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ORIGINAL ARTICLE

Non-atopic IgE and eosinophil cationic protein after allogeneic hematopoietic stem cell transplantation in children

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Abstract Allogeneic hematopoietic stem cell transplantation (HSCT) in childhood is associated with severe pulmonary complications, but the pathophysiologic mechanisms remain unclear. Our aim was to evaluate the association of total and specific IgE, eosinophil cationic protein (ECP) and eosinophilia in HSCT recipients with pulmonary complications. We prospectively measured total and specific serum IgE, eosinophils, and ECP before and 28, 100, and 180 days after HSCT. We included 30 children (age 2–17 years) undergoing HSCT. Nine patients had a history of previous atopy without being associated with pulmonary complications after HSCT until day +360. Specific IgE levels showed a decline after HSCT, associated with the absence of allergy symptoms, suggesting a reduction of atopy. Elevated total serum IgE levels occurred in seven patients on day +28 after HSCT. This elevation did not coincide with allergy symptoms. ECP showed no correlation with total allergy symptoms, eosinophilia, IgE levels, or pulmonary complications. There was a significant correlation (p=0.0367) between ECP levels on day +28 and concurrent acute graft-versus-host disease (GvHD). Non-atopic serum ECP and IgE levels are elevated on day +28 after HSCT in children, with ECP showing a potential relation to acute GvHD.

Keywords Immunoglobulin E · Eosinophil cationic protein · Pediatric stem cell transplantation · Atopy · Asthma · Graft versus host disease

Abbreviations

ADV	Adenovirus
ALL	Acute lymphoblastic leukemia
AML	Acute myeloic leukemia
ATG	Antithymocyte globulin
BMT	Bone marrow transplantation
BOOP	Bronchiolitis obliterans with organizing pneumonia
CML	Chronic myeloic leukemia
CMV	Cytomegalovirus
CSA	Cyclosporine A
CT	Computed tomography
ECP	Eosinophil cationic protein
FEV	Forced expiratory volume
FVC	Forced vital capacity
GvHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
IgE	Immunoglobulin E
IPS	Idiopathic pneumonia syndrome

Conception and design TF, NP, AL; Analysis and interpretation: UP, PE; Drafting the manuscript for important intellectual content: MGS, ZS, HG, CP

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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
PC	Pulmonary complication
RIC	Reduced intensity conditioning
TBI	Total body irradiation
TLC	Total lung capacity
VC	Vital capacity

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has emerged over the past decades as an important treatment option for several malignant and non-malignant diseases in childhood and adolescence. However, conditioning regimens are potentially limited by severe side effects contributing to significant morbidity and mortality rates. Of the patients, 40% to 60% experience infectious or non-infectious pulmonary complications, accounting for a significant percentage (24%) of transplant related deaths [27] during the first 3 months after transplantation [17]. Depending on whether they occur before or after day 100 posttransplantation, these complications are classified as early or late [13]. Historically, about 50% of all pneumonias observed after HSCT have been attributed to bacterial, viral, or fungal infections. Risk factors for bacterial pneumonia include neutropenia and myeloablative therapy [8]. Non-infectious pulmonary complications such as bronchiolitis obliterans (BO) develop in 30–60% of HSCT patients [2, 23]. Conditioning regimen and graft type has been described to be contributing factors. Yet, the exact underlying pathophysiologic mechanisms of these diseases remain unclear, which also makes appropriate treatment rather difficult [2, 13]. However, an association between late-onset non-infectious pulmonary complications (LONIPC) and chronic GvHD have been assumed [23]. Unfortunately, non-infectious lung pathology often responds poorly to standard therapeutic approaches [9], hence identification of predisposing risk factors is crucial for the development of preventive strategies [17].

