Delaying haematopoietic stem cell transplantation in children with viral respiratory infections reduces transplant-related mortality

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Running Title: HSCT delay for pre-transplant URTI infections

Summary

Viral respiratory infections (VRIs) contribute to the morbidity and transplant-related mortality (TRM) after allogeneic haematopoietic stem cell transplantation (HSCT) and strategies to prevent and treat VRIs are warranted. We monitored VRIs before and after transplant in children undergoing allogeneic HSCT with nasopharyngeal aspirates (NPA) and assessed the impact on clinical outcome. Between 2007 and 2017, 585 children underwent 620 allogenic HSCT procedures. Out of 75 patients with a positive screening NPA (12%), transplant was delayed in 25 cases (33%), while 53 children started conditioning with a VRI. Patients undergoing HSCT with a positive screening NPA had a significantly lower overall survival (54% vs 79%) and increased TRM (26% vs 7%) compared to patients with a negative NPA. Patients with a positive NPA who delayed transplant and cleared the virus before conditioning had improved OS (90%) and lower TRM (5%). Pre-HSCT positive NPA was the only significant risk factor for progression to a lower respiratory tract infection and was a major risk factor for TRM. Transplant delay, whenever feasible, in case of a positive screening NPA for VRIs can positively impact on survival of children undergoing HSCT.

Abbreviations: haematopoietic stem cell transplantation (HSCT), nasopharyngeal aspirate (NPA), viral respiratory infection (VRI), upper respiratory tract infection (URTI), lower respiratory tract infection (LRTI), bronchoalveolar lavage (BAL), cytomegalovirus (CMV), adenovirus (ADV), Epstein-Barr virus (EBV), respiratory syncytial virus (RSV), parainfluenza virus (PF), paediatric intensive care unit (PICU).

Introduction

Haematopoietic stem cell transplantation (HSCT) is curative for several haematological (Peters *et al*, 2010) and immunological disorders (Freeman, 2018), as well as an alternative approach to enzyme replacement therapy in some inborn errors of metabolism (de Ru *et al*, 2011). However, infections are a major cause of treatment failure and contribute significantly to transplant related mortality (TRM) (Atay *et al*, 2018). Viral infections are difficult to control in immunocompromised hosts such as transplanted patients, due to impaired humoral and cell-mediated immunity post-SCT because of *in vivo* (serotherapy) (Lamba *et al*, 2005) or *ex vivo* (alpha/beta and B cells depletion) lymphodepletion (Laberko *et al*, 2017) and immunosuppressants used to treat graft *versus* host disease (GvHD). Currently, there are no clear recommendations on screening and monitoring for respiratory viruses in children undergoing HSCT and there is very little evidence on efficacy of anti-viral drugs (Hirsch *et al*, 2013). Despite a lower prevalence compared to herpes family viruses or adenovirus reactivation, respiratory viruses can be responsible for severe lower respiratory tract infections that can negatively affect transplant outcome (Chemaly *et al*, 2014; Dokos *et al*, 2013; Boeckh, 2008). Epidemiological studies have shown an incidence of upper and lower respiratory infections in children undergoing HSCT of around 20-30% (Bredius *et al*, 2004; Verdeguer *et al*, 2011; Srinivasan *et al*, 2013, 2011b). Progression to a lower respiratory tract infection can be influenced by patients' age and lack of immune reconstitution after transplant (Srinivasan *et al*, 2011a; Gooskens *et al*, 2016; El Saleeby *et al*, 2008). Overall, there are few specific recommended anti-viral treatments, and for a large proportion of respiratory viruses there is no evidence on efficacy for prophylaxis or therapy (Hirsch *et al*, 2013; Dignan *et al*, 2016).

The impact of viral infections on outcome of children undergoing HSCT has not been fully explored, especially for patients that start a conditioning regimen with an active viral respiratory infection, as screening for respiratory pathogens in asymptomatic patients is not routine clinical practice. Viral respiratory infections can lead to high early transplant-related mortality within the first three months after transplant (Hutspardol *et al*, 2015). However, although ECIL guidelines recommend considering delaying HSCT in the presence of viral infections (Hirsch *et al*, 2013), there are no data on the efficacy of this approach on final outcome. In this retrospective study we analysed the prevalence of viral respiratory infections (VRIs) in children undergoing allogeneic HSCT and the prognostic significance of a positive nasopharyngeal aspirate (NPA) sample collected as part of the screening protocol prior to transplant.

