Clinical Effectiveness of Influenza Vaccination After Allogeneic Hematopoietic Stem Cell Transplantation: A Cross-sectional, Prospective, Observational Study

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The influenza virus has a significant impact on morbidity and mortality in allogeneic hematopoietic stem cell transplantation patients (allo-HSCT), leading to complications ranging from self-limited upper–respiratory tract infections to life-threatening or fatal pneumonias [1–4]. The particular threat influenza poses for allo-HSCT recipients was well documented during the 2009 influenza A/H1N1 pandemic, as well as during consecutive seasonal influenza epidemics. It showed increased risks of hospital admissions, mechanisms of seasonal influenza epidemics. It showed increased risks of hospital admissions, mechanisms of seasonal influenza epidemics. It showed increased risks of hospital admissions, mechanisms of seasonal influenza epidemics. It showed increased risks of hospital admissions, mechanisms of seasonal influenza epidemics. It showed increased risks of hospital admissions, mechanisms of seasonal influenza epidemics. 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was prospectively recorded at the time of their respiratory virus screening. In this study, we report the prevalence of influenza RTD according to the vaccination status over 5 consecutive influenza seasons in a consecutive series of allo-HSCT recipients with virologically-documented respiratory virus infections (RVIs).

**PATIENTS AND METHODS**

**Study Population**

This was a prospective, cross-sectional study of RVIs in adult (>18 years) allo-HSCT recipients that was conducted at 2 Spanish transplant centers. The whole cohort comprised consecutive allo-HSCT recipients with respiratory symptoms who were prospectively screened for respiratory viruses at the Hospital Clinic Universitari of Valencia (HCUV) between December 2013 and May 2016 and at the Hospital Universitari i Politècnic la Fe in Valencia (HLF) from June 2016 to May 2018.

**Characteristics of the Respiratory Virus Survey Protocol**

In December 2013 at the HCUV and in May 2016 at the HLF, we implemented the medical information/education for recipients and caregivers, explaining in detail the risks of having RVIs in the context of immunosuppression. The specific information provided included a description of the respiratory symptoms that merit urgent notification to the transplant team; recommendations concerning screening for respiratory viruses; details of available therapies; and infectious prevention control measures for patients and caregivers. A telephone number (on-call 24 h) for emergent conditions was also provided [20]. The local ethics committee approved the study protocol.

**Clinical and Biological Variables**

We prospectively recorded participants’ clinical and biological characteristics at the time of CARV polymerase chain reaction (PCR) test screening, including the month and year of vaccination and the influenza vaccination status at each flu season. Variables included the immunodeficiency scoring index (ISI) [21] and Basel immunodeficiency grading [22, 23] results; hospital admissions; the use of immunosuppressant drugs; the presence of signs or symptoms of acute or chronic GvHD; and transplant characteristics.

**Cohort Selection**

With the aim of retrospectively comparing the influenza RTD prevalence between vaccinated and non-vaccinated participants, we included 1 recipient/RV episode/vaccination status in each flu season, following the selection algorithm described in Figure 1. We designed the case selection as follows: the first inclusion criterion was to retrospectively select all RVI episodes (irrespective of the CARVs detected) with known vaccination statuses. The next step was to divide the CARV episodes into 2 groups according to the vaccination status at each RVI episode (vaccinated or unvaccinated, in each corresponding flu season). Following recommendations from the World Health Organization’s guidelines [24], we excluded the CARV episodes taking place outside the flu seasons (between June and July) and those that did not meet the selection criteria.

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**Figure 1.** Study selection criteria algorithm. Abbreviations: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; CARV, community-acquired respiratory virus; PCR, polymerase chain reaction; RVI, respiratory virus infection.
November) from both groups. We considered December to May as recruitment periods, according to our local and national epidemiological data, since influenza viruses were circulating in our community. The third step was to exclude episodes that occurred before day 90 after a stem cell infusion from the unvaccinated group, since the vaccine was not given to any recipients during that period and the first recorded influenza vaccine was given at day 91 after transplant. For both cohorts, we also excluded RVI episodes when baseline disease relapses occurred before the CARV episode. For recipients with more than 1 RVI episode in the same flu season, we applied the following criteria: recipients with more than 1 episode of CARV RVI other than influenza during the same flu season were computed only once and classed as not having an influenza infection in that season. Recipients with more than 1 episode of RVI and whose vaccination status changed between 2 consecutive CARV RVI episodes in the same season were computed twice: once in the non-vaccinated group and once (after vaccination) in the vaccinated group. If a recipient developed 2 RVI episodes caused by 2 different influenza viruses (ie, influenza A/H1/N1 and B) at different time intervals during the same flu season, both episodes were computed in the corresponding group. When a recipient developed 2 influenza virus RVI episodes in the same season and the same influenza virus was detected, only the first episode was computed. Finally, when a recipient developed 2 RVI episodes, 1 of them caused by the influenza virus, we only computed the influenza episode in that season.