Previous studies have shown that eosinophil granulocytes and serum IgE are elevated after HSCT in adults. Although elevated eosinophil counts have been reported in adults with chronic GvHD, no correlation with outcome could be shown [4]. However, in adults with acute GvHD after HSCT, elevated eosinophils were associated with a better overall survival [19]. Frankovich et al. [12] have reported an association of asthma and atopic history and non-infectious pulmonary complications after autologous HSCT in pediatric Hodgkin patients [11]. However, no prospective studies in a pediatric cohort of allogeneic HSCT patients have been published as yet. The development of asthma after allogeneic hematopoietic stem cell transplantation has been observed and described as a plausible phenomenon of transfer from donor to recipient [3, 15, 26]. Adjacent to the different cellular phenotypes, different clinical types of asthma are known, including non-atopic asthma. Some authors even call for regarding asthma as a syndrome rather than a disease because of heterogeneity in pathophysiologic mechanisms, clinical symptoms, and response to treatment [5].

In cases of sibling donor grafts, we evaluated donors' atopic history within the interview before transplant. After solid organ transplantation in children, it has been observed that Th2 specific immunologic response is preserved [10], hence Th2 cell related clinical reactions can be observed even in the presence of severe immunosuppression. Upon activation, eosinophilic granulocytes release cytotoxic cellular contents, such as eosinophil cationic protein (ECP), which has been shown to be a reliable marker for exacerbations in asthmatic patients and to be useful in follow-up of steroid treatment and assessment of asthma severity [20]. Yet, it is not very helpful in diagnosing asthma and its role in other allergic diseases such as rhinitis and atopic dermatitis still remains controversial [7, 18, 21]. ECP levels are not only increased in allergic conditions but also during viral infections and in patients with nasal polyps [22]. However, Koh et al. [20] propose that assessing ECP levels of asthmatic patients is limited to eosinophil patterns and subsequently atopic forms of disease.

We hypothesized that monitoring serum ECP and IgE levels may have a clinical value for early diagnosis of pulmonary complications after HSCT in childhood.

Patients and methods

Patients

We prospectively assessed the prevalence and underlying co-factors of pulmonary complications in 30 consecutive pediatric patients, who received either allogeneic bone marrow transplantation (BMT) or allogeneic peripheral blood stem cell transplantation (PBSCT) for malignant or non-malignant diseases at St. Anna Children's Hospital from February 2008 until March 2009. Patients were treated according to clinical protocols approved by the local investigation review board after informed consent was obtained from the patients, the patient's parents, or legal guardians. Medical records, spirometric data, and radiologic examinations were reviewed for all these patients. Patient characteristics are summarized in Table 1.

Table 1 Patient baseline characteristics

Patients (*n*)	30
Median age (years)	10, 54
Recipient sex:	
Male	20
Female	10
Diagnosis:	
ALL (acute lymphoblastic leukemia)	16
AML (acute myeloic leukomia)	3
HLH (hemophagocytic lymphohistiocytosis)	1
SCD (sickle cell disease)	1
SAA (severe aplastic anemia)	1
Fanconi anemia	1
β-thalassemia	2
SCID (severe combined immunodeficiency)	2
CML (chronic myelogenous leukemia)	1
MDS (myelodysplatic syndrome)	2
Stem cell donors:	
MSD (matched sibling donor)	12
MUD (matched unrelated donor)	18
Conditioning:	
Myeloablative	18
RIC (reduced intensity conditioning)	12
Atopy:	
Atopic	9
Non-atopic	21

Conditioning regimen

Eighteen patients underwent myeloablative conditioning. Sixteen patients received total body irradiation (TBI) of 12 Gy. TBI was hyperfractionated, and lung shielding was performed at 10 Gy. Two patients received busulfan 16 mg/kg plus either cyclophosphamide 120 mg/kg or etoposide 60 mg/kg. In addition, patients with matched unrelated donor (MUD) and mismatched unrelated donor conditioning regimen contained antithymocyte globulin (ATG) as in vivo T cell depletion. Twelve patients underwent reduced intensity conditioning (RIC) which consisted of fludarabin (180 mg/m^2) and melphalan (140 mg/m^2) plus ATG or MabCampath in most cases. Patients with severe combined immunodeficiency did not receive any chemotherapy, and conditioning was restricted to ATG or Campath.