Patients and Methods

We retrospectively included all children who underwent allogeneic haematopoietic stem cell transplantation in the Bone Marrow Transplantation Unit at Great Ormond Street Hospital for Children in London, between November 2007 and November 2017. All but two patients were screened with NPA for VRIs before starting the conditioning regimen. The decision to delay the transplant in case of a positive NPA was made according to patients' characteristics and the clinical background. Patients with malignant disorders delayed the transplant according to disease status and donor availability. For patients with PID transplant delay was considered if residual T cell immunity was observed and could reasonably allow viral clearance. We collected data on positive NPAs up to 90 days prior to transplant, in order to evaluate viral clearance in patients whose HSCT was delayed due to a positive NPA. We considered patients with active VRI at the time of transplant when they had a positive NPA before the start of the conditioning regimen. New occurrence of VRIs after HSCT (documented through NPA or bronchoalveolar lavage, BAL) was also recorded, and weekly NPA monitoring was routinely adopted during the early phases post-transplant. Moreover, we also retrieved data on symptomatic patients that were re-admitted or followed-up in the outpatient clinic up to 1 year after transplant.

We aimed to investigate the impact of viral respiratory infections on clinical outcome. Patients with severe respiratory symptoms or requiring PICU admission for respiratory distress and evidence of viral infection in the NPA or in the bronchoalveolar lavage (BAL), without any sign of other possible cause were considered as suffering from a viral LRTI. Mortality was considered to be attributable to VRIs when patients died from respiratory failure with positive specimens (NPA or BAL) for viral infections, and with no other ascertainable causes.

PCR for Respiratory Viruses

Viral DNA and RNA on NPA and BAL samples were analysed via PCR for the following viruses: CMV; ADV; respiratory syncytial virus A and B; influenza virus A and B; parainfluenza virus 1-4; human metapneumovirus. Extended viral PCR adopted from 2013 included rhinoviruses, enterovirus and human coronavirus strains.

HSCT procedures

HLA typing was performed by molecular typing for HLA class I and II loci; mismatch was defined as ≤ 9 out of 10 HLA identical for bone marrow and peripheral blood stem cell source and ≤ 5/6 for cord blood. Myeloablative conditioning included Busulfan-based conditioning receiving > 8 mg/kg cumulative dose and TBI-based regimens receiving ≥ 8Gy fractioned dose. The diagnosis of acute GvHD was made clinically, and confirmed pathologically with skin, mucosal or liver biopsy whenever possible. Grading of acute GvHD was performed according to the Seattle criteria (Martino *et al*,

1999). Immune reconstitution was evaluated through absolute count of total lymphocytes and lymphocyte subsets evaluated through flow cytometry (CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, CD16/56$^+$ cells) at 1, 3, 6 and 12 months after transplant. Significant CMV, EBV and ADV reactivation were considered when peripheral blood viral loads reached the cut-off of 10.000 IU/ml, 40.000 copies/ml, and 1.000 copies/ml, respectively.

Statistical analysis

Transplant features and demographic characteristics were included in descriptive statistics. Evaluated outcomes included overall survival (OS), transplant related mortality at day +100 after HSCT (defined as patients in CR deceased up to 100 days after the procedure), progression to LRTI (as previously defined) and rate of admission to ICU. Log rank test (Mantel-Cox) was performed to compare Kaplan-Meier survival curves and cumulative incidence of transplant related mortality between groups of patients. Univariate analysis for categorical variables using Fisher's exact test investigated risk factors for final outcome. Variables that showed a significant association with outcome in univariate analysis were included in logistic regression model for multivariate analysis. Threshold for significant results for all the analysis was set at p<.05. Data analysis and statistics were performed using Prism GraphPad software, version 6 (GraphPad Software Inc., La Jolla, CA, USA) and Epi Info™ 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) software.

Results

Transplant characteristics

Features of patients and transplant procedures are summarized in **Table I**. In this 10-years study period 586 children underwent 621 allogenic HSCT procedures. One patient was excluded from the analysis, as NPA samples were not collected at any time before and after transplant. Most patients (249/585, 43%) were referred to HSCT for a primary immunodeficiency disorder (PID), or haematological malignancy (198/585, 34%). The preferred stem cell source was bone marrow (BM=296/620, 47.7%) and most patients were fully HLA-matched with related (187/620, 30%) or unrelated (210/620, 34%) donors. The median age of patients in the cohort was 4.8 years (range 0.04-17.03). A small proportion of patients with SCID (37/620, 6%) did not receive any conditioning prior to HSCT.

