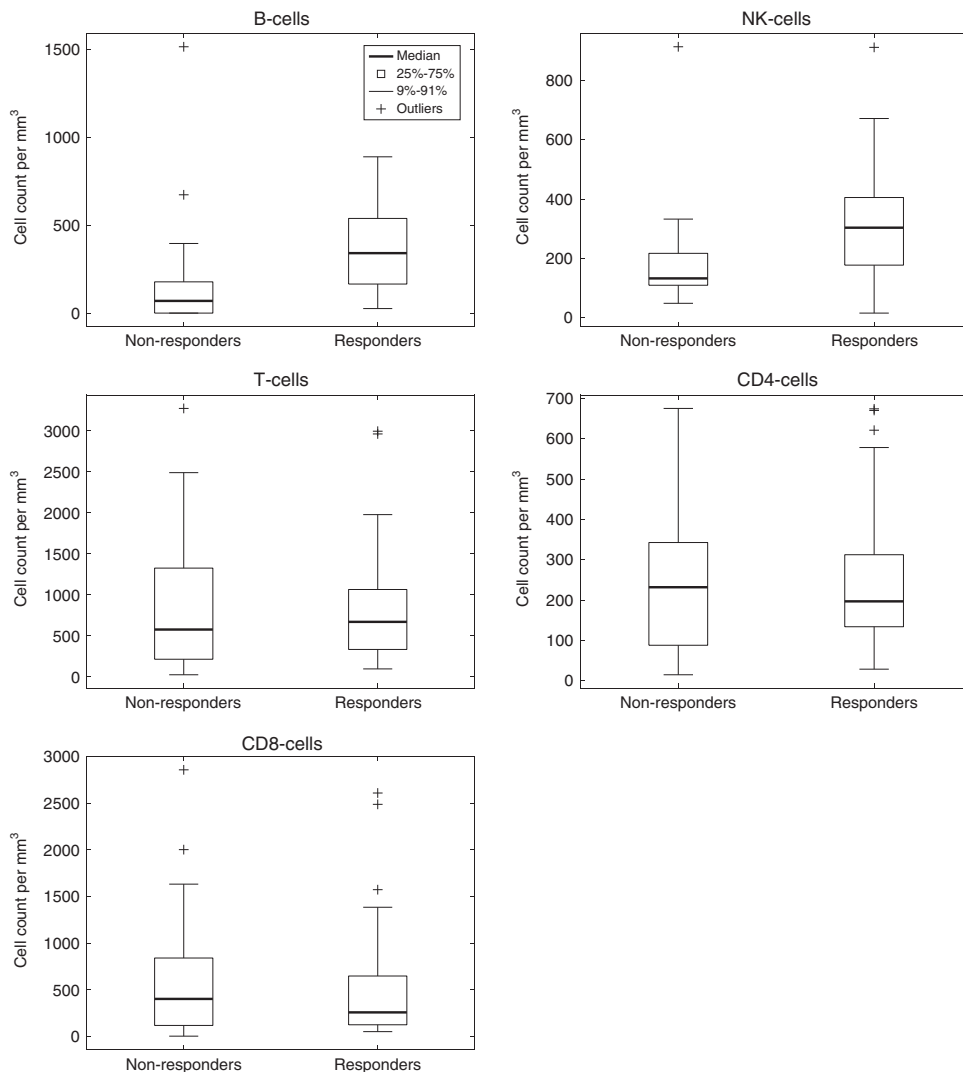


Fig. 2. Scatter of the immunological parameters for responders and non-responders. B-cells: mean difference -180.23 (± 64.68 , $P = 0.01$). NK-cells: mean difference -120.49 (± 42.88 , $P = 0.01$). T-cells: mean difference 59.44 (± 180.77 , $P = 0.77$). CD4-cells: mean difference -12.21 (± 40.99 , $P = 0.77$). CD8-cells mean difference 138.49 (± 154.38 , $P = 0.37$).

use of steroids (OR 4.74; $P = 0.01$), use of Rituximab (OR 14.39; $P = 0.09$), B-cells <135 per mm^3 (OR 11.33; $P = 0.00$) and NK-cells <170 per mm^3 (OR 7.89; $P = 0.00$) were associated with vaccine failure (Table 2).

