I, Christopher E Dandoy, hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
Early post-transplant echocardiographic screening identifies serious pathology in children and young adults

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This work and its defense approved by:

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Committee member: Sonata Jodele, M.D.
Early post-transplant echocardiographic screening identifies serious pathology in children and young adults

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In the Department of Environmental Health
Division of Epidemiology & Biostatistics
of the College of Medicine

by

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Medical Doctor, University of Utah, May 2007
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Committee: Erin Haynes, Ph.D., Sonata Jodele, MD, Adam Lane, PhD.
ABSTRACT

Cardiac complications after hematopoietic stem cell transplant (HSCT) can lead to significant morbidity and mortality. Cardiac evaluation during the first 100 days after HSCT is only performed if clinically indicated. It is not known if scheduled post-transplant echocardiographic screening would identify patients at risk, leading to early interventions and improved outcomes. We conducted a single center prospective clinical study to screen for cardiac complications in pediatric and young adult patients. One hundred consecutive HSCT patients underwent scheduled echocardiographic screening on days +7, +30 and +100 after transplantation independent of their clinical condition and were found to have a high incidence of pericardial effusion (PEF), elevated right ventricular (RV) pressure including pulmonary hypertension (PH), and left ventricular (LV) dysfunction. We found strong associations between transplant-associated thrombotic microangiopathy (TA-TMA), oxygen requirement, and severe hypertension with patients that developed a PEF or elevated RV pressure. Our data suggests that scheduled echocardiographic screening at day +7 and day +30 after HSCT in patients undergoing allogeneic transplant will identify those at risk in whom early interventions might be beneficial. Day +100 screening is indicated in autologous HSCT patients and those who received anthracyclines prior to HSCT to monitor for LV dysfunction.
ACKNOWLEDGEMENTS

I wish to thank Sonata Jodele and Stella Davies for their continual guidance and mentorship. Thank you to my oversight committee consisting of Sonata Jodele, Adam Lane, and Erin Haynes. I would like to thank the other co-authors on this work, including Russel Hirsch, Ranjit Chima, Zachary Paff, Michelle Cash, Thomas Ryan, Javier El-Bietar, and Kasiani Myers. I would like to thank the physicians, nurses, care managers, transplant coordinators, echocardiography technicians, and other care providers and staff at Cincinnati Children’s Hospital Medical Center who participated in this work. Finally, I would like to thank the patients and their families.
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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is an important and effective treatment strategy for many malignancies, marrow failure syndromes, and immunodeficiencies in children and young adults. The chemotherapy and radiation utilized in HSCT may cause significant cardiac and vascular endothelial toxicity resulting in complications after transplant including pericardial effusion (PEF), left ventricular (LV) dysfunction, and pulmonary hypertension (PH). As such, heart disease is a major cause of long term morbidity and mortality in childhood survivors of HSCT\(^1\).

Predicting the impact of the LV dysfunction, PEF and PH in pediatric patients after HSCT is challenging due to the lack of prospective studies evaluating these factors. LV dysfunction has been reported in patients after high dose cyclophosphamide and anthracyclines\(^2\)-\(^4\). Mo et al. found an incidence of LV dysfunction and heart failure in roughly 1.7% of adult patients after HSCT\(^5\). The mean time to development of LV dysfunction was 5 months after HSCT and 22% of these patients died from heart failure\(^6,7\). PEF, if left untreated, can cause cardiac tamponade acutely decreasing cardiac function\(^8\)-\(^12\). The reported incidence of PEF in retrospective cohorts has varied from 0.2% to 19% in patients after HSCT\(^6,13\). Age, gender, preparative regimen, graft versus host disease (GVHD), CMV and EBV viremia are suggested associated causes or risk factors\(^13\)-\(^19\). In our institution, we found a high incidence of PEFs in patients with transplant-associated thrombotic microangiopathy (TA-TMA) occurring in 15 of 33 consecutive patients\(^20\).