Viral respiratory infections before HSCT

Screening NPA results before starting the conditioning regimen were available for 618 transplants. Two patients were not investigated for VRIs at any time before transplant but were monitored in the early and late post-transplant follow-up. Overall, 75 patients (12.1%) presented with a viral pathogen in the NPA before transplant. Three patients received two HSCT with a positive NPA. Most of the children presented with symptoms of active URTI (47/75, 63%), while 24 (32%) were asymptomatic. Frequencies of different viral infections are reported in **Table II**. Eight patients presented pre-transplant with co-infection involving two viruses. The decision to delay HSCT was taken in 25 cases. Most of them were found positive for RSV (11/25, 44%), 6 had Influenza A or B, 5 Parainfluenza viruses, 2 Adenovirus and finally 1 Metapneumovirus. However, three patients did not clear the viral infection and overall 50 patients underwent 53 HSCT with an active viral URTI. These were Rhinovirus (18/53, 34%), Parainfluenza (12/53, 23%), RSV (6/53, 11%), Adenovirus (4/53, 7%), Influenza A/B (3/53, 5%), Metapneumovirus (2/53, 4%) and CMV (1/53, 2%). Seven patients presented with a viral co-infection: Coronavirus + Rhinovirus (2/53, 4%), CMV + Rhinovirus (1/53, 2%), Parainfluenza + Influenza (1/53, 2%), Adenovirus + Rhinovirus (1/53, 2%), RSV + CMV (1/53, 2%), RSV + Parainfluenza(1/53, 2%).*De novo viral infections after HSCT*

After transplant patients were routinely screened through NPA once a week during admission for HSCT and, following discharge, in case of respiratory symptoms suggestive of URTI. Eighty-six (14%) new viral respiratory infections were documented within the first year post-HSCT (**Table II**) and 60/86 (70%) children had respiratory symptoms. Seven patients were found positive with two viruses at the same determination (3/7 were Coronavirus co-infections). Viruses were detected from day +1 and up to 11 months after transplant, although 71% (61/86) of infections occurred within the first 50 days after transplant, and the median time to NPA positivity was 25 days. About half (45/86, 52%) of new VRI occurred in the first 30 days after transplant ("early infections").

Lower airways infections and TRM

Of the 161 patients with URTI at or within the first year after HSCT, 32 (20%) were diagnosed with LRTI, which was documented with a positive BAL in 19 cases and with a positive NPA in 13 patients. The most frequent virus responsible for LRTI was Parainfluenza virus (12/32, 37.5%), followed by RSV (8/32, 25%). Three patients experienced a co-infection with two viruses. Of the 32 patients, 21 (66%) had LRTI due to the same pathogen detected on pre-transplant screening. Admission to the Paediatric Intensive Care Unit (PICU) was necessary for 19 patients with respiratory failure. Overall 17/32 (53%) patients diagnosed with LRTI died, and in all (**Table S1**) but two cases the cause was attributed to viral respiratory infection (LRTI-associated mortality 47%, 15/32). A significantly higher rate of LRTI was observed in patients with a positive NPA prior to transplant, compared to those that experienced a new URTI after transplant (36% vs 15%, OR 3.13, p<.01). Moreover, patients with an active pre-HSCT infection required PICU admission more frequently (30% vs. 8%, OR 4.8, CI95 1.9-12.9, p<.005, **Fig 1**). Early infections (from day +1 to day +30) and late infections (after day +30) equally contributed to LRTI (6/48 vs 7/38, p=.55) in children with negative pre-transplant NPA.

Risk factors for OS, TRM and progression to LRTI

Overall survival (OS) in our cohort was 78%and 100-days TRM was 8.5%. We stratified survival rates according to four different groups: patients undergoing transplant with a positive NPA (pre-NPA+), patients with a newly positive NPA after transplant (post-NPA+), patients with a negative NPA before and after transplant (NPA-) and a group of patients whose transplant was delayed due to positive NPA and cleared the virus before conditioning (delayed-NPA+). We found a statistically higher survival rate in patients that delayed HSCT and started the conditioning regimen after resolving the VRI (OS=90.5%) compared to patients that started conditioning with an active viral respiratory infection (OS=53%), (HR 4.2, CI95 1.1-7.7, p<.05) (**Fig 2**). No significant difference in OS was noted between the delayed group and patients diagnosed with a new respiratory infection after transplant or who did not experience any viral respiratory infection (72.6% and 73.6%, respectively, p=.2).

