Chronic kidney disease ten years after pediatric allogeneic hematopoietic stem cell transplantation

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Chronic kidney disease (CKD) is an important sequela of hematopoietic stem cell transplantation (HSCT), but data regarding CKD after pediatric HSCT are limited. In this single center cohort study, we evaluated the estimated glomerular filtration rate (eGFR) dynamics, proteinuria and hypertension in the first decade after HSCT and assessed risk factors for CKD in 216 pediatric HSCT survivors, transplanted 2002-2012. The eGFR decreased from a median of 148 to 116 ml/min/1.73 m² between pre-HSCT to ten years post-HSCT. CKD (KDIGO stages G2 or A2 or more; eGFR under 90 ml/min/1.73m² and/or albuminuria) occurred in 17% of patients. In multivariate analysis, severe prolonged stage 2 or more acute kidney injury (AKI), with an eGFR under 60ml/min/1.73m² and duration of 28 days or more, was the main risk factor for CKD (hazard ratio 9.5, 95% confidence interval 3.4-27). Stage 2 or more AKI with an eGFR of 60ml/min/1.73m² or more and KDIGO stage 2 or more AKI with eGFR under 60ml/min/1.73m² but recovery within 28 days were not associated with CKD. Furthermore, hematological malignancy as HSCT indication was an independent risk factor for CKD. One third of patients had both CKD criteria, one third had isolated eGFR reduction and one third only had albuminuria. Hypertension occurred in 27% of patients with CKD compared to 4.4% of patients without. Tubular proteinuria was present in 7% of a subgroup of 71 patients with available β2-microglobulinuria. Thus, a significant proportion of pediatric HSCT recipients developed CKD within ten years. Our data stress the importance of structural long-term monitoring of eGFR, urine and blood pressure after HSCT to identify patients with incipient CKD who can benefit from nephroprotective interventions.

 METHODS

 Patients

Between January 2003 and December 2012, 320 children received an allogeneic HSCT in the Leiden University Medical Center as a treatment for hematological malignancy or severe nonmalignant diseases (bone marrow failure syndrome, hemoglobinopathy, or

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inborn error of immunity). Transplantations were performed according to European Society for Blood and Marrow Transplantation guidelines. Peripheral blood samples and urine samples were routinely obtained. Medical records were analyzed retrospectively. The study protocol was evaluated and approved by the institutional review board (G20.049).

A flowchart of patient inclusion is shown in Figure 1. Only patients surviving >1 year after HSCT were included in this study because of (i) time required to establish CKD and (ii) evaluated risk factors occurring during the first year after HSCT. A total of 216 patients survived the first year after HSCT without relapse, retransplantation, or death. A total of 155 patients reached the 10-year post-HSCT evaluation point. A total of 61 patients were censored at a median of 3.1 years (range, 1.0–5.5 years) after HSCT because of second transplantation (n = 6), relapse of malignancy (n = 9), or death (n = 11). A total of 18 patients were lost to follow-up. Because kidney function was evaluated every 3 to 5 years, the 10-year post-HSCT nephrologic evaluation was not yet performed at closure of this study in 17 patients.

Supportive care
During conditioning with any of the drugs busulfan, etoposide, fludarabine, treosulfan, and thiotepa, patients received oral and i.v. hydration (3 L/m²) and 2-mercaptethanesulfonate and i.v. hydration (2 L/m²). During cyclophosphamide conditioning, fludarabine, tacrolimus, and vancomycin. In patients with a reduced SCr in 3 consecutive hospital visits, or the use of antihypertensive medication. For the univariate and multivariate analysis of risk factors for the development of CKD after HSCT, we evaluated the impact of baseline patient characteristics (age, sex, and primary disease category) and HSCT characteristics (donor type, graft source and manipulation, serotherapy, chemotherapeutic agents, and TBI). The influence of the following major post-transplant events was evaluated: acute and chronic graft-versus-host disease (≥grade/score 2) and cytomegalovirus, Epstein-Barr virus, or adenovirus reactivation (viral load ≥10⁶ copies/ml at 2 consecutive measurements) and BK virus hemorrhagic cystitis (macroscopic hematuria with positive urine polymerase chain reaction). Furthermore, the impact of use of nephrotoxic drugs was applied in line with institutional recommendations.