PH is associated with increased pulmonary vascular resistance and subsequent elevated pulmonary artery pressures\(^21\)-\(^23\), if left untreated, increased pulmonary artery pressure leads to elevated right ventricular (RV) pressure, cardiac failure, and death\(^22,24,25\). The initial symptoms of PH can be vague, and respiratory complications after HSCT are common, making the diagnosis of PH difficult\(^22,26\). Jodele et al. reported a 2.3% incidence of PH with 80% mortality in a retrospective analysis of patients transplanted at our institution\(^27\). All 5 patients who developed PH had histologic evidence of thrombotic microangiopathy in pulmonary arterioles, suggesting that TA-TMA might be involved in pathogenesis of PH after HSCT.
There is no uniform approach for monitoring cardiac complications, since cardiac evaluations, especially early after HSCT, are usually performed when clinically indicated. We conducted a prospective single institution study to screen for PH; LV dysfunction; and PEF in pediatric and young adult patients undergoing HSCT and to examine the utility of scheduled cardiac screening during the first 100 days after HSCT. We hypothesized that scheduled post-transplant echocardiographic screening for cardiac complications would identify patients at risk leading to early interventions and improved outcomes.

METHODS

In January of 2012, uniform screening guidelines were established to monitor cardiac complications after HSCT in pediatric and young adult patients at Cincinnati Children’s Medical Center (CCHMC). All patients undergoing HSCT had echocardiographic evaluation within 30 days prior to starting their HSCT conditioning regimen (baseline echocardiography), on day +7, day +30 and day +100 after HSCT (scheduled screening independent of clinical condition). Patients who were admitted to the pediatric intensive care unit (PICU) for cardiorespiratory failure, TA-TMA, or had signs or symptoms of shock were additionally evaluated with echocardiography on arrival to PICU and then at a 7-14 day interval while receiving intensive care as clinically indicated (symptom based screening). The pediatric cardiology service evaluated patients with PEF or LV dysfunction on echocardiography while the pulmonary hypertension service evaluated those with elevated RV pressures or other signs concerning for PH.

The aim of this analysis was to determine the utility of the scheduled patient screening on day +7, +30 and +100 after HSCT in detecting elevated RV pressure, LV dysfunction, and PEF and predicting adverse outcomes. Our secondary aim was to identify risk factors associated with the development of PEF, elevated RV pressure and LV dysfunction.

Study population

The study population consisted of 100 consecutive children and young adults who received HSCT at Cincinnati Children's Hospital Medical Center (CCHMC) from January 2012 to March 2013. Data was
collected prospectively following institutional review board approval, data collected included patient demographics, echocardiography data, disease and therapy characteristics, transplant complications, and therapy outcomes. Patients receiving allogeneic and autologous HSCT were analyzed separately. Currently accepted clinical criteria were used for diagnosis of acute graft versus host disease (aGVHD), veno-occlusive disease of the liver (VOD), TA-TMA, viremias, and transplant related mortality (TRM). Respiratory failure was diagnosed in patients requiring endotracheal intubation and mechanical ventilation. One year overall survival (OS) was counted from day 0 (stem cell infusion) to 1-year post transplant or death.

**Echocardiography screening protocol**

Each HSCT patient received a comprehensive echocardiographic study at the time points listed above. Echocardiography screening included assessment of left heart function, PEF, and evaluation for PH. PH assessment was performed using the PH protocol as previously described.

A licensed technician performed all echocardiographic studies and pediatric cardiologists reviewed all evaluations. A dedicated pediatric pulmonary hypertension specialist reviewed abnormal PH-specific echocardiograms. Pulmonary artery pressure was estimated from a trans-tricuspid gradient calculated from the maximum velocity of continuous Doppler tricuspid regurgitation, using a modified Bernoulli equation and assuming right central venous pressure of 5 mmHg. PEF was identified from the separation of pericardial layers detected on echocardiography. LV function was evaluated by assessment of the LV ejection fraction and/or shortening fraction. Hemoglobin/hematocrit, oxygen requirement, respiratory failure, antihypertensive and vasopressor medications were documented at the time of each echocardiograph. Patients were categorized as having severe systemic hypertension if they required a continuous antihypertensive infusion and/or 3 or more antihypertensive medications to maintain systolic blood pressure below 95 percentile for age and height. All findings on post-transplant echocardiography were compared to the baseline pre-transplant echocardiograph.
Elevated RV pressure was defined as a RV pressure greater than 35% of the patient’s mean systemic pressure at time of echocardiography. Intraventricular septal flattening was also evaluated in the determination of elevated RV pressure. Echocardiography has a high sensitivity in predicting PH; however, the specificity is low until the estimated RV pressure approaches 50% systemic. Therefore, patients who had a documented increase in RV pressures after HSCT to 35-49% of systemic were classified as “at risk for PH” and those with RV pressures ≥50% of systemic were diagnosed with PH.