We also evaluated the stratification of 100-days transplant related mortality in these groups of patients (**Fig 3**). Children that presented with a screening positive NPA at the time of transplant had a significantly higher 100-days TRM (26.5%, HR 4.4, CI95 1.9-12, p<.001), while no significant difference in early mortality was observed in patients with new VRIs (7%) and no VRIs at the time of transplant due to delay of the procedure (5%) or no occurrence of respiratory infections (7%). Considering patients with positive NPA pre-HSCT, for11/14 (79%) deceased patients the cause of death was attributed to viral respiratory infection, while two died of fungal pneumonia and oneof lung GvHD/transplant-associated microangiopathy. Overall, LRTI-associated mortality was higher in patients with pre-HSCT active URTIs and in children that were found NPA positive after transplant

(24.6% vs 8.4%, HR 4.1, CI95 1.6-12, p<.005) (**Fig 4**). In order to evaluate the impact of different risk factors on patients that died due to transplant complications in the first 100 days after HSCT (100-days TRM) and progression to LRTI we performed a univariate analysis on different variables (**Table III**). NPA positivity at the time of transplant was a significant risk factor for early mortality related to transplant complications, as well as the use of UCB as stem cell source and the use of HLA mismatched donors. On multivariate analysis, only NPA positivity and the use of HLA mismatched donors were independently associated with early TRM (**Table III**). We also investigated the impact of rhinovirus infection on outcome (**Fig 5**). Early TRM was lower for patients with pre-transplant NPA positive for Rhinovirus (12%) as compared with patients with other VRI (34%) starting conditioning, although this was only partially significant (p=.07). However, no differences were noted with patients with pre-HSCT negative NPA (100d TRM 6%, p=.3). In univariate analysis the risk of progression to LRTI was significantly higher in children with pre-HSCT positive NPA (**Table III**).

Discussion

Viral respiratory infections represent a major cause of morbidity and mortality after haematopoietic stem cell transplantation, because of the possible evolution to respiratory failure and the lack of effective therapeutic strategies available. Screening for respiratory viruses is not routinely performed in Bone Marrow Transplant Units and the presence of an upper respiratory tract infection is not universally recognised as a risk factor that merits transplant delay. We extensively analysed the outcome of a sub-group of patients with a positive screening NPA before HSCT over a 10-years period. We found that pre-transplant viral infections had a profound impact on clinical outcomes, leading to lower OS and higher 100-days TRM, compared to patients that experienced a *de novo* viral infection after HSCT. Active viral infection at the time of transplant negatively influenced outcome of these patients who experienced respiratory infection-related mortality in almost 25% of cases.

A significant proportion of children included in our cohort was affected by PID. These patients could be more susceptible to recurrent and difficult to eradicate viral RTI. However, this did not result in an increased prevalence of pre-HSCT VRI as compared to the literature. Indeed, Campbell *et al* (Campbell *et al*, 2015) reported a higher prevalence of pre-transplant positivity of respiratory virus screening, especially in children. However, in their study only a minority of patients were < 18 years-old (52/458, 11%). Of note, in this study a higher mortality rate in children with pre-BMT viral infections was also reported, compared to adults. In our study newly occurring viral respiratory infections were mainly documented in the early phase after HSCT and prevalence was comparable to the one recently described in children receiving HSCT (16.6%) (Fisher *et al*, 2018). Interestingly, in this study early infections within the first 60 days after transplant were associated with higher morbidity and mortality, supporting the crucial impact of immune status for the outcome of patients with RVIs.

The main complication related to viral URTI is progression to LRTI. We showed that starting conditioning with an active upper airways respiratory infection can lead more frequently to pulmonary involvement, irrespective of background diagnosis, HLA matching, occurrence of GvHD. Use of UCB was only partially associated with higher rate of LRTI and this could be related to the lower cell dose and delayed lymphocytes engraftment (Kim *et al*, 2014; Nichols *et al*, 2004). Interestingly, a higher rate of intensive care need for patients with a positive screening NPA was also observed.

Importantly, children who delayed transplant presented similar background features to NPA+ transplanted patients but had a reduced early mortality after transplant compared to those patients that did not delay the transplant despite the active viral infection documented on admission. This difference is unlikely to reflect a selection bias for better risk patients: when we looked at early TRM, progression to LRTI was the main cause of graft failure in the NPA+ group, and three other patients died due to pulmonary complications. Accordingly, risk factors analysis showed that a pre-transplant

positive NPA was a major risk factor for early mortality. The more severe clinical course in patients with a respiratory virus documented in the upper airways before transplant could be related to the younger age of this sub-group of patients, since age < 2 years-old has been associated with dismal outcome of viral respiratory infection (El Saleeby *et al*, 2008). However, patients who experienced a viral infection after transplant were not significantly older, with a median age < 2 years.