Monitoring of kidney function
Routinely measured serum creatinine (SCr) values were used to monitor the glomerular function before the start of conditioning therapy (pre-HSCT), at 3 and 6 months and 1, 3, and 10 years after HSCT, or at latest stable follow-up before censoring. Urine analysis was performed, measuring creatinine, albumin, and β2-microglobulin in a portion of urine. Blood pressure was measured using automatic oscillometric blood pressure monitors at every hospital visit. Kidney function was evaluated every 3 to 5 years, and the moment closest to 10 years after HSCT was used for 10-year evaluation.

Definition of CKD
Kidney Disease: Improving Global Outcomes (KDIGO) guidelines were used for the classification of CKD.6–9 CKD was defined as an eGFR of <90 ml/min per 1.73 m² at ≥2 measurements over a period of at least 3 months (KDIGO stage G2 or lower) and/or albuminuria (KDIGO stage A2). For patients aged <18 years, the eGFR was calculated using the updated Schwartz formula with modification of the K for pubertal boys [(K × length)/SCr, 1] and/or albuminuria (KDIGO stage A2). For patients aged >18 years, the eGFR was calculated using the updated Schwartz formula with modification of the K for pubertal boys [(K × length)/SCr, 1] and/or albuminuria (KDIGO stage A2). For patients aged >18 years, the eGFR was calculated using the updated Schwartz formula with modification of the K for pubertal boys [(K × length)/SCr, 1].

Cohort 2003–2012

**Figure 1 | Patient inclusion.** Flowchart of patient inclusion. CKD, chronic kidney disease; FU TBA, follow-up to be assessed (10 years post–hematopoietic stem cell transplantation evaluation not reached); HSCT, hematopoietic stem cell transplantation; LTFU, lost to follow-up.
Table 1 | Patient Characteristics (n = 216)

| Characteristic                        | No.  | %    |
|---------------------------------------|------|------|
| Age at transplantation, yr            |      |      |
| 0–6                                   | 87   | 40.2 |
| 6–12                                  | 65   | 30.1 |
| 12–19                                 | 64   | 29.6 |
| Sex                                    |      |      |
| Female                                | 80   | 37.0 |
| Male                                  | 136  | 63.0 |
| Primary diagnosis                     |      |      |
| Nonmalignant diseases                 | 120  | 55.6 |
| Inborn error of immunity              | 40   | 18.5 |
| Bone marrow failure syndrome          | 44   | 20.4 |
| Hemoglobinopathy                      | 36   | 16.7 |
| Hematological malignancy              | 96   | 44.4 |
| Acute lymphoblastic leukemia          | 51   | 23.6 |
| Acute myeloblastic leukemia            | 18   | 8.3  |
| Other hematological malignancy        | 27   | 12.5 |
| Donor type                            |      |      |
| Identical related donor               | 70   | 32.0 |
| Matched unrelated donor               | 128  | 59.3 |
| Mismatched related donor              | 18   | 8.3  |
| Graft source                          |      |      |
| Bone marrow                           | 164  | 75.9 |
| Peripheral blood stem cells           | 35   | 16.2 |
| Cord blood                            | 17   | 7.9  |
| T-cell depletion of the graft         | 18   | 8.3  |
| Serotherapy                           |      |      |
| Anti-thymocyte globulin               | 143  | 66.2 |
| Alemtuzumab                           | 30   | 13.9 |
| No serotherapy                        | 51   | 23.6 |
| Conditioning regimen                  |      |      |
| Busulfan-based                        | 99   | 45.8 |
| Treosulfan-based                      | 27   | 12.5 |
| Total body irradiation–based          | 62   | 28.7 |
| Other regimens                        | 28   | 13.0 |
| Graft-versus-host disease             |      |      |
| Acute (≥ grade 2)                     | 33   | 15.3 |
| Chronic (≥ score 2)                   | 13   | 6.0  |
| Viral infections                      |      |      |
| Cytomegalovirus                       | 47   | 21.8 |
| Epstein-Barr virus                    | 34   | 15.7 |
| Human adenovirus                      | 20   | 9.3  |
| BK virus cystitis                     | 35   | 16.2 |
| Other major complications             |      |      |
| Veno-occlusive disease                | 14   | 6.5  |
| Thrombotic microangiopathy            | 0    | 0    |
| AKI, KDIGO definition                 |      |      |
| No AKI                                | 121  | 56.0 |
| Stage 2 AKI                           | 63   | 29.2 |
| Stage 3 AKI                           | 32   | 14.8 |
| AKI, KDIGO + lowest eGFR and duration\(^a\) |      |      |
| No AKI                                | 121  | 56.0 |
| Stage ≥2 AKI, eGFR ≥60 ml/min per 1.73 m\(^2\), >28 d or ≥28 d | 55 | 25.5 |
| Stage ≥2 AKI, eGFR <60 ml/min per 1.73 m\(^2\), ≤28 d | 26 | 12.0 |
| Stage ≥2 AKI, eGFR <60 ml/min per 1.73 m\(^2\), ≥28 d | 14 | 6.5 |
| Nephrotoxic medication                |      |      |
| Amphotericin B                        | 7    | 3.2  |
| Cyclosporine A                        | 207  | 96.8 |
| Cidofovir                             | 25   | 11.6 |
| Ganciclovir                           | 20   | 9.3  |
| Foscarnet                             | 31   | 14.4 |
| Furosemide\(^b\)                      | 134  | 62.0 |