Vaccination Policy and Vaccine Composition

Annual influenza vaccination was recommended to all patients at both transplant centers after the third month following allo-HSCT. Recipients received a seasonal inactivated trivalent influenza vaccine according to the national vaccination program that ran from November until February in each flu season. For patients with moderate to severe GvHD at the time of the vaccination program who had received gammaglobulin, anti-thymocytic globuline, or rituximab within the 3 months of the vaccination program, vaccination was given at day 91 after transplant. For both cohorts, we also excluded influenza virus RVI episodes when baseline disease relapses occurred before the CARV episode. For recipients with more than 1 RVI episode in the same flu season, we applied the following criteria: recipients with more than 1 episode of CARV RVI other than influenza during the same flu season were computed only once and classed as not having an influenza infection in that season. Recipients with more than 1 episode of RVI and whose vaccination status changed between 2 consecutive CARV RVI episodes in the same season were computed twice: once in the non-vaccinated group and once (after vaccination) in the vaccinated group. If a recipient developed 2 RVI episodes caused by 2 different influenza viruses (ie, influenza A/H1/N1 and B) at different time intervals during the same flu season, both episodes were computed in the corresponding group. When a recipient developed 2 influenza virus RVI episodes in the same season and the same influenza virus was detected, only the first episode was computed. Finally, when a recipient developed 2 RVI episodes, 1 of them caused by the influenza virus, we only computed the influenza episode in that season.

URTD was defined as a combination of upper-respiratory tract symptoms (rhinorrhea, sinusitis, otitis, or pharyngitis), as well as the positive identification of a CARV by a PCR test and the absence of lower-respiratory tract infection symptoms and/or any indication of pulmonary infiltrates in chest X-ray or computed tomography (CT) scan radiology results. We classified LRTD as possible, probable, or confirmed, as previously described [25]. There were no probable episodes, because we did not perform bronchoscopies in patients without the radiological proof of pulmonary involvement. We defined episodes as an URTD or LRTD according to European Conference on Infections in Leukaemia–4 recommendations [26]. Acute GvHD was diagnosed and graded according to the standard criteria [27].

Technical and Diagnostic Considerations

Patients with URTD symptoms underwent nasopharyngeal aspiration, nasopharyngeal swabs, or an induced sputum test, while bronchoalveolar lavage (BAL) was performed in patients with a LRTD whenever possible. CARV testing in BAL samples were performed with 2 real-time PCR multiplex platforms, as described elsewhere in detail [28]. At the HCUV, samples were tested by real-time PCR using the Luminex xTAG RVP Fast v1 assay (Luminex Molecular Diagnostics, Toronto, Canada), while at HLF the CLART PneumoVir DNA array assay (Genomica, Coslada, Spain) was performed and interpreted following the manufacturer’s recommendations. The CLART PneumoVir DNA array assay differs from the Luminex xTAG RVP Fast v1 assay in that it detects influenza C virus, but not alphacoronavirus NL63 virus or betacoronaviruses HKU1 and OC43. The CLART PneumoVir identifies the new influenza A/H1N1v.

Endpoints and Statistical Analysis

The primary objective of the study was to compare the prevalence of influenza URTDs and/or LRTDs and clinical characteristics among vaccinated and unvaccinated allo-HSCT recipients. Secondary endpoints included identifying the risk factors for both influenza RTD and progression of the influenza virus from an URTD to a LRTD.

Frequencies were compared using the $\chi^2$ test (Fisher exact test) for categorical variables. Differences between medians were compared using the Mann–Whitney U test. Univariate and multivariate analyses of the association of clinical and biological risk factors with the progression of influenza LRTD were calculated using logistic regression models. For multivariate analyses, only variables with parameter estimates showing a $P$ value $\leq .10$ in the univariate analysis were finally included. We reported 2-sided exact $P$ values, and $P$ values $\leq .05$ were considered statistically significant. The data were analyzed with the SPSS (version 20.0) statistical package.
RESULTS