European guidelines only provide weak recommendation for postponing conditioning in case of VRI (Hirsch *et al*, 2013) and transplant delay can be controversial, since some patients cannot postpone the procedure (e.g. patients affected by aggressive malignant disorders) or do not present immunological competence to clear the virus (e.g. patients with severe combined immunodeficiencies). Nevertheless, in case of Influenza or RSV infection, a delay of HSCT can allow pharmacological treatment (i.e. Oseltamivir and Ribavirin, respectively). Unfortunately, for most viruses there is no effective treatment available, and viral clearance is dependent on the efficacy of the host's immune system. Especially for patients with PID, that can more frequently present with chronic/refractory viral respiratory infections, balance of risk/benefit ratio is challenging since viral clearance is unlikely in SCID patients but can occur in the presence of a residual T cell immunity. Only one study reported the efficacy of transplant delay in 37 adults and children with RSV URTI before conditioning, showing that survival was significantly improved compared to those who encountered the virus after transplant (Peck *et al*, 2004). Whether to postpone HSCT because of a positive NPA for Rhinovirus is more debatable, since LRTI progression is not frequent. However, rhinovirus-associated lower airways infection before transplant can lead to a worsen outcome (Mowrer *et al*, 2018; Seo *et al*, 2017). However, our data, although retrospective and limited to a small cohort of patients, show that pre-HSCT rhinovirus infection is associated with a modestly lower TRM as compared to other viral strains involved in URTI, and is overall comparable to that of patients with negative screening NPA.

When transplant delay is not feasible, strategies aimed to improve lymphocytes recovery in the first weeks after transplant (e.g. targeted in vivo T-cell depletion (Admiraal *et al*, 2017), adoptive immunotherapy (Ciceri *et al*, 2009)) should be considered. Faster immune reconstitution is usually expected when *in vivo* T-cell depletion is omitted or administered early during conditioning (Lindemans *et al*, 2014). GvHD prophylaxis with Alemtuzumab results in delayed lymphocytes recovery when compared to ATG (Shah *et al*, 2007), and decision on *in vivo* T-cell depletion strategy should balance the risk of GvHD versus the burden of active viral infections. *Ex vivo* manipulation of the graft, selectively depleting alloreactive cells and allowing the presence of gamma/delta T cell in the graft, can tackle post-transplant infections (Locatelli *et al*, 2017; Bertaina *et al*, 2014), and turning-off T cell with an inducer of dimerization, have also been developed (Zhou *et al*, 2015). Finally, non-myeloablative and reduced-intensity conditioning regimens could limit organ toxicity and allow transplant also in patients with severe infections (i.e. PID patients), although the risk of engraftment failure with an active viral infection should be pondered. However, prospective data are necessary

to evaluate the impact of *in vivo* and *ex vivo* T cell depletion on VRIs and no recommendation on the best strategy can be made.

Conclusion

This retrospective study from the largest paediatric HSCT centre in the UK shows that screening for viral respiratory infections prior to transplant is of paramount importance, as outcomes are dismal when patients are transplanted with a positive screening NPA. Moreover, patients who delayed HSCT until clearance of viral respiratory infection had an improved outcome, suggesting that this screening strategy can reduce transplant-related mortality. Prospective studies are needed to explore novel strategies to treat viral infections in children eligible for HSCT.

Authors contributions

GO and RC designed the study, analysed the data and wrote the manuscript. KR, PA, GL, RE and PV interpreted the results and critically reviewed the manuscript. JSF, JB, OC and AL reviewed the manuscript. All authors reviewed and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest

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TABLES

Table I: Transplant characteristics (n=620) and patients demographics (n=585) of the study cohort (second column); HSCT (n=53) and patients' characteristics of children (n=50) transplanted with a positive NPA (third column); HSCT and patients' characteristics of children (n=22) who delayed the transplant and started conditioning with a negative NPA (fourth column).

	Study cohort n= 585	NPA+ transplanted patients n= 50	Delayed NPA+ patients n= 22	p value
Median age at BMT, years (range)	4.8 (0.04-17.03)	1.6 (0.07-12)	2.4 (0.2-8.8)	<.0001
Diagnosis				
Malignant disorders	198/585 (34%)	13/50 (26%)	8/22 (36%)	
ALL	93	7	4	
AML	64	3	2	
Others	41	3	2	
Non-malignant disorders	387/585 (66%)	37/50 (74%)	14/22 (64%)	.4
PIDs	249	31	9	
Metabolic disorders	47	2	4	
Haematological	41	0	0	
Autoimmunity/inflammatory	50	4	1	
Stem cells source				
BM	296/620 (47.7%)	22/53 (42%)	13/22 (59%)	
PB	215/620 (34.7%)	19/53 (36%)	6/22 (27%)	
UCB	107/620 (17.3%)	12/53 (22%)	3/22 (14%)	.5
BM+PB	2/620 (0.3%)			
HLA-matching				
Full matched	397/620 (64%)	31/53 (59%)	18/22 (82%)	
Mis-match	223/620 (36%)	22/53 (41%)	4/22 (18%)	.15
Conditioning				
None	37/620 (6%)	7/53 (13%)	1/22 (4%)	.1
Reduced intensity (RIC)	379/620 (61%)	34/53 (64%)	10/22 (46%)	
Myeloablative conditioning (MAC)	204/620 (33%)	12/53 (23%)	11/22 (50%)	
Serotherapy				
Alemtuzumab	281/620 (46%)	17/53 (32%)	9/22 (41%)	
ATG	102/620 (16%)	14/53 (26%)	3/22 (13%)	
Muromonab-CD3	5/620 (1%)	1/53 (2%)	0	
None	232/620 (37%)	21/53 (39%)	10/22 (46%)	.8
Number of transplants				
1st	568/620 (91%)	44/53 (83%)	22/22 (100%)	<.05