Table 1 | (Continued)

| Characteristic                        | No.  | %    |
|---------------------------------------|------|------|
| Gentamycin                            | 8    | 3.7  |
| Tacrolimus                            | 8    | 3.7  |
| Vancomycin                            | 198  | 91.7 |

AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; KDIGO, Kidney Disease: Improving Global Outcomes.

\(^a\)Patients with KDIGO stage ≥2 AKI were subdivided based on the lowest eGFR and duration: AKI with the lowest eGFR ≥60 ml/min per 1.73 m\(^2\); AKI with the lowest eGFR <60 ml/min per 1.73 m\(^2\) that recovered within 28 days; and AKI with the lowest eGFR <60 ml/min per 1.73 m\(^2\) and duration ≥28 days.

\(^b\)Only structural (>5 days) furosemide included.

Patient characteristics, transplantation variables, and post-transplant events and medication in 216 pediatric hematopoietic stem cell transplantation (HSCT) recipients who survived >1 year after HSCT without relapse or retransplantation. Occurrence of CKD after HSCT. All potential risk factors were included in a multivariate logistic regression analysis using backward stepwise elimination. In the adjusted model, hypertension (n = 14) and diabetes mellitus (n = 1) were added as risk factor in the evaluation. Patients were censored at the latest available data point in steady-state conditions, at least 1 month before the censoring events of death, retransplantation, or relapse, or at the latest visit before loss of follow-up. Parameters were tested for the proportional hazards assumption using the graphical approach (log minus log survival plots). P < 0.05 was considered statistically significant. Multinomial parameters were only considered significant if the Wald test had a P < 0.05 as well. Analysis was performed using SPSS Statistics 25 (IBM SPSS Inc). GraphPad Prism (version 6.05; GraphPad Software) was used to construct figures.

RESULTS

Patient characteristics

Between 2003 and 2012, 320 children received an allogeneic HSCT in our center. Two patients were excluded because of preexisting CKD. A total of 216 patients survived >1 year without relapse of malignancy or retransplantation and were included in this study (Figure 1). Detailed patient characteristics are listed in Table 1. At the time of HSCT, the median age was 7.8 years (range, 3 months–19 years). Median follow-up was 8.1 years (range, 1.0–14 years). Indications for HSCT were hematological malignancy (44%) or severe nonmalignant diseases (56%): bone marrow failure syndrome (20%), hemoglobinopathy (17%), and inborn error of immunity (19%). Patients received TBI-based conditioning (29%) or chemotherapy-based conditioning (70%). In the first year after HSCT, all patients received at least one of the nephrotoxic drugs listed in Table 1. A total of 84 patients had a significant reactivation of cytomegalovirus, Epstein-Barr virus, or human adenovirus. A total of 35 patients developed a hemorrhagic BK cystitis. In the first year after HSCT, KDIGO stage 2 and 3 AKI occurred in 63 and 32 patients, respectively. Of patients with stage ≥2 AKI, 55 (25.5%) had AKI with eGFR ≥60 ml/min per 1.73 m\(^2\), 26 (12%) had AKI with eGFR <60 ml/min per 1.73 m\(^2\) that recovered within 28 days, and 14 (6.5%) had prolonged severe AKI with eGFR <60 ml/min per 1.73 m\(^2\) and duration ≥28 days. In patients with
prolonged severe AKI, this was associated with fluid overload treated with diuretics (n = 6), nephrotoxic antiviral medication (cidofovir [n = 3] and/or foscarnet [n = 6]), veno-occlusive disease (n = 2), hemorrhagic BK virus cystitis with oliguria (n = 1), or prerenal with increased cyclosporine A blood levels (n = 3). In 4 cases, a combination of these factors was recognized (Supplementary Table S1). In 1 case, AKI occurred 6 months after HSCT without provoking factors; renal biopsy showed radiation nephropathy.