Patient Characteristics

Overall, 263 allo-HSCT recipients developed 601 episodes of upper- and/or lower-respiratory tract symptoms and were screened for RVIs. In total, 231 allo-HSCT recipients (87%) developed at least 1 episode of a virologically-documented RVI, accounting for 458 episodes (76%) of proven RVIs. According to the algorithm selection (Figure 1), we finally included 136 allo-HSCT recipients with 161 virologically-documented RVI episodes over 5 flu seasons. Out of 136 recipients, 8 were computed twice since they changed their vaccination status during the course of the study: 2 during the same season and 6 within 2 consecutive seasons. Thus, we finally included the characteristics of 144 recipient cases according to the subjects’ vaccination statuses (Table 1). There were 101 seasonally–non-vaccinated allo-HSCT recipients with 115 RVI episodes and 43 seasonally-vaccinated recipients with 46 RVI episodes who accomplished the selection criteria for comparison purposes. Of the 136 participants, 21 (15%) had computable RVI episodes in multiple flu seasons: specifically, 19 had 1 computable RVI episode in each of 2 consecutive seasons, whereas 2 recipients had 1 computable episode in each of 3 consecutive seasons.

Table 1. Patient Characteristics According to Flu Vaccine Status

| Characteristics                        | Non-vaccinated (n = 101) | Vaccinated (n = 43) | P Value |
|----------------------------------------|--------------------------|---------------------|---------|
| Age in years, median (range)           | 45 (18–70)               | 46 (18–72)         | .3      |
| Male, n (%)                            | 60 (60)                  | 28 (65)            | .6      |
| Baseline disease, n (%)                |                          |                     |         |
| AL/MDS                                 | 64 (64)                  | 25 (58)            | .7      |
| Lymphoid disorders                     | 32 (32)                  | 16 (37)            |         |
| Others                                 | 5 (5)                    | 2 (5)              |         |
| Disease status at transplant, n (%)    |                          |                     | .5      |
| CR                                     | 70 (70)                  | 34 (79)            |         |
| PR                                     | 15 (15)                  | 4 (9)              |         |
| Refractory/active disease              | 16 (15)                  | 5 (12)             |         |
| Prior ASCT, n (%)                      | 20 (20)                  | 10 (23)            | .7      |
| Period of transplant, n (%)            |                          |                     | .4      |
| 2016–2017                              | 36 (36)                  | 13 (30)            |         |
| 2014–2015                              | 34 (34)                  | 13 (30)            |         |
| 2012–2013                              | 22 (22)                  | 12 (28)            |         |
| 2009–2011                              | 9 (9)                    | 5 (12)             |         |
| Conditioning regimen, n (%)           |                          |                     |         |
| RIC                                    | 47 (47)                  | 22 (51)            | .7      |
| Type of donor, n (%)                   |                          |                     | .5      |
| HLA-identical sibling donor            | 31 (31)                  | 12 (28)            |         |
| Unrelated donor                        | 27 (27)                  | 10 (23)            |         |
| Umbilical cord blood                   | 26 (26)                  | 9 (21)             |         |
| Haploidentical family donor            | 17 (17)                  | 12 (28)            |         |
| Peripheral blood stem cell source, n (%)| 73 (73)                  | 33 (76)            | .8      |
| HLA fully-matched, n (%)               | 54 (54)                  | 20 (47)            | .5      |
| ATG as a part of the conditioning regimen, n (%) | 20 (20) | 10 (23) | .7 |
| GvHD prophylaxis, n (%)                |                          |                     | .5      |
| Sir-Tac                               | 15 (15)                  | 7 (16)             |         |
| CsA + MTX                             | 24 (24)                  | 11 (26)            |         |
| Post-CyPh                             | 32 (32)                  | 11 (26)            |         |
| CsA + PDN / Others                    | 30 (30)                  | 14 (32)            |         |
| Median time from allo-HSCT to CARV, days (range) | 343 (98–4578) | 592 (98–3700) | .1 |
| Number of recipients with CARVs per seasons, n (%) | | | |
| 2017–2018                              | 14                       |                     |         |
| 2016–2017                              | 7                        |                     |         |
| 2015–2016                              | 6                        |                     |         |
| 2014–2015                              | 14                       |                     |         |
| 2013–2014                              | 2                        |                     |         |
| Median F-Up after CARV, days (range)   | 370 (100–580)            |                     | .3      |

There were 136 recipients included in this study. However, 8 out of these 136 recipients changed their vaccination status during the course of the study. These 8 recipients were computed twice (once in each group), according to the vaccination status at the corresponding flu season. Thus, in total we show the characteristics of 144 cases, according to vaccination status.