Patients were diagnosed as having a PEF if a new or enlarging PEF was found after transplant. PEFs were clinically classified as “small” (no interventions), “moderate to large” (medical interventions required) and “tamponade” (surgical intervention required) per standard guidelines. Cardiac tamponade was diagnosed when echocardiography demonstrated: diastolic collapse of the anterior RV free wall, right atrial collapse, left atrial and/or LV collapse.

LV function was measured by ejection fraction (EF), which represents the volumetric fraction of blood pumped out of the ventricle and fractional shortening (FS), an additional sensitive and specific measurement to assess LV function. Patients found to have an EF of 50% or less and/or a FS level less than two standard deviations below the age adjusted mean were determined to have LV dysfunction.

Patients were identified as having an abnormal echocardiograph at day +7, +30, and +100 if they were found to have at least one of the above mentioned outcome measures: elevated RV pressure, PEF, and/or LV dysfunction.

**Statistical analysis**

Descriptive statistics were reported as medians, interquartile ranges (IQR), and frequencies. Differences in categorical and continuous variables were assessed with the Fisher exact and Wilcoxon rank sum tests, respectively. Associated odds ratios and their corresponding 95% confidence intervals were calculated using the Mantel-Haenszel method. One-year overall survival (OS) comparing patients with abnormal
echocardiography to those without was calculated with Kaplan-Meier with the associated p values calculated by Log-Rank analysis. Cumulative incidence of TA-TMA with death as a competing variable was calculated by Gray’s method. Cumulative incidence was calculated in R . All other data analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago IL).

RESULTS

Patient demographics (Table 1)

We analyzed echocardiographic screening data from 100 consecutive HSCT patients, with separate analyses for patients receiving allogeneic and autologous HSCT. The majority of the study patients were Caucasian with a median age of 5.4 years. Sixty one percent received transplantation for non-malignant disorders, mainly primary immune deficiencies (36%) and bone marrow failure syndromes (20%). Eighty three percent of patients underwent allogeneic HSCT and 86% of the allogeneic grafts were from unrelated donors. Bone marrow was the most common stem cell source, used in 58% of patients. Peripheral blood stem cells (PBSC) were used mainly for autologous stem cell transplantation (n=17) and patients with Fanconi anemia as an ex vivo T-cell depleted graft (n=11). Cord blood was used when a suitable bone marrow donor was not available. The conditioning regimen was myeloablative in 43% and reduced intensity in 57% of patients; 9% of patients received total body radiation.

Allogeneic graft recipients

Echocardiographic screening results in allogeneic HSCT recipients (n=83) (Figure 1)

Day +7: Twenty-eight of 83 (34%) screened patients had abnormal echocardiography at day +7. Of the 17 patients with PEF, one had a moderate to large PEF. Eleven patients (13%) had elevated RV pressure, all being at risk for PH. Five of the eleven patients (45%) with elevated RV pressure at day +7 had an oxygen requirement. Patients with abnormal echocardiography at day +7 had a 64% (18/28) OS (SE +/- 23%) compared to 78% (43/55) OS (SE +/- 11%) in patients with normal echocardiography (log-rank p=0.124). Two of the 3 patients with 2 abnormal findings on day +7 died prior to one year.
Day +30: Thirty-one of 82 (38%) screened patients had abnormal echocardiography at day +30. Three patients who had a small PEF on day +7 progressed to a moderate/large PEF by day +30. These 3 patients, and the patient with a moderate to large PEF at day +7, accounted for all of patients with moderate to large PEF. Nine patients (11%) had elevated RV pressure at day +30, 2 of them were diagnosed with PH (RV pressure >50% systemic). One of the 2 patients had normal day +7 echocardiography and the other had RV=40% systemic that progressed to PH by day +30 evaluation. Both patients had an oxygen requirement and TA-TMA prior to the diagnosis of PH. Overall, patients with abnormal echocardiography at day +30 had a 65% (20/31) OS (SE +/- 17%) compared to 80% (41/51 patients) OS (SE +/- 13%) in patients with normal echocardiography (log-rank p=0.120). Four of the six patients with two abnormal findings (all PEFs and elevated RV pressure) on day +30 echocardiography died prior to one year after HSCT.