**eGFR dynamics**
Before HSCT, the median eGFR was 148 ml/min per 1.73 m² (range, 81–311 ml/min per 1.73 m²; Figure 2). At 3 months after HSCT, the median eGFR decreased to 128 ml/min per 1.73 m² (range, 43–244 ml/min per 1.73 m²; P < 0.0001). In 21 of 28 patients (75%) with an eGFR <90 ml/min per 1.73 m² at 3 months after HSCT, this reduction was transient and the eGFR recovered during the first year after HSCT. At group level, the eGFR remained stable between 3 and 12 months after HSCT. After the first year, the eGFR decreased from a median of 131 ml/min per 1.73 m² to a median of 126 ml/min per 1.73 m² at 3 years after HSCT and a median of 116 ml/min per 1.73 m² at 10 years after HSCT (1 vs. 3 years after HSCT: P = 0.007; 1 vs. 10 years after HSCT: P < 0.0001; and 3 vs. 10 years after HSCT: P < 0.0001; Figure 2).

**Incidence, timing, and risk factors for CKD**
From the 216 children who survived >1 year after HSCT, 37 (17.1%) developed CKD during follow-up (median, 8.1 years; range, 1.0–14 years). CKD was defined as either an eGFR <90 ml/min per 1.73 m² (KDIGO stage ≥G2) or albuminuria (KDIGO stage ≥A2).30 The vast majority of CKD patients developed CKD >5 years after HSCT (Figure 3a). Median age of onset of CKD was 19 years (range, 4.0–29 years). Only 6 of 37 CKD patients were prepubertal at the moment they developed CKD.

We evaluated the impact of baseline patient characteristics, HSCT parameters, post-transplant events, and use of nephrotoxic medication on the development of CKD. The potential risk factors included in this analysis are listed in Table 2, which also shows their univariate and multivariate HRs for CKD. Classification of AKI based on KDIGO stages 2 and 3 AKI was not discriminative to identify patients at risk for CKD (Table 2). Further categorization of patients with KDIGO stage ≥2 AKI, based on the lowest eGFR and duration of AKI, revealed that prolonged severe AKI with eGFR <60 ml/min per 1.73 m² and duration ≥28 days had a strongly increased HR for CKD. In contrast, AKI with eGFR ≥60 ml/min per 1.73 m² and AKI with eGFR <60 ml/min per 1.73 m² that recovered within 28 days were not associated with the development of CKD. The use of cyclosporine A as graft-versus-host disease prophylaxis had a decreased HR for CKD (Table 2).

In multivariate analysis, patients who went through AKI with eGFR <60 ml/min per 1.73 m² and duration ≥28 days in the first year after HSCT had an increased risk of CKD (HR, 9.5; 95% confidence interval, 3.4–27; Table 2 and Figure 3b). Patients with hematological malignancy as HSCT indication had an increased HR for the development of CKD (HR, 3.5; 95% confidence interval, 1.4–8.6; Table 2 and Figure 3c). In contrast, a decreased HR for CKD was observed in patients who received cyclosporine A as graft-versus-host disease prophylaxis (HR, 0.2; 95% confidence interval, 0.1–0.3). As diabetes mellitus and hypertension are associated with proteinuria, correction for diabetes mellitus (n = 1) and hypertension (n = 14) was performed, which did not affect the outcome of the multivariate analysis (Supplementary Table S2).

The incidence of CKD was comparable between patients with different hematological malignancies. Within the more heterogeneous group of patients with nonmalignant diseases, patients transplanted for inborn errors of immunity had a lower incidence of CKD (5%) compared with patients with hemoglobinopathies (14%) or bone marrow failure syndromes (23%; P = 0.067; Supplementary Figure S1).

**Comprehensive evaluation of kidney function 10 years after HSCT**
Finally, we evaluated kidney function of the long-term HSCT survivors in more detail. A total of 155 patients survived without relapse or retransplantation and were available for follow-up at 10 years after HSCT (range, 7.5–14 years; median, 9.7 years; Figure 1). CKD, defined as KDIGO stage ≥G2 or ≥A2, was present in 33 of 155 patients (21.3%).

Twenty-one patients (13.5%) had an eGFR <90 ml/min per 1.73 m² at 10 years after HSCT. Three patients (2%) had an eGFR <60 ml/min per 1.73 m². One of them received renal replacement therapy and died from CKD while listed for
renal transplant. Early after HSCT, this patient went through a disseminated adenovirus infection and BK virus cystitis, treated with systemic cidofovir. This patient did not encounter AKI but developed obstructive uropathy and progressive CKD in the years after HSCT.

