Eur J Pediatr (2012) 171:1095–1101
DOI 10.1007/s00431-012-1692-x

ORIGINAL ARTICLE

Exhaled nitric oxide and pulmonary complications after paediatric stem cell transplantation

T. Fazekas · P. Eickhoff · A. Lawitschka · B. Knotek · U. Pötschger · C. Peters

Received: 18 October 2011 / Accepted: 2 February 2012 / Published online: 16 February 2012

Abstract Pulmonary complications are major causes of morbidity and mortality after haematopoietic stem cell transplantation (HSCT). We hypothesise that elevated exhaled nitric oxide (FeNO) levels early after HSCT in children are predictive for pulmonary complications. The present prospective study included 30 children (age, 4–18 years) before HSCT. FeNO levels were evaluated 10 days before transplant, at day 0, day +28 and day +60 after HSCT. During the follow-up period until day +100, pulmonary complications and lung function were assessed. Before HSCT, the mean FeNO levels were comparable in children with or without post-transplant pulmonary complications. However, they differed at day 0 and day +28 with a mean of 7 (±1.95) and 13 (±3.44) ppb at day 0 and a mean of 13 (±3.44) and 14 (±3.57) ppb at day +28, respectively. Conclusion: Children with pulmonary complications after day +28 have higher mean FeNO levels 28 days after HSCT than children without later pulmonary complications. Therefore, FeNO could be an important diagnostic tool for hyperinflammatory response in bronchial epithelium after paediatric HSCT.

T. Fazekas (✉) · P. Eickhoff · A. Lawitschka · B. Knotek · C. Peters
St. Anna Children's Hospital,
Kinderspitalgasse 6,
1090 Vienna, Austria
e-mail: tamas.fazekas@stanna.at

U. Pötschger
Children's Cancer Research Institute,
St. Anna Children's Hospital,
Vienna, Austria

Keywords Bone marrow transplantation · Exhaled nitric oxide · Graft versus host disease · Hematopoietic stem cell transplantation

Abbreviations

ADV	Adenovirus
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ARDS	Acute respiratory distress syndrome
ATG	Antithymocyte globulin
BMI	Body mass index
BMT	Bone marrow transplantation
BOOP	Bronchiolitis obliterans with organising pneumonia
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
CSA	Cyclosporine A
CT	Computed tomography
DLCO	Diffusion capacity of the lung for carbon monoxide
EBV	Epstein–Barr virus
EONIPC	Early-onset non-infectious pulmonary complications
FeNO	Fraction of exhaled nitric oxide
FEV	Forced expiratory volume
FVC	Forced vital capacity
GvHD	Graft versus host disease
HLA	Human leukocyte antigen
HSCT	Haematopoietic stem cell transplantation
HSV	Herpes simplex virus
IgE	Immunoglobulin E
iNOS	Inducible nitric oxide synthase
IPS	Idiopathic pneumonia syndrome

ITGV	Intrathoracal gas volume
MMF	Mycophenolate mofetil
MTX	Methotrexate
MSD	Matched sibling donor
MUD	Matched unrelated donor
PBSCT	Peripheral blood stem cell transplantation
PERDS	Peri-engraftment respiratory distress syndrome
PFT	Pulmonary function testing
ppb	Parts per billion
PC	Pulmonary complication
RIC	Reduced intensity conditioning
RSV	Respiratory syncytial virus
TBI	Total body irradiation
TLC	Total lung capacity
VC	Vital capacity

Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) is an important treatment option for several malignant and non-malignant diseases in childhood. However, 40% to 60% of the patients have pulmonary complications after HSCT, which contributes for a significant percentage to transplant-related mortality [9, 25, 27]. These complications are infectious or non-infectious and are classified as early or late depending on whether they occur before or after day 100 after transplantation [12]. Several risk factors for infectious complications including neutropenia and myeloablative therapy [8] have been published, while non-myeloablative conditioning regimen and graft source may predispose for non-infectious pulmonary complications such as pulmonary graft versus host disease (GvHD). Yet, reliable predisposing risk factors have not been established, partly due to insufficient understanding of the exact underlying pathophysiologic mechanisms of these diseases [1, 12]. The most common early-onset non-infectious pulmonary complications include peri-engraftment respiratory distress syndrome (PERDS), idiopathic pneumonia syndrome (IPS) and bronchiolitis obliterans with organising pneumonia (BOOP) [12, 30]. Therefore, measurement of the degree of bronchial inflammation is a potential key for early detection of HSCT-related pulmonary pathology. During inflammatory processes in the lungs, inducible nitric oxide synthase (iNOS) is known to be upregulated, leading physiologically to production of nitric oxide (NO) supporting a non-specific defence against micro-organisms. Furthermore, nitric oxide seems to be involved in airway and vascular calibre regulation, finally leading to bronchodilation [14]. Kanamori et al. [15] have shown that fraction of exhaled nitric oxide (FeNO) is elevated in adults with bronchiolitis obliterans after HSCT. We therefore hypothesised that FeNO may be a sensitive marker for early alloimmunologic pulmonary complications also in children and adolescents, but prospective data have not been published yet to support this hypothesis.

Patients and methods

Patients

In this first prospective study, we assessed the prevalence and underlying risk factors of pulmonary complications in paediatric patients after allogeneic bone marrow transplantation or allogeneic peripheral blood stem cell transplantation for malignant or non-malignant diseases at St. Anna Children's Hospital between 1 October 2007 and 30 November 2009. Inclusion criteria were age above 3 years and below 18 years, indication for allogeneic stem cell transplantation and signed informed consent by patients and/or legal guardians. Patients were excluded if they had severe respiratory distress and if they were unable to cooperate or did not comply with the age requirements. We prospectively included 30 consecutive children after allogeneic HSCT: age, 3–17 years (mean 12.79 (±5.2) years); mean BMI, 20.3; 11 female. All these patients had performed a total of four FeNO measurements pre- and post-HSCT. For the underlying diseases and baseline patient characteristics see Table 1; the respective subgroups are displayed in the flow chart of Fig. 1. For all these patients, medical records, spirometric data and radiologic examinations were reviewed.

The study has been approved by the local IRB Ethikkommission St. Anna Kinderspital (project approval number 190109), and has been conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Prior to any study-related screening procedures being performed on patients, informed consent was obtained from the patients or their legal guardians.

Transplantation details

A total of 23 (77%) patients underwent myeloablative conditioning. Eighteen patients received total body irradiation of 12 Gy. Six patients received busulfan 16 mg/kg plus either cyclophosphamide 120 mg/kg or etoposide 60 mg/kg. Children with matched unrelated donor (MUD) received a conditioning regimen containing antithymocyte globulin (ATG) as in vivo T cell depletion. Seven patients underwent reduced intensity conditioning which consisted of fludarabine (180 mg/m^2) and melphalan (140 mg/m^2), plus ATG in most cases. Three of these children received additional thiotepa (10 mg/kg). Twenty-eight children received bone marrow with a mean of 9.2×10^6 CD34+ cells/kg and 38.4×10^6 CD3+ cells/kg body weight. Two children were transplanted with peripheral blood stem cells with a mean of 13.8×10^6 CD34+ cells/kg and 47×10^7

Table 1 Baseline characteristics of patients undergoing HSCT

Patients:	
n	30
Mean age:	
Years	11.94
Range	4.36–18.33
Gender:	
Male	19
Female	11
Diagnosis:	
ALL (acute lymphoblastic leukaemia)	17
AML (acute myeloid leukaemia)	3
CML (chronic myeloid leukaemia)	2
Aplastic anaemia	1
β-Thalassemia	1
Biphenotypic leukaemia	2
Ewing's sarcoma	1
Fanconi's anaemia	1
Myelodysplastic syndrome	1
Congenital neutropenia	1
Stem cell source:	
MSD (matched sibling donor)	14
MUD (matched unrelated donor)	16
Conditioning:	
Myeloablative	23
RIC (reduced intensity conditioning)	7
Atopy:	
Atopic	1
Non-atopic	29

CD3+ cells/kg body weight (BW). One child received cord blood stem cells, with 0.09×10^6 CD34+ cells/kg BW. Sixteen patients were transplanted from matched unrelated donors; 14 had matched sibling donors. None of our patients underwent HLA-mismatch transplantation.