Day +100: Twenty-three of 76 (30%) allogeneic patients screened at day +100 had abnormal findings. One patient had a moderate to large PEF that persisted from day +30. Five patients (6%), had elevated RV pressures, none were diagnosed with PH. Five (6%) patients had depressed LV function on day +100, 2 of them had LV dysfunction prior to HSCT. Three patients developed new LV dysfunction during the first 100 days: one was diagnosed at day +30, and the other two on day +100 screening. Four of the five (80%) patients with LV dysfunction during the first +100 days had previously received anthracyclines. Patients with abnormal echocardiography at day +100 had a 65% (15/23) OS (SE +/- 19%) compared to 85% (45/53) OS (SE +/- 8%) in patients with normal echocardiography (log-rank p=0.043).

Cumulative incidence of TA-TMA in patients with elevated RV pressure and PEF on Day +7 after HSCT (Figure 2)

TA-TMA and Day +7 echocardiography: Twenty-eight allogeneic patients (34%) developed TA-TMA at a median of 26 days after HSCT (IQR 16-41 days). All patients were diagnosed with TA-TMA in the first 100 days after HSCT, and all but 2 patients developed TA-TMA after day +7 echocardiography. The cumulative incidence of TA-TMA within 100 days was significantly higher in patients with elevated RV
pressure (figure 2a), and PEF (figure 2b) at day +7 than patients without these findings. Seventy-three percent (8/11) of patients with elevated RV pressure at day +7 developed TA-TMA compared to 28% (20/72) of patients that did not have elevated RV pressure at day +7 (p=0.0013). Fifty three percent (9/17) of patients with PEF at day +7 developed TA-TMA compared to 29% (19/66) in patients that did not have PEF at day +7 (p=0.043).

Autologous graft recipients

Echocardiographic screening results in autologous HSCT recipients (n=17) (Figure 3)

Seventeen patients underwent screening after autologous stem cell transplantation. There were no abnormal findings on pretransplant echocardiography. There were no documented PEFs, one patient (6%) developed PH, and six patients (35%) had LV depression documented on the 100-day screening. Day +7: Two patients (13%) had elevated RV pressure. One of these patients had an estimated RV pressure of 36% with an associated oxygen requirement on day +7. She developed TA-TMA shortly after, and progressed to PH by day +30. Day +30: Two patients (13%) had decreased LV function on day +30 screening. Both patients received anthracyclines prior to HSCT, and continued to have decreased LV function at day +100. Day +100: Six patients (38%) had low LV function; of these, 5 had previous anthracycline exposure. There was no statistical difference in survival for autologous patients with and without abnormal day +7, +30, +100 echocardiography.

Echocardiography results and associated outcomes (Table 2)

Elevated right ventricular pressure

Three patients had elevated RV pressures prior to transplant. None of the three developed PH after HSCT, and all had resolution of their increased RV pressures by day +100. Nineteen patients had newly identified elevated RV pressures and were designated at risk for PH during the +100 day screening period, two others were found to have RV pressure elevation upon PICU admission. The 3 patients that developed PH during the first 100 days were diagnosed with TA-TMA prior to PH diagnosis, and each
had an oxygen requirement at the time of diagnosis. Of note, one patient with normal echocardiography during the first 100 days developed PH after the screening period at day +352. This patient developed bronchiolitis obliterans with secondary PH that was diagnosed in the PICU. No patient underwent cardiac catheterization because of concerns for unforeseen morbidity, and all were treated with PH directed therapy including nitric oxide, bosentan, and/or sildenafil. Two of the four patients with PH died from cardiopulmonary failure.