Abbreviations: AL, acute leukemia; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ASCT, autologous stem cell transplantation; ATG, anti-thymocytic globuline; CARV, community-acquired respiratory virus; CR, complete remission; CsA, cyclosporine A; F-Up, follow-up; GvHD, graft-versus-host disease; HLA, human leucocyte antigen; MDS, myelodysplastic syndrome; MTX, methotrexate; PDN, prednisone; Post-CyPh, post-transplant cyclophosphamide; PR, partial remission; RIC, reduced intensity conditioning; Sir-Tac, sirolimus and tacrolimus.
Table 2. Clinical and Biological Characteristics of Patients at the Time of Respiratory Virus Infection Episodes According to Influenza Vaccination Status

| Characteristic                                                                 | CARV Episodes, Non-vaccinated (n = 115) | CARV Episodes, Vaccinated (n = 46) | P Value |
|-------------------------------------------------------------------------------|----------------------------------------|-----------------------------------|---------|
| Immunodeficiency scoring index, n (%)<sup>a</sup>                            |                                        |                                   |         |
| ANC < 0.5 × 10<sup>9</sup>/L, 3pts                                           | 8 (7)                                  | 1 (2)                             | .3      |
| ALC < 0.2 × 10<sup>9</sup>/L, 3pts                                           | 27 (23)                                | 7 (9)                             | .2      |
| Age ≥ 40 y, 2pts                                                             | 77 (67)                                | 36 (78)                           | .18     |
| Myeloablative conditioning regimen, 1pt                                      | 58 (50)                                | 23 (50)                           | 1       |
| GVHD, acute or chronic, 1pt                                                  | 69 (60)                                | 17 (37)                           | .009    |
| Corticosteroids, 1pt                                                         | 48 (41)                                | 10 (22)                           | .02     |
| Recent or pre-engraftment allo-HSCT, 1pt                                     | 0                                      | 0                                 |         |
| ISI, n (%)                                                                   |                                        |                                   |         |
| Low risk: 0–2                                                                | 39 (34)                                | 19 (41)                           |         |
| Moderate risk: 3–6                                                            | 65 (56)                                | 26 (56)                           | .2      |
| High risk: 7–12                                                               | 11 (9)                                 | 1 (2)                             |         |
| Basel immunodeficiency grading score, n (%)<sup>a</sup>                      |                                        |                                   |         |
| Allo-HSCT ≤6 months                                                          | 29 (25)                                | 6 (13)                            | .1      |
| T-cell or B-cell depletion ≤3 months                                         | 2 (2)                                  | 0                                 | .1      |
| GVHD grade ≥2 or extensive chronic                                          | 41 (36)                                | 9 (19)                            | .06     |
| ANC < 0.5 × 10<sup>9</sup>/L                                                  | 8 (7)                                  | 1 (2)                             | .3      |
| ALC < 0.1 × 10<sup>9</sup>/L                                                  | 10 (9)                                 | 1 (2)                             | .2      |
| IgG < 4 g/L                                                                  | 34 (30)                                | 6 (13)                            | .03     |
| Basel score, n (%)                                                            |                                        |                                   |         |
| Moderate                                                                      | 55 (48)                                | 31 (67)                           |         |
| Severe                                                                       | 38 (33)                                | 13 (28)                           | .04     |
| Very severe                                                                  | 22 (19)                                | 2 (4)                             |         |
| Other characteristics<sup>b</sup>                                             |                                        |                                   |         |
| On IS, n (%)                                                                 | 91 (90)                                | 25 (54)                           | .003    |
| ALC < 0.5 × 10<sup>9</sup>/L, n (%)                                          | 27 (23)                                | 8 (19)                            | .5      |
| RVI characteristics and clinical consequences                                |                                        |                                   |         |
| CARV LRTD, n (%)                                                             | 38 (33)                                | 8 (19)                            | .05     |
| Hospital admission, n (%)                                                    | 32 (27)                                | 4 (9)                             | .01     |
| Type of donor, n (%)                                                          |                                        |                                   |         |
| HLA-identical sibling donor                                                   | 36 (31)                                | 12 (26)                           |         |
| Unrelated donor                                                              | 30 (26)                                | 11 (24)                           | .6      |
| Umbilical cord blood                                                         | 28 (24)                                | 10 (22)                           |         |
| Haploidentical family donor                                                   | 21 (18)                                | 13 (28)                           |         |
| RVI episodes/Influenza RVI per seasons, n (%)                                 |                                        |                                   | 1       |
| 2017–2018                                                                     | 34 / 21                                | 14 / 9                            |         |
| 2016–2017                                                                     | 14 / 6                                 | 9 / 2                             |         |
| 2015–2016                                                                     | 17 / 11                                | 6 / 0                             |         |
| 2014–2015                                                                     | 35 / 14                                | 15 / 3                            |         |
| 2013–2014                                                                     | 15 / 7                                 | 2 / 1                             |         |
| Flu-RTD, n (%)                                                                | 59 (51)                                | 15 (32)                           | .036    |
| Antiviral therapy, n (%)                                                      | 44 (39)                                | 13 (26)                           | .5      |
| Median time from allo-HSCT to Flu-RTD, days (range)                          | 374 (91–4763)                          | 724 (98–3280)                     | .02     |
| Median time from allo-HSCT to flu vaccination, months (range)                | 163 (3–107)                            |                                   |         |
| Flu-LRTD, n (%)                                                               | 18 (16)                                | 1 (2)                             | .01     |
| Possible                                                                     | 9 (8)                                  |                                   |         |
| Proven                                                                       | 9 (8)                                  | 1 (2)                             | .04     |
| Flu-related hospital admission, n (%)                                        | 16 (14)                                | 1 (2)                             |         |
| Overall mortality rate, n (%)                                                | 12 (23)                                | 5 (10)                            | .026    |
| Day + 30                                                                      | 4 (3)                                  | 1 (2)                             | .1      |
| Day + 60                                                                      | 9 (8)                                  | 1 (2)                             | .3      |
| Day + 90                                                                      | 11 (10)                                | 1 (2)                             | .1      |
| Microbiological findings                                                      |                                        |                                   |         |
| Respiratory virus, n                                                         |                                        |                                   |         |
| Influenza type<sup>c</sup>                                                    |                                        | 2                                 |         |
Clinical and Biological Characteristics According to Vaccination Status at the Time of Respiratory Virus Infection Episodes