Pulmonary follow-up

Prior to transplantation, all children underwent routine clinical and laboratory examination, chest X-ray and lung function testing, if possible. During the first 4 weeks after HSCT, daily clinical and laboratory examinations, within the first 3 months weekly, and then monthly examinations were performed until 1 year post-transplant. Additional PCR screening for cytomegalovirus (CMV), EBV, ADV, RSV and influenza was performed in case of respiratory symptoms or fever. Additional chest X-rays or high-resolution CT scan was done in case of persisting respiratory symptoms with unknown aetiology. Active or passive tobacco smoke exposure was not recorded systematically.

Pulmonary infections were defined according to the diagnostic criteria following the guidelines of the European Organization for Research and Treatment of Cancer/Mycoses Study Group, distinguishing between proven (by culture), probable (typical CT signs) and possible (criteria for a host factor and a clinical criterion but without microbial criteria) infections. Infectious pulmonary complications were defined as pulmonary infection with clinical signs of tachydyspnea, crackles and radiologic infiltrates. Non-infectious pulmonary complications were defined according to clinical, spirometric and radiologic findings as described previously [12].

FeNO measurements

Exhaled nitric oxide was measured with the portable NIOX MINO® device (Aerocrine AB, Sweden), following published recommendations [2–4, 6, 16]. After inhalation of NO-free air, children exhaled from total lung capacity with a flow rate of 50 ml/s against a flow resistance created by the device in order to close the soft palate to avoid any nasal NO contamination. Repeated exhalations (three that agree within 10% or two within 5%) were performed with at least 30-s intervals and mean NO was recorded [6]. FeNO was

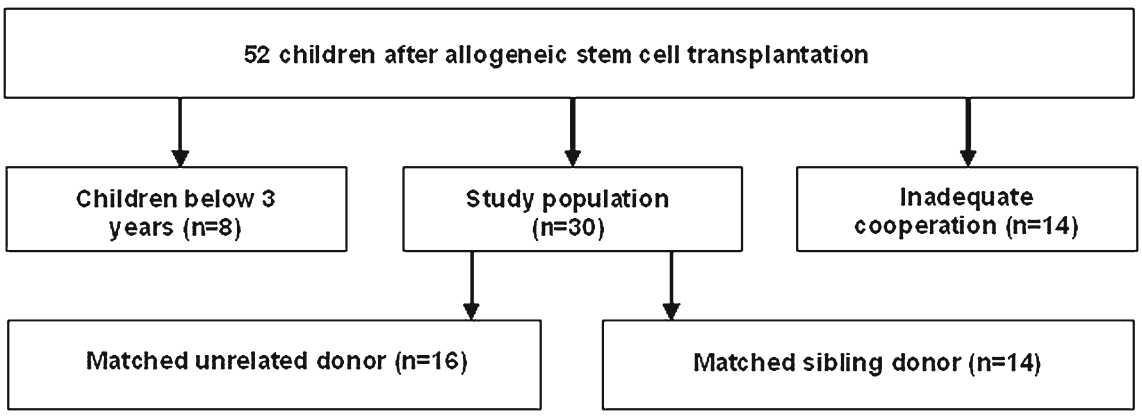

Fig. 1 Subgroups of the study population

measured 10 days before HSCT, at day 0, day +28 and day +60 after HSCT. FeNO measurements were always performed before spirometry or physical activity.