Our data analysis showed that patients with elevated RV pressure during the screening period were more likely to develop TA-TMA (OR 7.3, 95% CI: 2.5-20.9), have an oxygen requirement in the first 100 days (OR 18.1, 95% CI: 5.3-61.8), and have severe hypertension during the first 100 days (OR 3.0, 95% CI:1.1-8.2) with these associations being statistically significant (Table 2). Transplant related mortality (TRM) was 29% in patients with elevated RV pressures, however, this was not statistically significant.

Pericardial effusion

Thirty-six patients (36%) had a PEF during the first 100 days, and three patients were found to have a previously undetected small PEF upon later PICU admission. Six of the 39 (15%) patients had a small PEF prior to transplant. PEF were found at a median of 30 days (range 7-178 days) after HSCT. Our data analysis showed that patients with PEF during the screening period were more likely to develop TA-TMA (OR 6.0, 95% CI: 2.4-15.0), have an oxygen requirement in the first 100 days (OR 5.3, 95% CI: 2.1-13.1), have severe hypertension during the first 100 days (OR 3.1, 95% CI: 1.3-8.1), have adenovirus viremia (OR 5.6, 95% CI: 1.8-17.4), or have an increased TRM (OR 3.9, 95% CI: 1.2-12.4). These associations were statistically significant and are listed in Table 2. Acute GVHD, engraftment syndrome, and chronic GVHD were not significantly associated with the development of PEF.

Four patients developed moderate to large PEF, and all four received medical intervention and required pericardiocentesis for cardiac tamponade. Two of the 4 patients had aGVHD and 2 were diagnosed with TA-TMA. All patients with a moderate to large PEF survived to 1 year.
Decreased left ventricular pressure

Twelve patients (12%) had low LV function during the first 100 days, and two additional patients with normal screening were found to have low LV function in subsequent echocardiography during the first year. Two of the 12 patients (16.7%) with decreased LV function during the first 100 days had decreased function prior to transplant. In the 12 patients that did not have decreased LV function prior to transplant, decreased LV function was found at a median of 90 days (IQR 45-108 days). Malignancy and autologous stem cell source were significantly associated with the development of decreased LV function likely secondary to anthracycline use. Thirty patients had previous anthracycline exposure prior to HSCT. Anthracycline exposure was significantly associated with the development of LV dysfunction as 9 of the 14 (64%) patients had previous anthracyclines (p=0.005, OR 5.6, CI: 1.7-18.5). No other significant associations were found in patients developing LV dysfunction at time of echocardiography.

DISCUSSION

This is the first study to prospectively investigate the incidence of cardiac complications in pediatric and young adult patients undergoing HSCT. We performed echocardiographic screening in all HSCT patients on day +7, +30 and +100 after HSCT independent of their clinical condition and documented much high incidence of echocardiographic abnormalities affecting post-transplant non-relapse mortality, than previously reported.

More than one third of HSCT patients had at least one echocardiographic abnormality at each screening point that was associated with lower 1-year post transplant survival as compared to patients without detected abnormalities on echocardiograms. PH and moderate to large PEF were noted mainly in allogeneic HSCT recipients early after HSCT (day +7 and +30 screening), while LV dysfunction was documented in both allogeneic and autologous transplant recipients at 100 days post-HSCT, mainly in, but not limited to patients with the history of anthracycline use prior to transplantation.
We documented elevated RV pressure in 21% of patients and a 4% cumulative incidence of PH during the first year after HSCT that was associated with 50% OS. Oxygen requirement was documented in all patients prior to developing PH and 3 of the 4 patients later had respiratory failure requiring intubation and mechanical ventilation. PH targeted treatment was provided to all four patients, but two patients died from respiratory failure. We believe that PH is underdiagnosed in HSCT patients since the echocardiographic finding of RV pressure 35-49% of systemic has sensitivity of 83% and specificity of 72%, likely missing less severe cases of PH [32]. The gold standard diagnostic test for PH – cardiac catheterization- should be considered in certain patients after thorough risk-benefit ration assessment.