Patients’ clinical and biological characteristics at the time of their RVI episodes are summarized in Table 2 according to their vaccination statuses. As expected, the non-vaccinated group had significantly higher rates of conditions related to poor serological influenza vaccine responses. Active GvHD, steroid therapy, ongoing immunosuppression therapy, and hypogammaglobulinemia were significantly over-represented in the non-vaccinated group as compared to the vaccinated group (60% vs 37%, 41% vs 22%, 90% vs 54%, and 30% vs 13%, respectively; \(P < .05\) for all comparisons). Of note, the rate of low total lymphocyte counts at the time of RVIs at different cut-offs (<500, <200, and <100) were not statistically different among groups.

Influenza Virus Infection Characteristics

We accounted for 74 proven influenza RVI episodes over 5 flu seasons in 70 recipients, with a median time of onset of 511 days after stem cell infusion (range 98–4752). We observed a higher number of influenza RVI episodes in 2 seasons—2014–2015 (n = 19) and 2017–2018 (n = 30)—as shown in Table 2 and Figure 2. Together, these 2 seasons represent 64% of all influenza RVIs.

Prevalence of Influenza Respiratory Infection and Clinical Consequences According to Vaccination Status

The observed 5-season prevalence of influenza RVIs was significantly higher in the non-vaccinated (51%) compared to the vaccinated group (36%; \(P = .036\)). This statistical difference was even higher regarding the influenza LRTD prevalence (16% in non-vaccinated vs 2% in the vaccinated group, \(P = .01\)). The progression rates of URTDs to LRTDs in recipients with influenza RVIs were 30% (18 out of 59) in the non-vaccinated group compared to 7% in the vaccinated group (1 out of 15; \(P < 0.01\)). Influenza-related hospital admissions were more common in the non-vaccinated group compared to the vaccinated group (14% vs 2%, \(P < .05\)).

Risk Factors for Influenza Infection and for Progression to Lower–Respiratory Tract Disease

Logistic regression univariate and multivariate analyses of risk factors for influenza virus respiratory infections and progressions to LRTDs are shown in Table 3.

In order to identify the conditions associated with influenza virus infections, we studied the 161 evaluable recipient/episode pairs. A multivariate analysis identified the flu vaccine as the main factor associated with a reduced risk of influenza virus infection (odds ratio [OR] 0.39, 95% confidence interval [CI] 0.18–0.8, \(P = .01\)).

To analyze the risk factors for LRTD progression of an influenza virus infection, we focused the analysis on A and B were detected in the same episode/sample and were classed as co-infections.
A multivariate analysis identified 2 independent conditions associated with LRTD progression. Again, the flu vaccine was associated with a lower probability of LRTD progression (OR 0.12, 95% CI 0.014–1, \( P = .05 \)). In contrast, a high-risk ISI score predicted a higher probability of the influenza virus LRTD progression (OR 36, 95% CI 2.26–575, \( P = .011 \)).