Spirometry

Whole body plethysmography was routinely performed for detection of underlying ventilatory abnormalities and for assessment of baseline lung function for comparison with post-transplant pulmonary function. Parameters were expressed as a percentage of predicted values in each patient, to make lung function variables comparable. Obstructive lung disease was defined as decreased forced expiratory volume in the first second (FEV1) less than 80% or the ratio of forced expiratory volume in 1 s and forced vital capacity (FVC) less than 70%, i.e. FEV1/FVC less than 70. Restrictive lung disease was defined as total lung capacity (TLC) less than 80% of the predicted value. Pulmonary function testings (PFTs) were routinely done before HSCT and 100 days after transplant, performed according to the American Thoracic Society Guidelines [4, 22]. The following parameters were measured: FEV1, FVC, FEV1/FVC ratio, ITGV, TLC and VC. Diffusion capacity of the lung for carbon monoxide was not performed routinely in our study population.

Graft versus host disease

GvHD was diagnosed and graded according to international criteria [11, 23]. First-line GvHD treatment included prednisone 2 mg/kg/day, non-responders were treated either with FK506 instead of CSA or MMF as add-on. Extensive chronic skin GvHD was additionally treated with phototherapy or extracorporeal photopheresis.

Statistics

Results were expressed as mean (range). Wilcoxon two-sample test was used to compare FeNO levels, FEV1% and FVC%, for patients with and without history of infectious or non-infectious complications or GvHD. p values less than 0.05 were considered as statistically significant. Calculations were performed using SPSS/PC+13.0 (SPSS Inc., Chicago, IL, USA).

Results

Pulmonary complications

Within the first 100 days after HSCT, pulmonary complications have been observed in 25 of 30 study patients (83%). Demographic data of patients with or without pulmonary complications are shown in Table 2. Before day +100 after HSCT, infectious pulmonary complications occurred in 21 patients (70%), including viral pneumonia in 8 cases, bacterial pneumonia in 11 cases and one case of pulmonary aspergillosis. Non-infectious complications were observed in four patients, including PERDS in one case and IPS in three cases. None of the children suffered from acute respiratory distress syndrome or needed mechanical ventilation or died during the study period. Children undergoing MUD and MSD transplantation received a comparable dose of systemic steroids. Children with a matched unrelated donor and later pulmonary complication (PC) received a mean cumulative steroid dose of 188 mg/kg (without PC, 33 mg/kg) prednisone, children with a matched sibling donor and later PC received 117 mg/kg (without PC, 95 mg/kg) prednisone. Children with PC after day 60 had a cumulative mean of 152 mg/kg steroids; those without early PC had 114 mg/kg.

Table 2 Demographics of patients with or without pulmonary complications

	No PC	PC until day +28	PC after day +60
n	5	21	4
Age (mean)			
Female	3	6	2
MAC	6	10	7
RIC	2	3	2
FeNO −10 (ppb mean ± SD)	9±3.03	8±0.97	10±1.83
FeNO 0 (ppb mean ± SD)	7±1.95	8±0.98	13±3.44
FeNO +28 (ppb mean ± SD)	8±1.33	10±2.09	14±3.57
FeNO +60 (ppb mean ± SD)	9±2.16	10±2.01	10±2.69

Pulmonary function testing, radiology and risk factors

Whole body plethysmography was performed pre-HSCT in 28 of 30 children in the study population, showing clinically relevant pathologies with an FEV1 of less than 80% of the predicted value in nine children (30%), mainly due to a history of severe pulmonary infections in the past. One hundred days after HSCT, 20 children were able to perform lung function testing, leading to pathologic results in nine cases (45%), with five children showing a restrictive pattern. No correlation could be found between lung function parameters and FeNO levels before or after HSCT in our study cohort. Overall, 11 children had pathologic chest radiographs before HSCT, predominantly revealing pulmonary infiltrates. After HSCT, 23 of 30 children had pathologic chest radiographs at any time point. Causes of radiologic pulmonary infiltrates were predominantly infections (21/23), while two of the pathologies were non-infectious of origin. As risk

factors increased generally for transplant-related mortality [10, 20], 23 of 30 children (77%) received myeloablative conditioning, 6 of 20 children (20%) received busulfan during conditioning, 16 patients (53%) had a matched unrelated donor and 19 children (63%) underwent total body irradiation with 12 Gy. Twenty-five subjects (83%) had a malignant underlying disease, 7 patients (23%) had one mismatch in their graft, 1 had two mismatches. Increased risk for CMV infection was assumed if the recipient was CMV IgG positive but the donor CMV IgG negative, which could be observed in seven children (23%).