An interesting observation in our study was the elevation of RV pressure and subsequent development of TA-TMA and hypertension, 73% of patients with RV pressure elevation on day +7 later developed TA-TMA and hypertension, likely indicating that pulmonary vascular injury occurs very early after transplantation, but it is not evident by currently used TA-TMA diagnostic criteria\textsuperscript{30}. Our data suggest that pulmonary vascular pathology leading to significant post-transplant complications is likely underestimated in HSCT population, especially in children, and may lead to poor outcomes if not treated.

The cumulative incidence of PEF (39%) and a new LV dysfunction (9%) within first 100 days after transplant was also higher than previously reported in the literature\textsuperscript{2,5,13-15,43}. All patients with a moderate and large PEF were diagnosed during screening, and medical or surgical interventions were initiated before patients had clinical deterioration likely affecting overall outcome. Development of PEF was strongly associated with oxygen requirement, severe hypertension, TA-TMA, intubation, and TRM (p=0.023). LV depression mainly occurred in patients with prior anthracycline use.

Our data shows that day+7 and day+30 echocardiography screening identifies a significant number of cardiac abnormalities within first 100 days after HSCT likely amenable to early interventions. TRM in this prospective cohort was possibly affected by prompt interventions offered to patients with PH and clinically significant PEF. A strong association between PH, PEF and TA-TMA should be further explored with the goal to determine common diagnostic biomarkers and potential targeted therapies. Our
group previously noted evidence of PH and PEF in patients with prolonged symptoms of TA-TMA and speculated that untreated thrombotic microangiopathy may affect pulmonary or cardiac vessels resulting in high mortality. Our current prospective screening data suggests that PEF and elevated RV pressure maybe very early indicators of TA-TMA that occurs due to vascular injury from chemotherapy, radiation or donor graft and can be identified by echocardiography prior to the evidence of microangiopathic hemolysis in the blood. Current TA-TMA diagnostic criteria like elevated LDH and schistocytes in peripheral blood may be late markers of vascular injury after HSCT.

**Recommended echocardiographic screening in children and young adults after HSCT (Figure 4)**

In summary, we recommend considering a comprehensive echocardiographic screening including RV pressure estimation in allogeneic transplant recipients on Day +7 and +30 after transplantation, especially in those with oxygen requirement, unexplained respiratory failure, severe hypertension, and/or TA-TMA. Repeat echocardiography should be definitely considered at day +30, in patients with abnormal findings on day +7 screening. Patients with elevated RV pressure, PEF, or LV depression should undergo prompt evaluation by cardiologists and/or pulmonary hypertension specialists. For patients with the history of the anthracycline exposure, echocardiography should be repeated at day +100. Autologous transplant recipients should receive echocardiographic screening at day +100 after HSCT to evaluate for LV depression. Day +30 screening should be reserved for autologous transplant recipients who received anthracyclines for their cancer therapy. A proposed screening algorithm is outlined in Figure 4. Future prospective studies should be dedicated to validate cardiac screening algorithms and to identify novel biomarkers that can aid in early diagnosis of cardiac complication after HSCT and serve as a potential therapeutic targets for PEF, PH and TA-TMA.
Works Cited


Table 1: Demographics of patients who underwent echocardiographic screening on days +7, +30, +100 after HSCT