Graft

Twenty-six children received unmanipulated bone marrow with a median of 7.4×10^6 CD34+ cells/kg and 45.6×10^6 CD3+ cells/kg body weight. Four children were transplanted with peripheral blood stem cells with a median of 10.2×10^6 CD34+ cells/kg and 51×10^7 CD3+ cells/kg body weight. Grafts from mismatched unrelated donors (n=3) underwent ex vivo T cell depletion either by CD34+ selection or by CD3+/CD19+ depletion. One child received cord blood stem cells, with 0.09×10^6 CD34+ cells/kg body weight.

Graft-versus-host disease and rejection prophylaxis

GvHD prophylaxis consisted of cyclosporin A (CSA) (matched sibling donors) or CSA plus methotraxate (unrelated donors) or CSA plus mycophenolate mofetil (MMF) for patients with reduced intensity conditioning. CSA was started at day −1 with serum levels of 60–80 ng/ml and tapered between day +60 and +120 depending on donor and underlying disease.

Supportive therapy

All patients were treated in sterile laminar air flow or HEPA-filter units until a leukocyte count of more than 1,000/μl was reached. All blood products were filtered and platelets from cytomegalovirus (CMV)-IgG negative single donors were used. Cotrimoxazole was given as pneumocystis prophylaxis from day −10 until 3 months after cessation of any immunosuppressive therapy. All HSV IgG positive patients received antiviral prophylaxis with acyclovir from day −7 until day +28, and CMV IgG positive patients with CMV IgG negative donors received prophylactic gancyclovir instead. Preemptive anitiviral treatment was started in case of positive PCR results for CMV or adenovirus in peripheral blood and continued until two negative results were obtained.

GvHD

GvHD was diagnosed and graded according to international consensus criteria [12, 25]. First-line GvHD treatment consisted of prednisone 2 mg/kg/day, non-responders received either FK506 instead of CSA or MMF as add-on. Patients with extensive chronic GvHD were additionally treated with phototherapy or extracorporeal photopheresis.

Clinical follow-up with special focus on pulmonary function

All children underwent routine clinical and laboratory examination prior to transplantation, weekly during the first 3 months after transplantation, and monthly until 1 year post-transplant. Follow-up time was 360 days after HSCT for all patients. Routine chest X-ray and lung function test were performed on day +100, +180, and +360. Additional thorax X-ray and PCR screening for respiratory viruses were performed in case of respiratory symptoms. In case of persisting respiratory symptoms with unknown etiology, a high resolution CT scan was performed.

Clinical and apparative definitions of pulmonary impairment and disease

Any pulmonary pathology such as clinical respiratory distress, radiologic chest abnormalities, or decreased lung function (see below) at 3 months post-transplant was recorded as complication. Active or passive tobacco smoke exposure was not recorded systematically.

Spirometry

Pulmonary function tests (PFT) were routinely performed in cooperative children before HSCT and after 100 and 180 days, then repeated following clinical appearance. Restrictive lung disease was defined as total lung capacity (TLC) <80% of the predicted value, severe obstructive lung disease was defined as the ratio of forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) less than 70%, i.e., FEV1/FVC<70. All tests were performed according to the American Thoracic Society guidelines [6]. The following parameters were evaluated: FEV1, FVC, FEV1/FVC ratio, intrathoracal gas volume (ITGV), total lung capacity (TLC), and vital capacity.

Definition of atopy

Due to previous immunosuppression in case of malignant disease, patients were considered atopic, if there was either a positive result for specific IgE on day −10 before HSCT or if they had a history of allergic symptoms. Due to the anonymity of stem cell donors which is protected by law, it was not possible for us to assess the donors' history of asthma and atopy.

Serum analysis

Blood samples were collected within routine diagnostic procedures at scheduled routine time points (day −10, day 0, day +28, and day +100). Total blood count including eosinophils, total IgE, ECP, and specific IgE levels were assessed.