Three patterns of eGFR dynamics could be distinguished in patients with CKD at 10 years after HSCT (Figure 4a). Of 21 patients, 5 had a >30% reduction of eGFR within the first months after HSCT, which did not recover thereafter. Eight patients had a >30% eGFR reduction within the first months after HSCT, which recovered at 1 to 3 years after HSCT, but then decreased in the following years. In another 8 patients, eGFR reduction occurred after the first year post-HSCT (Figure 4a).

Urine samples were available in 132 of 155 long-term HSCT survivors. A total of 18 patients (13.6%) had albuminuria. Tubular protein loss was evaluated in a subgroup of 71 patients. β2-Microglobulinuria was observed in 5 patients (7%). Two patients with CKD had biochemical signs of tubular kidney disease and required supplementation of bicarbonate, magnesium, and/or phosphate at 10 years after HSCT.

Ten years after HSCT, blood pressure measurements were available for analysis in 146 of 155 patients. In total, 14 patients (9%) had hypertension. Three patients had both systolic and diastolic hypertension, 1 patient had isolated systolic hypertension, and 5 patients had isolated diastolic hypertension. Five patients were using antihypertensive medication. The incidence of hypertension was 6 times higher in patients with CKD than in patients without CKD; 9 of 33 CKD patients (27%) had hypertension compared with 5 of 113 non-CKD patients (4.4%; \( P < 0.0001 \); Figure 4b).

**DISCUSSION**

We performed longitudinal follow-up of kidney function in a large cohort of 216 pediatric HSCT recipients. One out of 6 patients (17%) developed CKD within 10 years after HSCT. One-third of the CKD patients had both an eGFR < 90 ml/min per 1.73 m² as well as albuminuria, one-third had isolated eGFR reduction, and one-third only had albuminuria. Independent risk factors for CKD were hematological malignancy and prolonged severe AKI with eGFR < 60 ml/min.
### Table 2 | HRs from Cox proportional hazards model