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<td>Unrelated Donor</td>
<td>14 (67%)</td>
<td>57 (62%)</td>
<td></td>
<td>33 (84%)</td>
<td>38 (62%)</td>
<td></td>
<td>8 (57%)</td>
<td>63 (73%)</td>
<td></td>
</tr>
</tbody>
</table>
Demographics with associated outcomes: elevated RV pressure, PEF, and LV dysfunction. Statistics were done with Wilcoxon Ranked Sums test and Fischer Exact Test. Abbreviations: IQR=interquartile range; PBSC=peripheral blood stem cells; LV=left ventricular; PEF=pericardial effusion; RV=right ventricular
### Table 2: Echocardiography results and associated outcomes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Elevated RV pressure (n=21)</th>
<th>Normal RV pressure (n=79)</th>
<th>p value</th>
<th>PEF (n=39)</th>
<th>No PEF (n=61)</th>
<th>p value</th>
<th>LV Dysfunction (n=14)</th>
<th>No LV Dysfunction (n=86)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aGVHD (n=33)</td>
<td>5 (23%)</td>
<td>28 (35%)</td>
<td>0.435</td>
<td>15 (39%)</td>
<td>18 (30%)</td>
<td>0.389</td>
<td>7 (50%)</td>
<td>26 (30%)</td>
<td>0.219</td>
</tr>
<tr>
<td>cGVHD (n=4)</td>
<td>1 (5%)</td>
<td>3 (4%)</td>
<td>1.000</td>
<td>1 (3%)</td>
<td>3 (5%)</td>
<td>1.000</td>
<td>1 (7%)</td>
<td>0</td>
<td>0.140</td>
</tr>
<tr>
<td>VOD (n=1)</td>
<td>0</td>
<td>1 (1%)</td>
<td>1.000</td>
<td>0</td>
<td>1 (2%)</td>
<td>1.000</td>
<td>0</td>
<td>4 (5%)</td>
<td>1.000</td>
</tr>
<tr>
<td>TA-TMA (n=31)</td>
<td>14 (67%)</td>
<td>17 (21%)</td>
<td>&lt; 0.001</td>
<td>21 (54%)</td>
<td>10 (16%)</td>
<td>&lt; 0.001</td>
<td>2 (14%)</td>
<td>29 (34%)</td>
<td>0.215</td>
</tr>
<tr>
<td>Adenovirus (n=18)</td>
<td>4 (19%)</td>
<td>14 (18%)</td>
<td>1.000</td>
<td>13 (33%)</td>
<td>5 (8%)</td>
<td>0.003</td>
<td>2 (14%)</td>
<td>16 (19%)</td>
<td>1.000</td>
</tr>
<tr>
<td>CMV (n=24)</td>
<td>5 (24%)</td>
<td>19 (24%)</td>
<td>1.000</td>
<td>9 (23%)</td>
<td>15 (25%)</td>
<td>1.000</td>
<td>1 (7%)</td>
<td>23 (27%)</td>
<td>0.177</td>
</tr>
<tr>
<td>EBV (n=40)</td>
<td>9 (43%)</td>
<td>31 (40%)</td>
<td>0.805</td>
<td>15 (39%)</td>
<td>25 (41%)</td>
<td>0.837</td>
<td>5 (36%)</td>
<td>35 (41%)</td>
<td>0.778</td>
</tr>
<tr>
<td>Severe Hypertension (n=25)</td>
<td>9 (43%)</td>
<td>16 (20%)</td>
<td>0.047</td>
<td>15 (39%)</td>
<td>10 (16%)</td>
<td>0.018</td>
<td>3 (21%)</td>
<td>22 (26%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Oxygen during first 100 days (n=32)</td>
<td>17 (81%)</td>
<td>15 (19%)</td>
<td>&lt; 0.001</td>
<td>21 (54%)</td>
<td>11 (18%)</td>
<td>&lt; 0.001</td>
<td>5 (36%)</td>
<td>27 (31%)</td>
<td>0.763</td>
</tr>
<tr>
<td>Respiratory Failure (n=21)</td>
<td>9 (43%)</td>
<td>12 (15%)</td>
<td>0.013</td>
<td>16 (41%)</td>
<td>5 (8%)</td>
<td>&lt; 0.001</td>
<td>4 (29%)</td>
<td>17 (21%)</td>
<td>0.485</td>
</tr>
<tr>
<td>1 year TRM (n=15)</td>
<td>6 (29%)</td>
<td>9 (11%)</td>
<td>0.080</td>
<td>10 (26%)</td>
<td>5 (8%)</td>
<td>0.023</td>
<td>2 (14%)</td>
<td>13 (15%)</td>
<td>1.000</td>
</tr>
<tr>
<td>1 year OS (n=25)</td>
<td>8 (38%)</td>
<td>17 (22%)</td>
<td>0.156</td>
<td>14 (36%)</td>
<td>11 (18%)</td>
<td>0.059</td>
<td>6 (43%)</td>
<td>19 (22%)</td>
<td>0.108</td>
</tr>
</tbody>
</table>