Graft versus host disease

Eighteen children had a classic acute skin GvHD after HSCT, additionally with three cases with gut involvement, and two with liver GvHD. Classic chronic GvHD occurred in five children; none of whom with an overlap syndrome as described by the NIH criteria [11].

FeNO levels before and after HSCT

In children after HSCT, FeNO showed no significant correlation with lung function parameters, age, gender, myeloablative conditioning, conditioning with busulfan, CMV risk profile, graft source and number of HLA mismatches and underlying disease or acute GvHD. Conditioning with busulfan showed no significant correlation with pulmonary complications.

Children with matched sibling donors had significantly higher (mean 12.5 ppb) FeNO levels at day +28 (p=0.005) compared to MUD at day +28 (5.5 ppb), but with comparable levels at day 0 (p=0.454) and day +60 (p=0.064).

Children at higher risk for CMV infections showed no significantly elevated FeNO results (p=0.054). Finally, there was a significant correlation between FeNO levels before and immunoglobulin E (IgE) levels 100 days after HSCT (p=0.019), whereas no significance could be shown for the other time-points (p=0.378). None of our children had a history of atopy or elevated IgE levels before HSCT.

FeNO and pulmonary complications

Figure 2 shows an overview over all FeNO measurements and their respective results at four time-points, whereas Fig. 3 compares FeNO levels of children with or without pulmonary complications until day +100. Children with any kind of pulmonary complications until day 100 after HSCT had higher FeNO levels at days 0 (mean, 13 ppb; ±3.44) and +28 (mean, 14 ppb; ±3.57) than children without early respiratory pathology, at days 0 (mean, 7 ppb; ±1.95) and +28 (mean, 8 ppb, ±1.33), respectively (Table 2 shows the mean FeNO levels for all subgroups). No significant correlation between pulmonary infections after HSCT and FeNO levels at day 0 (p=0.389), day +28 (p=0.471) and day +60 (p=0.746) could be shown. A subanalysis of 17 children with acute lymphoblastic leukaemia (eight patients with MUD, nine patients with MSD) was performed. All children with acute lymphoblastic leukaemia (ALL) received myeloablative conditioning; 13 suffered from pulmonary complications after HSCT. FeNO levels in this subcohort were higher at day +28 (mean, 13 ppb; ±1.55) in patients with pulmonary complications after day +60 compared with children without early pulmonary pathology (mean, 7 ppb; ±1.96); FeNO levels were comparable in both groups at day −10, day 0 and day +60, respectively.

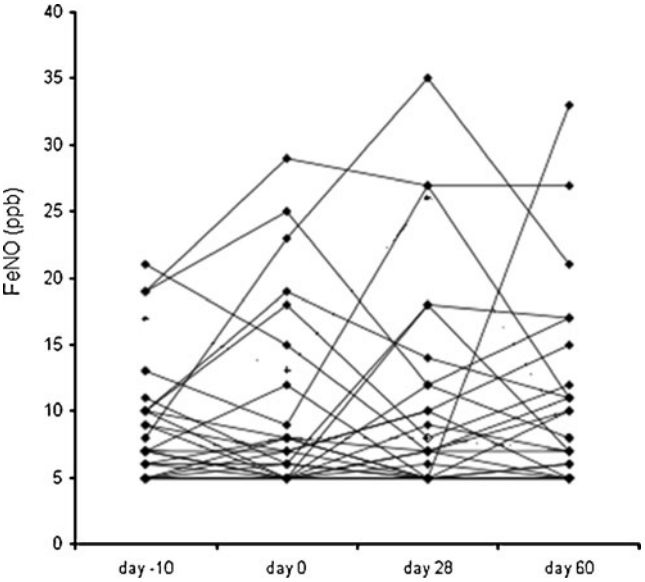

Fig. 2 FeNO levels in the study population before and after HSCT

Discussion

For the assessment of the individual risk for developing pulmonary complications, measurement of nitric oxide in exhaled