**DISCUSSION**

This study shows that, irrespective of the flu season, a trivalent influenza inactivated vaccine given after allo-HSCT was the most important factor associated with the lower prevalence of influenza RVIs among recipients with proven CARV RVIs. In allo-HSCT recipients with influenza RVIs, a multivariate analysis showed that influenza vaccination was associated with a lower probability of the influenza virus progressing to LRTD. We also observed that a high-risk ISI score was highly predictive of influenza LRTD progression in our cohort of patients.

Although this study was not designed to assess the vaccination rate in our population, only 28% of the recipients with proven CARV RVIs received the influenza vaccine over 5 flu seasons. This agrees with prior epidemiological studies and enquiries, where the vaccination rate has ranged from 20–60% after allo-HSCT [29–31]. However, some factors merit consideration when interpreting the reported vaccination rate. We did not detect all vaccinated recipients in our prospective respiratory virus survey, since those who were vaccinated may have had lower incidences of CARV RVIs and a lower propensity to seek medical attention or testing. However, the lack of consensus across current guidelines [12–15] regarding the timing and conditions in which the influenza vaccine should be administered, in particular when immunosuppressant therapy is required to treat active/uncontrolled moderate-to-severe GvHD, is likely the most important contributor to this apparently low vaccination rate. In these scenarios, physicians may decide to defer vaccination. Thus, based on our findings, we currently recommend flu vaccination at 3 months post-transplant to all of our recipients, irrespective of their immunosuppression status.

In our selected cohort, half (51%) of the unvaccinated recipients developed influenza RVIs, with moderate to severe clinical consequences, as reflected in higher hospital admission and LRTD progression rates. To address the clinical efficacy of influenza vaccination in recipients with GvHD, corticosteroid therapy, hypogammaglobulinemia, or with ongoing immunosuppressant use, we compared the influenza RVI prevalence in vaccinated recipients according to the presence or absence of such conditions. Although the number of cases was limited, we did not find statistical differences regarding vaccinated recipients with or without GvHD (36 % vs 31%, respectively, \( P = .9 \)), corticosteroids (40% vs 31%, \( P = .7 \)), immunosuppressants (36% vs 29%, \( P = .7 \)), or hypogammaglobulinemia (50% vs 30%, \( P = .1 \)). In fact, our multivariate analysis revealed that vaccination status was the main condition associated with a lower influenza RVI prevalence in recipients with at least 1 episode of a CARV RVI. This is an important finding, since we provide clinical evidence that seasonal influenza vaccines could be clinically beneficial in allo-HSCT recipients, even when a significant number of vaccinated recipients had GvHD (19%), used immunosuppressants (54%), or used corticosteroids (22%).

Although the serological vaccination response in such recipients is poor, we can speculate that vaccination may also exert a cellular-mediated response. Influenza vaccination was able to mediate peripheral blood T-cell responses, characterized by production of the Th1 cytokine IFN-gamma by CD4+ cells,
both in patients vaccinated more than 6 months after transplantation and those vaccinated earlier, after stem cell transplantation [10]. These data suggest that, even in cases where the expected serological response could be suboptimal (allo-HSCT <6 mo, GvHD, immunosuppressant use, or corticosteroid use), the influenza vaccine could elicit a clinical benefit through a cellulary-mediated effect, reducing the influenza infection prevalence and/or its severity.

Although the influenza RVI prevalence in vaccinated recipients was still substantial (36%), it should be noted that the risk factors for influenza LRTD progression identified vaccination as a condition associated with a reduced rate of LRTD progression. Furthermore, vaccination was also associated with a lower hospital admission rate. Given these findings, we speculate that vaccination could mitigate the severity of influenza RVIs, even in the presence of conditions related to decreased serological responses.

Another relevant finding was the usefulness of the ISI score in stratifying the LRTD progression risk of influenza RVIs, even when we analyzed episodes occurring after day 90 after stem cell infusion. ISI was developed by investigators from the MD Anderson Cancer Center to predict outcomes in allo-HSCT recipients with respiratory syncytial viruses [21]. The same investigators demonstrated its value in predicting LRTD progression in the setting of influenza RVIs [32]. Therefore, the use of the ISI could be applied to assess the need for prophylactic/therapeutic intervention with several doses of the influenza vaccine and/or with high doses of antiviral drugs in prospective studies.
Regarding influenza virus epidemiological data from the current study, most influenza RVI cases occurred in the 2014–2015 and 2017–2018 flu seasons. These data are in accordance with Spanish epidemiological data, where the influenza prevalence was significantly higher in 2014–2015 (350 cases/100 000 hab) and 2017–2018 (450 cases/100 000 hab) as compared to the 2013–2014, 2015–2016, and 2016–2017 flu seasons (270, 190, and 220 cases/100 000 hab, respectively) [33, 34]. These observations confirm that influenza RVI prevalence in our allo-HSCT recipients mimicked influenza virus prevalence in the general population.