**Table 2 Legend:** Outcome associations: elevated RV pressure, PEF, and LV dysfunction. Statistics were done with Wilcoxon Ranked Sums test and Fischer Exact Test. Abbreviations: aGVHD=acute graft versus host disease; cGVHD=chronic graft versus host disease; LV=left ventricular;
OS=overall survival; PEF=pericardial effusion; RV=right ventricular; VOD=veno-occlusive disease; TA-TMA=transplant associated thrombotic microangiopathy; TRM=transplant related mortality
Figure 1: Echocardiographic screening results in allogeneic HSCT recipients (n=83)
Figure 1 Legend: Data of 83 consecutive allogeneic transplant patients who were screened by echocardiography during first 100 days after HSCT is shown in this figure. Of the 28 patients with abnormal echocardiography on day +7, 11 (40%) had abnormal pre-transplant echocardiography. In the cohort of patients with abnormal day +7 echocardiography, one patient was diagnosed with a moderate to large PEF on day +7, the other 3 were diagnosed with their day +30 echocardiograph. Pulmonary hypertension was diagnosed in one patient with an abnormal day +7 echo (day +26) and in one patient with normal day +7 echocardiography (day +39). Four of the patients with abnormal echocardiography on day +7 died prior to 100 days (day +14, 53, 74, 78), 3 of the 4 were from transplant related causes. Two patients with normal echocardiography on day +7 died of disease progression prior to day +100 (day +74 for both). Abbreviations: HSCT=hematopoietic stem cell transplant; LV=Left ventricle dysfunction; PEF=Pericardial effusion; RV= elevated right ventricular pressure (pulmonary hypertension + at risk); T= 2 or more abnormal findings on echocardiography.
Figure 2: Cumulative incidence of TA-TMA in patients with elevated RV pressure and PEF on Day +7 after HSCT

Figure 2 Legend: Figure shows a cumulative incidence of TA-TMA, with death as a competing risk, in patients with and without abnormal echocardiography on day +7 after HSCT that was calculated by Gray’s method. a) Cumulative incidence of TA-TMA in patients with and without elevated RV pressure on day +7 echocardiography. Eight of the eleven patients (73%) with elevated RV pressure on day +7 after HSCT developed TA-TMA (one patient without TA-TMA died prior to day 100). Twenty of the 72 patients (28%) without elevated RV pressure developed TA-
TMA in the first 100 days (2 patients without TA-TMA died prior to day +100). b) Cumulative incidence of TA-TMA in patients with and without PEF on day +7 echocardiography. Nine of the seventeen patients (53%) with a PEF at day +7 developed TA-TMA (one patient without TA-TMA died prior to day 100). Nineteen of the 66 patients (29%) without a PEF developed TA-TMA in the first 100 days (2 patients without TA-TMA died prior to day +100). Abbreviations: HSCT=hematopoietic stem cell transplant; PEF=Pericardial effusion; RV=right ventricular pressure; TA-TMA=transplant associated thrombotic microangiopathy
Figure 3: Echocardiographic screening results in autologous HSCT recipients (n=17)
**Figure 3 Legend:** Data of 17 consecutive autologous transplant patients who were screened by echocardiography during first 100 days after HSCT is shown in this figure. No patient had abnormal echocardiography prior to transplant. One patient developed pulmonary hypertension at day +30. This patient had elevated RV pressure at day +7 as well as an oxygen requirement. There were no deaths prior to day +100.

**Abbreviations:** HSCT=hematopoietic stem cell transplant; LV=Left ventricle dysfunction; PEF=Pericardial effusion; RV= elevated right ventricular pressure (pulmonary hypertension + at risk); T= 2 or more abnormal findings on echocardiography.
Figure 4: Recommended echocardiographic screening in children and young adults after HSCT

Abbreviations: LV=left ventricular; PEF=pericardial effusion; RV=right ventricular; TA-TMA=transplant associated thrombotic microangiopathy