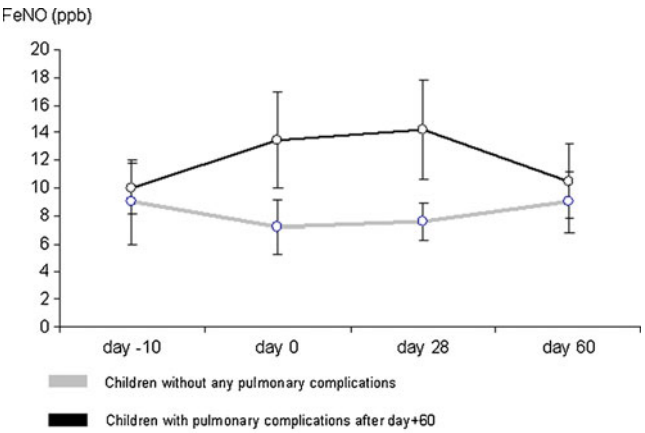

Fig. 3 Mean FeNO levels before and after HSCT in children with and without pulmonary complications

air (FeNO) has been proposed amongst various other factors in adults. Kanamori and co-workers [15] have published a case series of adults with BOOP after HSCT with FeNO levels above 36 ppb, suggesting that elevated FeNO may be predictive for pulmonary complications after HSCT. In a recent study with adult patients after lung transplantation, FeNO has been shown to be a valuable tool for risk stratification for bronchiolitis obliterans syndrome [21]. In contrast to immunocompetent children [7, 19], no data exist on normal FeNO levels in immunosuppressed children or on the predictive value of elevated FeNO for pulmonary complications after paediatric allogeneic HSCT. FeNO levels were generally lower in our cohort than in the adult study by Kanamori or in adult patients following autologous HSCT after breast carcinoma [24], reflecting the fact that norm values in immunocompetent children are also lower than in adults. Notably, FeNO levels did not correlate significantly with pulmonary function parameters such as FEV1, FVC or TLC. This finding may be due to the fact that the role of PFT as a predictive factor for pulmonary complications after HSCT is discussed controversially [30] and may be rather a secondary parameter for respiratory impairment.

A possible role for bronchial dysfunction after HSCT may have myeloablative conditioning and the wide use of systemic corticosteroids in our population, which is known to reduce FeNO levels significantly [26]. Immunologic inactivation of iNOS may therefore be responsible for the missing correlation of FeNO with pulmonary infections in our immunosuppressed study population. We hypothesised that inactivation of NO production could be reversed by alloimmunologic mechanisms as described for non-infectious pulmonary complications after HSCT [13, 17, 25, 28, 29]. We could show that children with pulmonary complications after day +60 had a trend towards higher FeNO levels at day 0 and day +28 than children without PC (in a subcohort of ALL patients only at day +28), without reaching statistical significance. These data suggest a possible role of FeNO as an easily accessible, non-invasive marker preceding PC in children. Therefore, we suggest measuring FeNO at least at day +28 for assessment of the individual risk for PC and for early detection of pulmonary pathology after HSCT in childhood.

FeNO levels did not show any significant correlation with acute GvHD in our cohort. In contrast, other studies [18, 27] have shown a significant correlation of FeNO levels with acute GvHD, with a supposedly immunologic underlying mechanism. However, considering our observation that elevated FeNO levels precede early PC rather than acute GvHD, it is still possible that FeNO would be predictive for chronic pulmonary GvHD, which has to be proven in further studies. Of interest, children with matched sibling donors had higher FeNO levels at day +28 than children with matched unrelated donors, which may be attributed to different GvHD prophylaxis. However, therapy with corticosteroids surprisingly did not influence the findings of our study. One could expect that children without pulmonary complications had lower FeNO levels simply because they received a higher cumulative dose of systemic steroids. In contrast, children without any PC and low FeNO levels received lower cumulative doses of steroids (114 mg/kg prednisone) until day +60 than children with PC (152 mg/kg prednisone) after day +60 and high FeNO levels.