In multivariate analysis, viral reactivations during follow-up ($P = 0.03$, OR 6.53, 95% CI (1.22–34.95)), B-cells <135 per mm^3 ($P = 0.00$, OR 7.24, 95% CI (1.89–27.68)) and NK-cells <170 per mm^3 ($P = 0.00$, OR 11.06, 95% CI (2.39–51.07)) were predictive for vaccine failure (Table 2). The AUC of this multivariate model was 0.871 (95% CI 0.79–0.96), indicating an 87.1% chance of predicting vaccine failure correctly. Additionally, outliers in timing of measurement of immunologic parameters were excluded and repetitive analyses were performed. This did not change the results. Finally, explorative analyses were performed for the outcome measure; predictive parameters remained similar.

DISCUSSION

This study is the first study describing vaccine responses to a full vaccination schedule post-alloHSCT and assessing both clinical and immunological parameters as predictors for vaccine failure. Antibody responses were analysed per vaccine and predictors for

overall response were studied. Only 40.6% of post-alloHSCT patients had an optimal vaccine response (adequate response to all six vaccines), whereas 27.1% of all patients had vaccine failure (adequate response to less than four out of six vaccines).

Responses varied per vaccine, which is supported by previous studies. In general, our responses are comparable to those reported on post-alloHSCT patients previously [22–28]. When comparing post-HSCT responses to healthy individuals, these are remarkably poor (Fig. 2). Of note, tetanus and diphtheria vaccinations induce best responses in both populations, whereas meningococcal C and pertussis induce poorest.

Evaluating predictors for vaccine failure, B-cells <135 per mm^3 (OR 7.24; $P = 0.00$) and NK-cells <170 per mm^3 (OR 11.06; $P = 0.00$) at vaccination were correlated significantly. To the best of our knowledge, this is the first study in post-alloHSCT patients showing that B-cells and NK-cells could be used as predictive immunological parameters for vaccine failure. B-cells are required for obtaining antibody responses; patients with B-cell deficiencies [29, 30] or using Rituximab [10, 22] were previously found to have decreased vaccine responses, which is supported in our univariate analysis (OR 14.39; $P = 0.09$). For the role of NK-cells, fewer studies have been conducted. There is increasing evidence showing a role

Table 2. Univariate and multivariate analysis for vaccine failure.

Parameter	Student's <i>t</i> test		Univariate analysis		Multivariate analysis	
	Mean difference (±SD)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
aGVHD grade ≥ II within 3 months prior to vaccination			9.00 (0.89–90.84)	0.06		
Infections during follow-up						
Respiratory viruses			2.84 (1.01–7.98)	0.04		
Viral reactivations during follow-up			3.34 (1.13–9.86)	0.02	6.53 (1.22–34.95)	0.03
Epstein-Barr virus			2.68 (1.06–6.82)	0.04		
Total number of infections and reactivations	−0.85 (±0.43)	0.05				
Total of infections and reactivations > 4			3.20 (0.93–11.04)	0.08		
Usage of relevant immunosuppressive drugs						
Steroids			4.74 (1.46–15.44)	0.01		
Rituximab			14.39 (0.67–310.25)	0.09		
Cell counts at first vaccination						
B-cells < 135 per mm ³	180.23 (±64.68)	0.01	11.33 (3.64–35.34)	0.00	7.24 (1.89–27.68)	0.00
NK-cells < 170 per mm ³	120.49 (±42.88)	0.01	7.89 (2.65–23.53)	0.00	11.06 (2.39–51.07)	0.00

Parameters investigated in univariate analysis included patient characteristics (age at hematopoietic stem cell transplantation (HSCT), sex, hematological diagnosis), data on HSCT (donor type, cell source, conditioning, anti-thymocyte globuline (ATG), total body irradiation (TBI), T-cell depletion), data on follow-up period (acute graft-versus-host disease (aGVHD), infections and reactivations, usage of relevant immunosuppressive drugs), data on vaccination (time between HSCT and first vaccination, cell counts at the moment of vaccination). $P < 0.10$ in univariate analysis were included in multivariate logistic regression analysis. Hosmer and Lemeshow test $P = 0.966$ for this multivariate model. *This OR was calculated by using the Haldane-Anscombe correction.

for NK-cells as driver for an optimal adaptive immune response. NK-cells can indirectly modulate the adaptive immune response by either augmenting or dampening antigen-presenting cell (APC) and T-cell effector functions. Hereby they can enhance B-cell activation and thus influence both the quality and the magnitude of adaptive immune responses [31, 32] and contribute to vaccine-induced immunity [33, 34]. Although the median time for measuring cell constitution was 5 days pre-vaccination, some of the samples had a measuring moment post-vaccination. We cannot exclude the potential effect of vaccination itself on cell counts. However, if vaccination would have increased cell counts, the actual counts would have been lower pre-vaccination and thereby we would have overestimated these, increasing the potential effect of the counts on vaccine response.