2nd	49/620 (8%)	8/53 (15%)		
3rd	3/620 (1%)	1/53 (2%)		

Abbreviations: ALL= acute lymphoblastic leukemia; AML= acute myeloid leukemia; BM= bone marrow; PB= peripheral blood; PID= primary immunodeficiency; UCB= unit cord blood; ATG= anti-thymocyte globulin.

Table II: Viral pathogens detected in nasopharyngeal aspirates of children screened for respiratory infections before starting conditioning (eight patients presented with a viral co-infection) and *de novo* respiratory viral infections after transplant, caused by viruses that were not detected at the time of pre-transplant screening (seven patients presented with a viral co-infection).

Viral pathogen	Pre-HSCT positive NPA n=75 (%)	Post-HSCT positive NPA n=86 (%)
Rhinovirus	22/75 (29%)	22/86 (26%)
Parainfluenza (PF1, PF2, PF3)	17/75 (23%)	23/86 (27%)
RSV	17/75 (23%)	19/86 (22%)
Influenza (A/B)	10/75 (13%)	4/86 (5%)
ADV	8/75 (11%)	16/86 (19%)
CMV	4/75 (5%)	1/86 (1%)
Metapneumovirus	3/75 (4%)	3/86 (3%)
Coronavirus	2/75 (3%)	4/86 (5%)
Enterovirus	0	1/86 (1%)

Abbreviations: ADV= Adenovirus; CMV= Cytomegalovirus; RSV= respiratory syncytial virus; PF= parainfluenza; NPA= nasopharyngeal aspirate.

Table III: univariate analysis (Fisher's exact test) of risk factors for 100-days TRM in children receiving HSCT (n=620) and for progression to LRTI in patients with a positive NPA pre-HSCT conditioning (n=53) versus patients with a documented URTI after HSCT (n=86) and multivariate analysis (logistic regression) for 100-days TRM.

Univariate analysis (Fisher's exact test)				
	100d-TRM OR (95CI)	p value	LRTI OR (95CI)	p value
Pre-NPA+ vs. Post-NPA+	4.3 (1.5-12.5)	<.001	3.1 (1.3-6)	<.01
UCB vs. BM/PBSC	2.3 (1.2-4.2)	<.05	2.8 (1.1-7)	.052
aGvHD II-IV vs. aGvHD 0-I	0.7 (0.4-1.3)	.3	0.8 (0.3-1.8)	.7
HLA ≤9/10 vs. 10/10	3 (1.5-6)	<.005	1.3 (0.6-3)	.5
PID diagnosis vs. Other	1.2 (0.7-2)	.7	1.8 (0.8-4)	.2
Viral reactivation vs. None viral reactivation	1 (0.6-1.8)	1		
CD3$^+$ cells at 1 mo <200/mm^3 vs. >200/mm3	1.2 (0.6-2.2)	.6	1.4 (0.6-3)	.5
2nd/3rd HSCT vs 1st HSCT	1.15 (0.43)	.8		
Logistic regression for 100-days TRM				
pre-NPA+	4.8 (2.4-9.8)	<.001		
HLA mismatch ≤9/10	2.7 (1.4-5)	<.005		
UCB	1.5 (0.7-2.9)	.2		