Finally, we observed a positive correlation of elevated FeNO and total serum IgE, which may reflect a systemic alloimmunologic pro-inflammatory upregulation leading to stimulation of B cells and iNOS [5] equally. The primary aim of this study was to describe the patterns of FeNO levels in children after HSCT. We expected a trend towards a higher prevalence of pulmonary complications in children with higher FeNO and found some suggestive data at day 0 and day +28. Compared with the reference values for non-transplant children, FeNO levels were generally lower in our immunosuppressed population, mainly due to high doses of systemic corticosteroids in the course of HSCT. However, our data show that even with severe immunosuppression, the bronchial epithelium reacts to injury by elevated FeNO levels, as an early marker for the start of respiratory pathology.

In conclusion, elevated FeNO levels after HSCT in children seem to precede early pulmonary complications. The clinical relevance of our observations has to be fostered by follow-up of our study group and by multi-centre studies measuring FeNO in children after HSCT.

What is already known on this topic:

- FeNO is elevated in adults with pulmonary complications after HSCT,
- which have a high mortality also in childhood.

What this study adds:

- FeNO is elevated in children with pulmonary complications after HSCT,
- therefore representing a novel and important diagnostic tool for this population.

Conflict of interest/disclosure The authors declare no conflict of interest. No honorarium, grant or other form of payment was given to anyone to produce the manuscript.

References

1. Afessa B, Litzow MR, Tefferi A (2001) Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. Bone Marrow Transplant 28(5):425–434
2. American Thoracic Society (1999) Recommendations for standardized procedures for the online and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. Am J Respir Crit Care Med 160:2104–2117