Finally, we acknowledge that this study has some important limitations, such as the retrospective nature of the analyses, the small sample size, the somewhat heterogeneous vaccination policy, and the cohort selection method. In addition, the use of 2 different PCR methods, which differed (minimally) in their analytical performance, can be viewed as a limitation. In spite of this, our study has strengths that merit consideration. We used molecular testing. Our prospective CARV survey mirrored national epidemiological data in influenza RVIs in each flu season. We provided data encompassing 5 complete flu seasons, limiting the distortion likely introduced by the vaccination coverage variability between seasons.

In conclusion, we provide clinical evidence that influenza vaccination after allo-HSCT is associated with a lower prevalence of influenza RVI and a lower severity of the disease.

Note
Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References
1. Ljungman P, Ward KN, Crooks BN, et al. Respiratory virus infections after stem cell transplantation: a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 2001; 27:7–21.
2. Champlin RE, Whimbey E. Community respiratory virus infections in bone marrow transplant recipients: the M.D. Anderson Cancer Center experience. Biol Blood Marrow Transplant 2004; 7:35–41.
3. Bowden RA. Respiratory virus infections after marrow transplant: the Fred Hutchinson Cancer Research Center experience. Am J Med 1997; 102:27–30; discussion 42–3.
4. Ljungman P. Respiratory virus infections in bone marrow transplant recipients: the European perspective. Am J Med 1997; 102:4–7.
5. Choi SM, Boudreault AA, Xie H, Englund JA, Corey L, Boeckh M. Differences in clinical outcomes after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. Blood 2011; 117:5056–6.
6. Ljungman P, de la Camara R, Perez-Bercoff I, et al; Infectious Diseases Working Party, European Group for Blood and Marrow Transplantation; Infectious Complications Subcommittee, Spanish Group of Haematopoietic Stem-cell Transplantation. Outcome of pandemic H1N1 infections in hematopoietic stem cell transplant recipients. Haematologica 2011; 96:1231–5.
7. Engelhard D, Nagler A, Hardan I, 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; 3:111–5.
8. Gribbins DA, Panayiotidis P, Boussiotis VA, Hannoun C, Pangalis GA. Influenza virus vaccine in B-cell chronic lymphocytic leukemia patients. Acta Haematol 1994; 91:115–8.
9. Pauksen K, Linde A, Hammarström V, et al. Granulocyte-macrophage colony-stimulating factor as immunomodulating factor together with influenza vaccination in stem cell transplant patients. Clin Infect Dis 2000; 30:342–8.
10. Avestisy 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; 86:257–63.
11. Hilgendorf I, Freund M, Illg W, et al. Vaccination of allogeneic hematopoietic stem cell transplant recipients: report from the international consensus conference on clinical practice in chronic GVHD. Vaccine 2011; 29:2825–33.
12. Ljungman P, Cordonnier C, Einsele H, et al.; Center for International Blood and Marrow Transplant Research; National Marrow Donor Program; European Blood and Marrow Transplant Group; American Society of Blood and Marrow Transplantation; Canadian Blood and Marrow Transplant Group; Infectious Disease Society of America; Society for Healthcare Epidemiology of America; Association of Medical Microbiology and Infectious Diseases Canada; Centers for Disease Control and Prevention. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant 2009; 44:521–6.
13. Rubin LG, Levin MJ, Ljungman P, et al.; Infectious Diseases Society of America. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis 2013; 56:e4–100.
14. 7th European conference on infections in leukaemia guidelines for vaccination of patients with hematological malignancies and HSCT recipients [Internet]. 2017. Available at: http://www.ecvl-leukaemia.com.
15. Engelhard D, Mohty B, de la Camara R, Cordonnier C, Ljungman P. European guidelines for prevention and management of influenza in hematopoietic stem cell transplantation and leukemia patients: summary of ECIL-4 (2011), on behalf of ECIL, a joint venture of EBMT, EORTC, ICHS, and ELN. Transpl Infect Dis 2013; 15:239–32.
16. Machado CM, Cardoso MR, da Rocha IE, Boas LS, Dulley FL, Pannuti CS. The benefit of influenza vaccination after bone marrow transplantation. Bone Marrow Transplant 2005; 36:897–900.
17. Natori Y, Shiotsuka M, Slomovic J, et al. A double-blind, randomized trial of high-dose versus standard-dose influenza vaccine in adult solid-organ transplant recipients. Clin Infect Dis 2018; 66:1698–704.
18. de Lavallade H, Garland P, Sekine T, et al. Repeated vaccination is required to optimize seroprotection against H1N1 in the immunocompromised host. Haematologica 2011; 96:307–14.
19. Ambati A, Emardottier S, Magalhaes I, et al. Immunogenicity of virosomal adjuvanted trivalent influenza vaccination in allogeneic stem cell transplant recipients. Transpl Infect Dis 2015; 17:371–9.
20. Piñana JL, Madrid S, Pérez A, et al. Epidemiologic and clinical characteristics of coronavirus and bocavirus respiratory infections after allogeneic stem cell transplantation: a prospective single-center study. Biol Blood Marrow Transplant 2018; 24:563–70.
21. Shah DP, Ghanotii SS, Ariza-Heredia EJ, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. Blood 2014; 123:3263–8.
22. Spahr V, Tschudin-Sutter S, Baertig V, et al. Community-acquired respiratory paramyxovirus infection after allogeneic hematopoietic stem cell transplantation: a single-center experience. Open Forum Infect Dis 2018; 5:sof077.
23. Khanna N, Steffen I, Studt JD, et al. Outcome of influenza infections in outpatients after allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis 2009; 11:100–5.
24. The World Health Organization. Evaluation of influenza vaccine effectiveness: a guide to the design and interpretation of observational studies [Licence: CC BY-NC-SA 3.0 IGO]. Geneva, Switzerland: World Health Organization; 2017.
25. Seo S, Xie H, Campbell AP, et al. Parainfluenza virus lower respiratory tract disease after hematopoietic cell transplant: viral detection in the lung predicts outcome. Clin Infect Dis 2014; 58:1357–68.
26. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. Clin Infect Dis 2013; 56:258–66.
27. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974; 18:295–304.
28. Costa E, Rodriguez-Dominguez M, Clari MÁ, Giménez E, Galán JC, Navarro D. Comparison of the performance of 2 commercial multiples PCR platforms for detection of respiratory viruses in upper and lower tract respiratory specimens. Diagn Microbiol Infect Dis 2015; 82:40–3.
29. Kumar D, Humar A, Plevneshi A, et al.; Toronto Invasive Bacterial Diseases Network. Invasive pneumococcal disease in adult hematopoietic stem cell
transplant recipients: a decade of prospective population-based surveillance. Bone Marrow Transplant 2008; 41:743–7.