Supplementary tables

Table S1: features of 15 children who died due to viral LRTI

Diagnosis	Age at HSCT (years)	Conditioning regimen	Donor	Respiratory virus	Timing of NPA (days)
HLH	1.5	Treosulfan/Fludarabine/Alemtuzumab	MMUD	Adenovirus	d+321
MHC Class II Deficiency	1.6	Fludarabine/Melphalan/Alemtuzumab	MMFD	Adenovirus	d+35
SCID	0.4	None	Halplo	Cytomegalovirus	Pre-HSCT
ALL	13.3	TBI/Etoposide/ATG	MUD	Metapneumovirus	Pre-HSCT
SCID	0.7	Treosulfan/Fludarabine/ATG	MMUD	Parainfluenza 2	Pre-HSCT
SCID	1.2	Fludarabine/Melphalan/Alemtuzumab	MMUD	Parainfluenza 3	Pre-HSCT
HLH	1.6	Treosulfan/Fludarabine	MMUD	Parainfluenza 3	Pre-HSCT
HLH	1.8	Treosulfan/Fludarabine	MMUD	Parainfluenza 3	Pre-HSCT
SCID	0.4	None	MUD	Parainfluenza 3	Pre-HSCT
SCID	0.3	Treosulfan/Cyclophosphamide	MMUD	Parainfluenza 3	Pre-HSCT
ALL	12.4	TBI/Cyclophosphamide/Fludarabine	Haplo	Parainfluenza 3 + Influenza B	Pre-HSCT
RAG 1 - Omenn's syndrome	1.4	Treosulfan/Fludarabine	MMUD	Parainfluenza 3	d+10
MHC Class II Deficiency	2.4	Treosulfan/Fludarabine/Thiotepa/ATG	MUD	Adenovirus	Pre-HSCT
SCID	0.6	Busulfan/Fludarabine	MSD	Respiratory syncytial virus	Pre-HSCT
HLH	0.6	Treosulfan/Fludarabine	MMUD	Respiratory syncytial virus	d+280

Abbreviations: ALL= acute lymphoblastic leukemia; ATG= anti-thymocyte globulin; HLH= hemophagocytic lymphohistiocytosis; MHC= major histocompatibility complex; MMFD= mis-matched family donor; MMUD= mis-matched unrelated donor; MUD= matched unrelated donor; MSD= matched sibling donor; SCID= severe combined immunodeficiency.
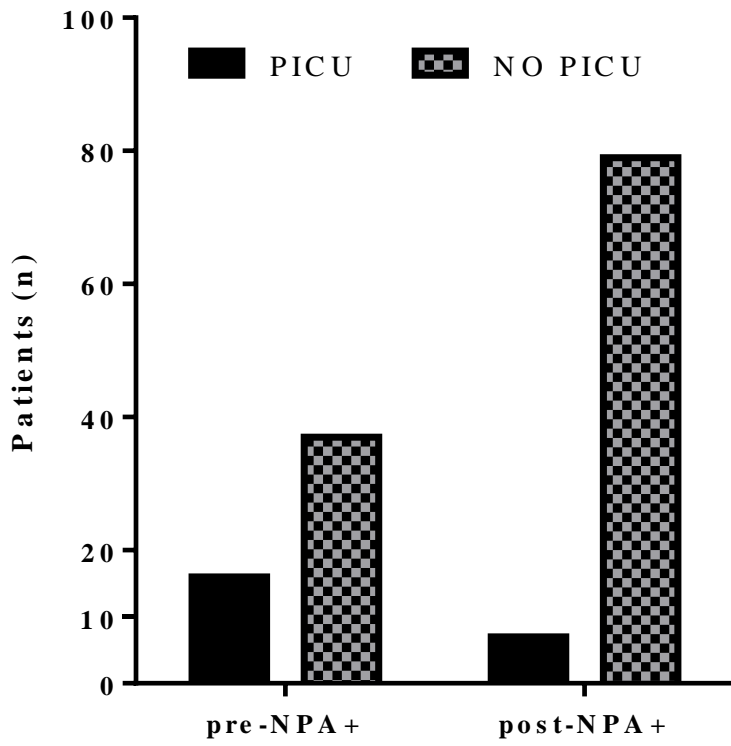
Figures legends

Fig 1: Admission to PICU was more frequent when NPA screening was positive at time of transplant (p<.005).

Fig 2: (a) Kaplan-Meier estimator for overall survival (OS) and log rank comparison for patients with pre-HSCT NPA positivity (53%), post-HSCT NPA positivity (72.6%), NPA negativity (73.6%) and patients with delayed HSCT (90.5%) (p<.05). **(b)** Cumulative incidence of 100d-TRM and log rank comparison for patients with pre-HSCT NPA positivity (26.5%), post-HSCT NPA positivity (7%), NPA negativity (7%) and patients with delayed HSCT (5%) (p<.001).