| Characteristic                                      | CKD, no. | Univariate Cox regression | Multivariate Cox regression |
|-----------------------------------------------------|----------|--------------------------|-----------------------------|
|                                                     | No (n = 179) | Yes (n = 37) | HR (95% CI) | P value | HR (95% CI) | P value |
| Age at transplantation, yr                         |          |                          |                           |         |             |         |
| 0–6                                                 | 79       | 8                        | 1.0                       | 0.065   |              |         |
| 6–12                                                | 54       | 11                       | 2.0 (0.8–4.9)             | 0.140   |              |         |
| 12–19                                               | 46       | 18                       | 2.7 (1.2–6.2)             | 0.019   |              |         |
| Sex                                                 |          |                          |                           |         |             |         |
| Female                                              | 66       | 14                       | 1.0                       |         |              |         |
| Male                                                | 113      | 23                       | 0.8 (0.4–1.6)             | 0.590   |              |         |
| Primary diagnosis                                   |          |                          |                           |         |             |         |
| Nonmalignant diseases                               | 103      | 17                       | 1.0                       |         |              |         |
| Hematological malignancy                            | 76       | 20                       | 1.5 (0.8–2.9)             | 0.218   | 3.5 (1.4–8.6) | 0.006   |
| Donor type                                          |          |                          |                           |         |             |         |
| Identical related donor                             | 61       | 9                        | 1.0                       |         |              |         |
| Matched unrelated donor                             | 103      | 25                       | 1.7 (0.8–3.7)             | 0.172   |              |         |
| Mismatched related donor                            | 15       | 3                        | 1.3 (0.4–4.9)             | 0.676   |              |         |
| Graft source                                        |          |                          |                           | 0.515   |              |         |
| Bone marrow                                         | 136      | 28                       | 1.0                       |         |              |         |
| Peripheral blood stem cells                         | 27       | 8                        | 1.3 (0.6–2.7)             | 0.569   |              |         |
| Cord blood                                          | 16       | 1                        | 0.4 (0.1–2.8)             | 0.343   |              |         |
| T-cell depletion of the graft                       | 15       | 3                        | 0.9 (0.3–3.0)             | 0.905   |              |         |
| Serotherapy                                         |          |                          |                           |         |             |         |
| Anti-thymocyte globulin                             | 121      | 22                       | 0.9 (0.5–1.8)             | 0.846   |              |         |
| Alemtuzumab                                         | 19       | 11                       | 2.0 (1.0–4.1)             | 0.055   |              |         |
| Conditioning regimen                                |          |                          |                           | 0.342   |              |         |
| Busulfan-based                                      | 87       | 12                       | 1.0                       |         |              |         |
| Treosulfan-based                                    | 23       | 4                        | 1.3 (0.4–3.9)             | 0.685   |              |         |
| Total body irradiation–based                        | 48       | 14                       | 1.5 (0.7–3.3)             | 0.305   |              |         |
| Other regimens                                      | 21       | 7                        | 2.4 (0.9–6.0)             | 0.073   |              |         |
| Graft-versus-host disease                           |          |                          |                           |         |             |         |
| Acute (≥ grade 2)                                   | 27       | 6                        | 1.2 (0.5–2.9)             | 0.691   |              |         |
| Chronic (≥ score 2)                                 | 9        | 4                        | 2.0 (0.7–5.8)             | 0.175   |              |         |
| Viral infections                                    |          |                          |                           |         |             |         |
| Cytomegalovirus                                     | 36       | 11                       | 1.6 (0.8–3.3)             | 0.169   |              |         |
| Epstein-Barr virus                                  | 26       | 8                        | 1.5 (0.7–3.4)             | 0.278   |              |         |
| Human adenovirus                                    | 16       | 4                        | 1.5 (0.5–4.1)             | 0.481   |              |         |
| BK virus cystitis                                    | 27       | 8                        | 1.6 (0.7–3.5)             | 0.235   |              |         |
| Other major complications                           |          |                          |                           |         |             |         |
| Veno-occlusive disease                              | 11       | 3                        | 1.1 (0.3–3.7)             | 0.840   |              |         |
| Thrombotic microangiopathy                          | 0        | 0                        | NA                        |         |              |         |
| AKI, KDIGO definition                              |          |                          |                           | 0.707   |              |         |
| No AKI                                              | 104      | 17                       | 1.0                       |         |              |         |
| Stage 2 AKI                                         | 50       | 13                       | 1.3 (0.7–2.7)             | 0.640   |              |         |
| Stage 3 AKI                                         | 25       | 7                        | 1.3 (0.5–3.0)             | 0.251   |              |         |
| AKI, KDIGO + lowest eGFR and duration<sup>a</sup>   |          |                          |                           | 0.001   | <0.0001      | 0.001   |
| No AKI                                              | 104      | 17                       | 1.0                       | 0.001   | <0.0001      | 0.001   |
| Stage ≥2 AKI, eGFR ≥60 ml/min per 1.73 m<sup>2</sup>,<br>&lt;28 d or ≥28 d | 47   | 8                        | 0.9 (0.4–2.1)             | 0.854   | 0.8 (0.3–1.9) | 0.608   |
| Stage ≥2 AKI, eGFR ≥60 ml/min per 1.73 m<sup>2</sup>, &lt;28 d | 23   | 3                        | 0.7 (0.2–2.5)             | 0.569   | 0.8 (0.2–2.9) | 0.711   |
| Stage ≥2 AKI, eGFR &lt;60 ml/min per 1.73 m<sup>2</sup>, ≥28 d | 5    | 9                        | 4.4 (2.0–10.0)            | &lt;0.0001 | 9.5 (3.4–26.9) | &lt;0.0001 |
| Nephrotoxic medication                              |          |                          |                           |         |             |         |
| Amphotericin B                                      | 7        | 0                        | NA                        |         | NA          |         |
| Cyclosporine A                                      | 174      | 33                       | 0.2 (0.1–0.6)             | 0.005   | 0.2 (0.1–0.3) | 0.005   |
| Cidofovir                                           | 19       | 6                        | 1.6 (0.7–3.9)             | 0.274   |              |         |
| Ganciclovir                                         | 15       | 5                        | 1.6 (0.6–4.1)             | 0.332   |              |         |
| Foscarnet                                           | 23       | 8                        | 1.7 (0.8–3.6)             | 0.205   |              |         |
| Furosemide<sup>b</sup>                              | 116      | 18                       | 0.7 (0.4–1.3)             | 0.266   |              |         |
| Gentamycin                                          | 6        | 2                        | 1.2 (0.3–4.8)             | 0.850   |              |         |
| Tacrolimus                                          | 5        | 3                        | 3.1 (1.0–10.3)            | 0.059   |              |         |
| Vancocycin                                          | 164      | 34                       | 1.0 (0.3–3.3)             | 0.981   |              |         |

AKI, acute kidney injury; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; KDIGO, Kidney Disease: Improving Global Outcomes; NA, not analyzed.