30. Lerchenfeldt SM, Cronin SM, Chandrasekar PH. Vaccination adherence in hematopoietic stem cell transplant patients: a pilot study on the impact of vaccination cards and reminder telephone calls. Transpl Infect Dis 2013; 15:634–8.

31. Ariza-Heredia EJ, Gulbis AM, Stolar KR, et al. Vaccination guidelines after hematopoietic stem cell transplantation: practitioners’ knowledge, attitudes, and gap between guidelines and clinical practice. Transpl Infect Dis 2014; 16:878–86.

32. Kmeid J, Vanichanan J, Shah DP, et al. Outcomes of influenza infections in hematopoietic cell transplant recipients: application of an immunodeficiency scoring index. Biol Blood Marrow Transplant 2016; 22:542–8.

33. Centro Nacional de Epidemiología, Instituto de Salud Carlos III. Informe de Vigilancia de la Gripe en España. Temporada 2016-2017. http://www.isciii.es/ISCIII/es/contenidos/fd-servicios-cientifico-tecnicos/fd-vigilancias-alertas/fd-enfermedades/fd-gripe/fd-informes-semanales-vigilancia-gripe/pdfs_2016_2017/Informe_Vigilancia_GRIPE_2016-2017_v.27septiembre2017.pdf. Accessed 2017.

34. Centro Nacional de Epidemiología, Instituto de Salud Carlos III. Informe semanal de Vigilancia de la Gripe en España. Nº 546. http://www.isciii.es/ISCIII/es/contenidos/fd-servicios-cientifico-tecnicos/fd-vigilancias-alertas/fd-enfermedades/fd-gripe/fd-informes-semanales-vigilancia-gripe/pdfs_2017-2018/grn202018.pdf. Accesed 24 May 2018.