Fig 3: **(a)** Cumulative incidence of mortality due to LRTI in patients with a positive NPA at the time of transplant was higher than patients who experience a new URTI after BMT (p<.005). **(b)** Cumulative incidence of 100d-TRM and log rank comparison for patients with pre-HSCT rhinovirus (12%), other pre-HSCT URTI (34%) and no pre-HSCT URTI (6%)
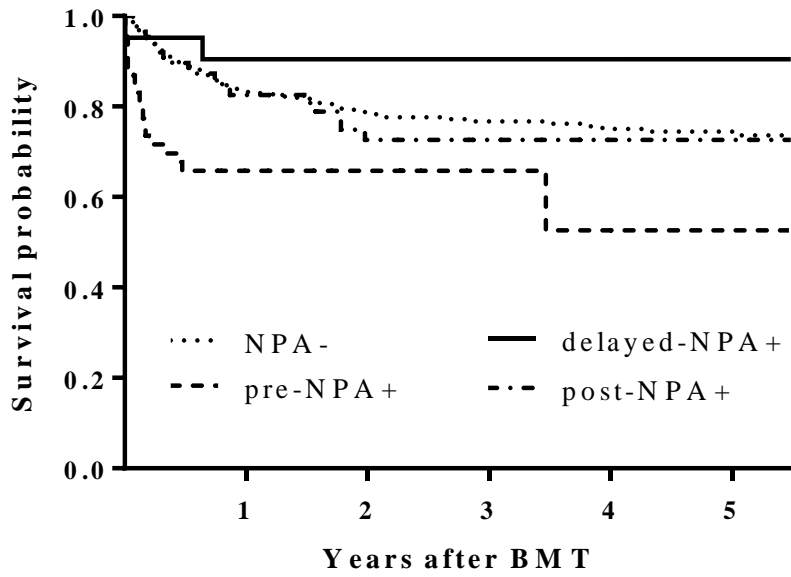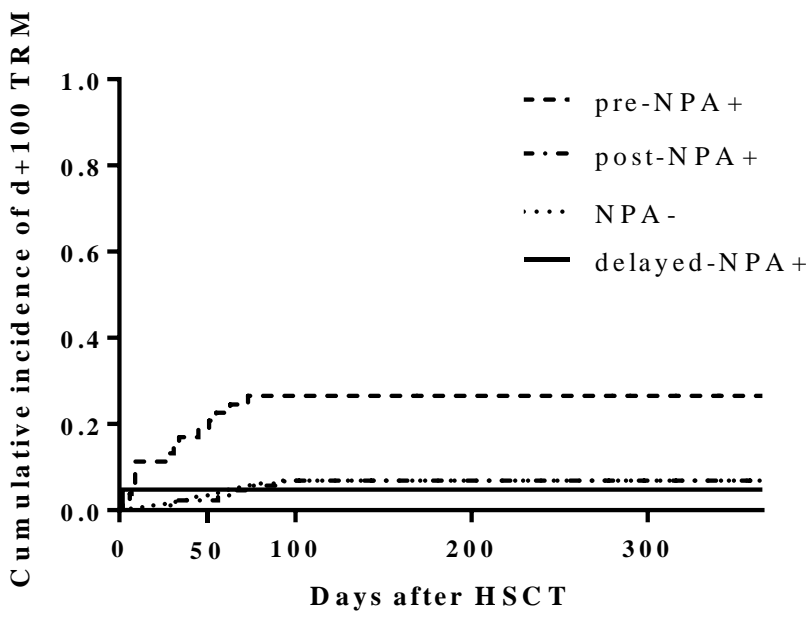
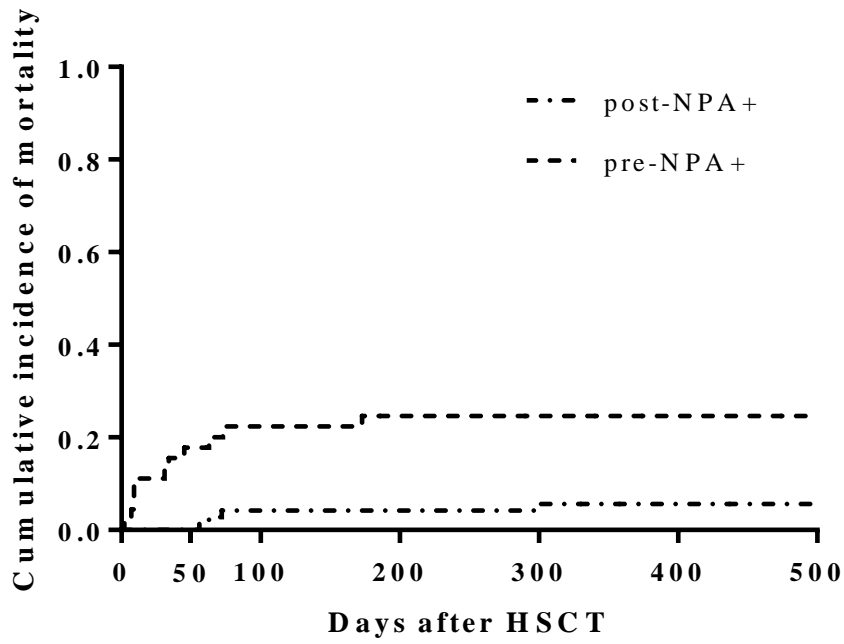**FIGURES**

Fig 1

Fig 2

a

b

Fig 3

a

b