<sup>a</sup>Patients with KDIGO stage ≥2 AKI were subdivided based on lowest eGFR and duration: AKI with the lowest eGFR ≥60 ml/min per 1.73 m<sup>2</sup>; AKI with the lowest eGFR <60 ml/min per 1.73 m<sup>2</sup> that recovered within 28 days; and AKI with the lowest eGFR <60 ml/min per 1.73 m<sup>2</sup> and duration ≥28 days.

<sup>b</sup>Only structural (>5 days) furosemide included.

Risk factors for CKD evaluated in univariate and multivariate Cox proportional hazards model. CKD was defined as KDIGO stage ≥G2 or ≥A2. HR, 95% CI, and P value are shown. For multinomial parameters, the P value from Wald test is shown as well. A total of 216 survivors of hematopoietic stem cell transplantation were included, of whom 37 developed CKD and 133 were censored. All listed parameters were included in a stepwise backward elimination multivariate Cox proportional hazards model. Only parameters that were retained in the multivariate model are shown in the multivariate table.
Figure 4 | Estimated glomerular filtration rate (eGFR) dynamics of chronic kidney disease (CKD) patients and incidence of hypertension. (a) Dynamics of eGFR in 21 patients with CKD. Kidney Disease: Improving Global Outcomes (KDIGO) stage G2 at 10 years after hematopoietic stem cell transplantation (HSCT). Red triangles: rapid eGFR reduction without recovery. Green squares: rapid eGFR reduction with initial recovery but subsequent eGFR reduction. Blue diamonds: stable eGFR in first year, but subsequent eGFR reduction. Horizontal lines: cutoff values for CKD. KDIGO stage ≥G2 (90 ml/min per 1.73 m²) and KDIGO stage ≥G3 (60 ml/min per 1.73 m²). (b) Incidence of AKI in 146 HSCT recipients at 10 years after HSCT: 123 patients without CKD and 33 patients with CKD. Bars = patients using antihypertensive medication (red), patients with systolic hypertension (green), patients with diastolic hypertension (blue), or patients with both systolic and diastolic hypertension (black). Statistics: ****P < 0.0001.

per 1.73 m² and duration ≥28 days. In contrast, AKI with eGFR ≥60 ml/min per 1.73 m² and AKI with eGFR <60 ml/min per 1.73 m² that recovered within 28 days were not associated with the development of CKD.

We were able to monitor the kidney function of HSCT recipients for 10 years after HSCT and into adulthood, and used KDIGO criteria to uniformly grade the severity of CKD. This long-term follow-up is crucial, because many patients developed CKD > 5 years after HSCT and after they had reached the pubertal age. The incidence of CKD in our cohort was higher than in most other studies describing CKD in pediatric HSCT recipients (Supplementary Table S3). These studies generally described small patient groups, used varying definitions of CKD, and had a short follow-up period, which is reflected in the large variation in reported CKD incidence after pediatric HSCT. In general, severe diseases in childhood, especially malignancies and intensive care unit admissions with AKI, are associated with CKD, which is often progressive in adulthood.

The eGFR dynamics in our cohort were largely comparable to studies reporting eGFR dynamics after adult HSCT. However, in adults, the eGFR was around 30% lower at all time points. Although the median eGFR was well above the cutoff for CKD at all time points, a 22% reduction of eGFR occurred between pre-HSCT and 10 years after HSCT. Half of this eGFR reduction occurred in the first year after HSCT, during which AKI occurred in 44% of patients. In concordance with the high incidence of AKI early after HSCT, the eGFR at 3 months after HSCT was affected by patients with— or recovering from—AKI. However, at later time points, only steady-state eGFR measurements were included. Remarkably, the other 50% of eGFR reduction occurred between 1 and 10 years after HSCT. Although this reduction is not necessarily clinically significant for an individual patient, the ongoing eGFR reduction in the stable period between 3 and 10 years after HSCT should raise awareness of potential further loss of kidney function in upcoming decades in these young adults and teenagers.

A weakness of this study is the use of SCr-based eGFR formulas. Early after HSCT, poor feeding status, hyperfiltration, and reduced muscle mass might lead to an overestimation of the eGFR calculated with SCr-based formulas. As a result, the increase of muscle mass in the years following HSCT and especially in puberty can reveal CKD that was not recognized at earlier time points. In future studies, the addition of cystatin C might improve the evaluation of kidney function after HSCT, although this marker can be affected by inflammation and the use of steroids early after HSCT.