Viral reactivations post-HSCT are associated with vaccine failure (OR 6.53; $P = 0.03$). Previous studies reported on viral reactivation being an indicator for poor immune recovery post-HSCT [35–37]. Therefore, these reactivations might predict an impaired vaccine response and thus postponing vaccination would be indicated. Also, we found a correlation between vaccine failure and specifically respiratory virus infection (OR 2.84; $P = 0.04$) and EBV reactivation OR 2.68; $P = 0.04$ in univariate analysis. For EBV, the treatment of reactivation with Rituximab might play an important role [38]. Furthermore, frequency of reactivations and infections (more than four during follow-up) was correlated with vaccine failure (OR 3.20; $P = 0.08$). Reactivation or infection closer to the vaccination would also likely have a higher impact on immunological response and would thereby decrease vaccine responses even more. However, as only 3 patients (3.1%) in our population had an infection within 3 months prior to vaccination, our results did not show this impact. Moreover, present data show that infections and reactivations in the first year and not only short before revaccination need to be taken in account to predict vaccine failure.

In previous studies, different predictors for post-HSCT vaccine response were reported. First, T-cells were reported to influence response [32, 39–41], especially CD4-cells [31, 42]. In our analyses, T-cells were not associated with vaccine responses. This is possibly due to our vaccination regimen: in patients with active GVHD, high dose of immunosuppressants or active infection, vaccination was postponed. This postponement might have led to exclusion of patients with lowest T-cell counts. However, still thirty-two

patients (33.3%) had not completely recovered T-cells (absolute cell count <500 per mm³) at vaccination. Furthermore, aGVHD was previously reported to delay T-cell recovery and to negatively influence responses [6, 8, 22, 42, 43]. Despite postponement of vaccination due to aGVHD in six patients, influence of aGVHD was supported: univariate analysis showed influence of both aGVHD (OR 9.00, $P = 0.06$) and steroids (OR 4.74; $P = 0.01$) on response.

The present study is one of the first analyzing clinical and immunological parameters together as predictors for vaccine failure post-alloHSCT. Furthermore, we took into account timing and duration of the immunosuppressive effect of medication. This resulted in more precise and clinically relevant outcomes. The current study also has potential limitations. First, no pre-vaccination titers were measured which might have caused overestimation of the responses. Second, the primary outcome measure, namely vaccine failure defined as an adequate response to less than four out of six vaccines, might have been chosen differently. However, this outcome was considered to be clinically relevant and usable, and adjustment of this definition did not influence the results. Furthermore, it was common practice in our center at time of this study to initiate vaccination at one year post-HSCT (despite recommendations [3, 5] to initiate vaccination at three till six months post-HSCT under certain conditions) due to our (more severe) conditioning regimes and to postpone vaccination in patients who had severe GVHD or an active infection. This postponement could have influenced results, although vaccination was only postponed in seven patients (7.3%). Furthermore, data on the immunological parameters were incomplete: complete immune cell counts were unavailable in 22.9% of the patients. Finally, this study only focused on antibody titers as a measure for vaccine-induced immunity. Other measurements for immunity such as T-cell responses or functional assays were not addressed.

In conclusion our study showed vaccine failure in 27.1% of patients who were vaccinated at one year post-alloHSCT. Only 40.6% of all patients obtained optimal response, which is remarkably poor compared to healthy populations. Consequently, antibody responses must be evaluated post-vaccination and improving these responses is highly desirable. Viral reactivation post-HSCT might be a clinical indicator of poor immune recovery and thereby predict vaccine failure. Impaired reconstitutions of

B-cells and NK-cells were associated with vaccine failure and could potentially be used to guide an individual approach of vaccination post-HSCT to optimize vaccine responses.

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AUTHOR CONTRIBUTIONS

MJ and AB designed the study and wrote the manuscript. RJW collected clinical data, FVL collected samples for immunological data. NN and GS performed the Multiplex

Immuno Assays on collected samples and DVB supervised these assays. MJ analyzed and interpreted the clinical and immunological data. AB, RR, DVB and JK gave critical input. All authors critically revised the manuscript, approved the final version to be published and agreed to accountability of the work.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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