3. American Thoracic Society (2005) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. Am J Respir Crit Care Med 171:912–930
4. American Thoracic Society Workshop Report (2006) ATS workshop proceedings: exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate: executive summary. Am J Respir Crit Care Med 173:811–813
5. Arany I, Brysk MM, Brysk H, Tyring SK (1996) Induction of iNOS mRNA by interferon gamma in epithelial cells is associated with growth arrest and differentiation. Cancer Lett 110:93–96
6. Baraldi E, Jongste JC (2002) Measurement of exhaled nitric oxide in children, 2001. Eur Respir J 20:223–237
7. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Pijnenburg MW, Silkoff PE, Bisgaard H (2005) Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. J Allergy Clin Immunol 115:1130–1136
8. Collaco MJ, Gower AW, Mogayzel P Jr (2007) Pulmonary dysfunction in pediatric hematopoietic stell cell transplant patients: overview, diagnostic considerations and infectious complications. Pediatr Blood Canc 49:117–126
9. Cooke KR, Yanik G (2004) Acute lung injury after allogeneic stem cell transplantation: is the lung a target of acute graft-versus-host disease? Bone Marrow Transplant 34:753–765
10. Copelan EA, Penza SL, Theil KS, Elder PJ, Bechtel TP, Tighe MB, Ezzone SA, Scholl MD, Belt PS, Young DC, Avalos BR (2000) The influence of early transplantation, age, GVHD prevention regimen, and other factors on outcome of allogeneic transplantation for CML following BuCy. Bone Marrow Transplant 26 (10):1037–1043
11. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J, Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers ME (2005) National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 11(12):945–956
12. Gower AW, Collaco JM, Mogayzel P Jr (2007) Pulmonary dysfunction in pediatric hematopoietic stem cell transplant patients: non-infectious and long-term complications. Pediatr Blood Canc 49(3):225–233
13. Haddad IY, Panoskaltsis-Mortari A, Ingbar DH, Yang S, Milla CE, Blazar BR (1999) High levels of peroxynitrite are generated in the lungs of irradiated mice given cyclophosphamide and allogeneic T cells: a potential mechanism of injury after marrow transplantation. Am J Respir Cell Mol Biol 20:1125–1135
14. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G (1987) Endothelium derived relaxing factor produced and released from artery and vein in nitric oxide. Proc Natl Acad Sci U S A 84:9265–9269
15. Kanamori H, Fujisawa S, Tsuburai T, Yamaji S, Tomita N, Fujimaki K, Miyashita A, Suzuki S, Ishigatsubo Y (2002) Increased exhaled nitric oxide in bronchiolitis obliterans organizing pneumonia after allogeneic bone marrow transplantation. Transplantation 74:1356–1358
16. Kharitonov S, Alving K, Barnes PJ (1997) Exhaled and nasal nitric oxide measurements: recommendations. Eur Respir J 10:1683–1693
17. Khurshid I, Anderson LC (2002) Non-infectious pulmonary complications after bone marrow transplantation. Postgrad Med J 78:257–262
18. Langrehr JM, Murase N, Markus PM, Cai X, Neuhaus P, Schraut W, Simmons RL, Hoffman RA (1992) Nitric oxide production in host-versus-graft and graft-versus-host reaction in the rat. J Clin Invest 90:679–683
19. Malmberg LP, Peatays T, Haahtela T, Laatikainen T, Jousilathi P, Vartiainen E, Mäkelä MJ (2006) Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values. Pediatr Pulmonol 41:635–642
20. Matthes-Martin S, Pötschger U, Bergmann K, Frommlet F, Brannath W, Bauer P, Klingebiel T (2008) Risk-adjusted outcome measurement in pediatric allogeneic stem cell transplantation. Biol Blood Marrow Transplant 14(3):335–343
21. Neurohr C, Huppmann P, Leuschner S, von Wulffen W, Meis T, Leuchte H, Ihle F, Zimmermann G, Baezner C, Hatz R, Winter H, Frey L, Ueberfuhr P, Bittmann I, Behr J, Munich Lung Transplant Group (2011) Usefulness of exhaled nitric oxide to guide risk stratification for bronchiolitis obliterans syndrome after lung transplantation. Am J Transplant 11:129–137
22. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J (2005) Interpretative strategies for lung function tests. ATS/ERS task force: standardisation of lung function testing. Eur Respir J 26:948–968
23. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED (1995) 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant 15:825–828
24. Qureshi MA, Girgis RE, Dandapantula HK, Abrams J, Soubani AO (2004) Increased exhaled nitric oxide following autologous peripheral hematopoietic stem-cell transplantation: a potential marker of idiopathic pneumonia syndrome. Chest 125:281–287
25. Sakaida E, Nakaseko C, Harima A, Yokota A, Cho R, Saito Y, Nishimura M (2003) Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. Blood 102(12):4236–4242
26. Smith AD, Cowan JO, Brassett KP, Filsell S, McLachlan C, Monti-Sheehan G, Peter Herbison G, Robin Taylor D (2005) Exhaled nitric oxide: a predictor of steroid response. Am J Respir Crit Care Med 172(4):453–459
27. Soubani AO, Miller KB, Hassoun PM (1996) Pulmonary complications of bone marrow transplantation. Chest 109(4):1066–1077
28. Vora A, Monaghan J, Nuttall P, Crowther D (1997) Cytokine-mediated nitric oxide release: a common cytotoxic pathway in host-versus-graft and graft-versus-host reactions? Bone Marrow Transplant 20(5):385–389
29. Weiss G, Schwaighofer H, Herold M, Nachbaur D, Wachter H, Niederwieser D, Werner ER (1995) Nitric oxide formation as predictive parameter for acute graft-versus host-disease after human allogeneic bone marrow transplantation. Transplantation 60:1239–1244
30. Wieringa J, van Kralingen KW, Sont JK, Bresters D (2005) Pulmonary function impairment in children following hematopoietic stem cell transplantation. Pediatr Blood Canc 45(3):318–323