In adult HSCT, repeatedly reported risk factors for CKD are the occurrence of AKI, older age, cyclosporine A use, TBI, and chronic graft-versus-host disease. Little is known about the risk factors for CKD after pediatric HSCT (Supplementary Table S3). In our study, prolonged severe AKI and hematological malignancy were independent risk factors for CKD. AKI is identified as a risk factor for CKD in most studies in adult HSCT. Although the studies that used the most severe definition of AKI had the highest odds ratio for CKD, no studies investigated the severity of AKI in relation to the development of CKD. The use of KDIGO stages without further specification of eGFR and duration was not discriminative in our cohort of pediatric HSCT recipients. We hypothesize that this is related to hyperfiltration or low muscle mass in a subgroup of patients, allowing a 2-fold or 3-fold increase of creatinine within the normal range of eGFR.
When patients with KDIGO stage ≥2 AKI were further categorized based on lowest eGFR and duration of AKI, a strongly increased risk for CKD was found for patients with prolonged severe AKI. Patients with AKI with eGFR ≥60 ml/min per 1.73 m² and even patients with AKI with eGFR <60 ml/min per 1.73 m² but recovery within 28 days did not have an increased risk for CKD. As AKI is a frequently occurring complication after pediatric HSCT, this observation helps to identify the children who are at risk for CKD.

In contrast to adult HSCT, hematological malignancy is a major, but no longer the predominant, indication for HSCT in children. Compared with patients with inborn errors of immunity and nonmalignant hematological diseases, patients with hematological malignancy usually receive more potentially nephrotoxic drugs during their treatment before HSCT, which likely explains the increased incidence of CKD. The lowest incidence of CKD was observed in patients with inborn errors of immunity. These patients were young (median, 3 years of age) at the time of HSCT and generally received less intensive conditioning before HSCT. Most of these children had not reached pubertal age at the time of evaluation. Therefore, longer follow-up is needed to validate this observation.

In contrast to previous reports in pediatric and adult HSCT, we observed a reduced HR of CKD in patients treated with cyclosporine A. However, in the small number of patients who did not receive cyclosporine A, this was due to various clinical reasons. Therefore, no firm conclusions can be drawn from this observation.

Few studies have evaluated proteinuria long-term after HSCT. The incidence of albuminuria in our cohort (14%) was comparable to our earlier study in pediatric HSCT recipients but much higher than the pediatric cohort described by Patzer et al. In adults, a higher incidence of proteinuria has been described, corresponding with the lower eGFR reported in adults. With 7% of patients having β2-microglobulinuria, tubular damage was not a major problem. We observed hypertension in 9% of long-term HSCT survivors, which is lower than reported in other studies among pediatric or adult HSCT survivors (17%–34%). In concordance with a study in adult HSCT recipients by Kersting et al., patients with CKD significantly more often had hypertension compared with patients without CKD. Hypertension and diabetes, which are major causes of proteinuria in adults, are frequent early complications of HSCT but often recover within 2 years. Whereas kidney disease is the main cause of hypertension in children, hypertension is most often not of renal origin in adults. In this cohort of teenagers and young adults, correction for hypertension and diabetes mellitus did not affect the results of our multivariate analysis.

Despite therapeutic drug monitoring, avoidance and replacement of nephrotoxic drug combinations, reduction of TBI-based conditioning, and close monitoring of kidney function early after HSCT, we did not observe a reduction of CKD among long-term pediatric HSCT survivors compared with earlier studies. We hypothesize that, because of the longer follow-up and strict definitions of CKD, we have recognized more patients with CKD. Recent guidelines for late effects follow-up after pediatric HSCT for both malignant and nonmalignant indications recommend annual evaluation of the renal function in all pediatric HSCT patients. Our data support the annual monitoring of eGFR using creatinine and cystatin C, as well as glomerular and tubular proteinuria and blood pressure after pediatric HSCT to identify patients with incipient CKD who could benefit most from nephroprotective interventions, like renin-angiotensin-aldosterone system inhibition and “Dietary Approaches to Stop Hypertension” diet. Because CKD may still become evident many years after the initial damage, follow-up of pediatric HSCT survivors into adulthood is essential for a better understanding of long-term renal complications after pediatric HSCT.

DISCLOSURE
All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL
Supplementary File (PDF)
Table S1. Overview of patients with prolonged severe acute kidney injury (AKI).
Table S2. Cox proportional hazards model with correction for diabetes and hypertension.
Table S3. Literature overview of chronic kidney disease after pediatric allogeneic hematopoietic stem cell transplantation.
Figure S1. Covariate-adjusted survival curves for separate nonmalignant HSCT indications.

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