For serum ECP samples, the standardized radioimmunoassay technique was used as described by Peterson et al. [24]. Specific IgE measurements were conducted with all serum samples. Allergens tested for included house dust mite, grasses mix, trees mix, food mix, and animal mix. Additional allergens were included if indicated by the patient's history. Specific and total IgE levels in peripheral blood were determined using the CAP-fluoroenzyme immunoassay by Phadia AB (Uppsala, Sweden).

Ethics

The study has been approved by the local ethics committee and has been conducted in accordance with the study protocol, International Conference on Harmonization (ICH) guidelines, applicable regulations and guidelines governing clinical study conduct, and the ethical principles that have their origin in the Declaration of Helsinki. The investigator has explained the nature of the study to the patients or their parents and answered all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent form has been reviewed and signed and dated by the subject and/or their parents and the person who administered the informed consent.

Statistics

Results were expressed as median (range). Wilcoxon two-sample test was used to compare IgE levels, absolute eosinophils, FEV1%, FVC%, ECP for patients with and without history of atopy, infectious and non-infectious complications, GvHD. Spearman rank correlation and associated p values were calculated for the analysis of the association between absolute eosinophil counts with ECP levels. Fisher's exact test was used to analyze the association between history of atopy and infectious and non-infectious complications. Calculation of the required sample size was based on previous results obtained in adult patients [14], which indicated that data from 30 individuals would be sufficient to obtain significant results at 80% power. 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

In a prospective single-center epidemiologic study from February 2008 until March 2009, we included 30 consecutive children after allogeneic HSCT. Statistical analysis was done including all 30 (age 0.3–17 years, median 10.4 years) patients, all of whom had been tested at least four times for total IgE and eosinophil count, and twice for specific IgE and ECP pre- and post-HSCT. For the underlying diseases and patient characteristics, see Table 1. None of the patients had been diagnosed with asthma before allogeneic HSCT. Yet, nine patients had a history of previous atopy, as reported by the parents.

Twenty-two children had a classic acute GvHD after HSCT, all of them with skin manifestations, additionally with four cases with gut involvement, and seven with liver

GvHD. None of these children had received ex vivo T cell depletion. Classic chronic GvHD occurred in five children, none of whom with an overlap syndrome as described by the NIH criteria [12]. Patient 1 developed chronic skin GvHD (NIH global score 2) on day +218, with additional gut involvement (NIH grade 1) from day +240, with an overall grade of 2. Patient 2 showed signs of chronic skin GvHD (NIH grade 3) from day +196, with additional liver involvement (NIH grade 1) beginning on day +204, with an overall grade of 3. Patient 3 had chronic skin GvHD (NIH grade 3) from day +286, with additional liver involvement (NIH grade 3) starting from day +311, with an overall grade of 3. Patient 4 developed chronic skin GvHD (NIH grade 2) on day +227, with additional liver involvement (NIH grade 2) from day +301, with an overall grade of 2. Patient 5 suffered from chronic isolated liver GvHD (NIH grade 2) from day +249.

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. At the time of blood sampling for ECP and eosinophils, some children received systemic steroids. None of the children at day −10, 12 children at day +28, 2 children at day +100, and 1 patient at day +180 after HSCT.

Pulmonary complications after HSCT

Table 2 shows early and late pulmonary complications. We found a significant correlation between atopy, and infectious pulmonary complications before day +100 was found (p=0.029), but not after day +100 (p=0.375). There was no significant correlation between the history of atopy and non-infectious pulmonary complications. No significant correlation was found between the presence of both infectious or non-infectious pulmonary complications and elevated serum IgE or eosinophilia (data not shown). We found a significant correlation between lower FVC pre-transplant and non-infectious pulmonary complications after day +100 (p=0.028). However, only three patients (10%) suffered from non-infectious pulmonary complications after day +100. Two children died after day +180 due to pulmonary complications: one after pulmonary aspergillosis and one due to CMV pneumonia.

IgE

Three patients showed elevated specific IgE levels pre-transplant, two of whom had been mono-sensitized to food mix and grasses mix, respectively. One patient had shown poly-sensitization to food mix, grasses mix, and trees mix. This corresponded to previous symptoms, only the poly-sensitized patient continued to have increased specific IgE after transplant, showing a steady decrease. Six of the children with an atopic history did not show elevated IgE or eosinophil levels. None of the patients showed allergic symptoms at any time after transplant. Median total IgE at the time points −10, 0, +28, and +180 were 74.9, 80.76, 15.39, and 13.17 U/L, respectively. Elevated total serum IgE levels occurred in four patients on day +28 after HSCT (see Fig. 1), indicating a peak in the first month after transplant. IgE only correlated significantly with pre-HSCT history of atopy on day +180 (p=0.001).

Table 2 Early (before day +100 after HSCT) and late (after day +100) pulmonary complications in children after HSCT

Pulmonary complication	Early	Late
Bacterial pneumonia	6	3
CMV pneumonia	2	3
Viral bronchiolitis	1	2
PERDS	4	0
ARDS	1	0
TRALI	1	0
PVOD	1	0
BOOP	0	2
CVD	0	1

CMV cytomegalovirus; *PVOD* pulmonary veno-occlusive disease, *TRALI* transfusion-related acute lung injury, *PERDS* peri-engraftment respiratory distress syndrome, *ARDS* acute respiratory distress syndrome, *BOOP* bronchiolitis obliterans with organizing pneumonia, *CVD* combined ventilatory disorder

Eosinophil granulocytes

Elevated relative eosinophil levels (measured in percent of leukocytes) were found in five patients before transplant, seven patients on day +28, five patients on day +100, and

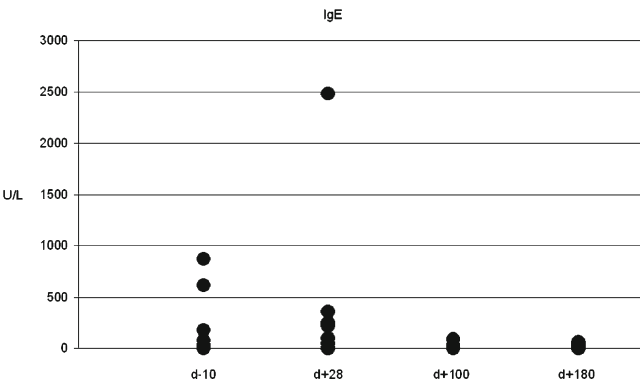

Fig. 1 Total IgE serial measurements. *d −10*: 10 days before HSCT, *d +28*: 28 days after HSCT, *d +100*: 100 days after HSCT, *d +180*: 180 days after HSCT

six patients 180 days after transplant. Six patients showed elevated relative eosinophil levels at more than one time of measurement, one of these patients presented with acute skin GvHD and one with chronic liver GvHD. Neither relative nor absolute eosinophil count correlated with history of atopy at any date of measurement.

ECP

Serum levels of eosinophilic cationic protein at four time points after HSCT are shown in Fig. 2. In contrast to serum eosinophilia without correlation with acute or chronic GvHD, there was a significant correlation (p=0.0367) between ECP levels on day +28 and acute skin GvHD (Fig. 3). However, no correlation was found between early ECP values and later development of chronic GvHD, neither for ECP levels at day +10 (p=0.502), day +28 (p=0.832), or day +100 (p=0.495). ECP showed no significant correlation with atopic history, allergy symptoms, IgE levels, PFT parameters, or pulmonary complications at any of the dates of measurement. Of interest, ECP levels correlated with eosinophilia at day −10 (p=0.006) and at day +180 (p=0.005), but not at days +28 and +100. Of interest, the mean onset of acute GvHD was day 38 (range 16–82), while in those patients with elevated ECP levels, onset of acute GvHD was before day +40.

Discussion

In our observational study, ECP did not correspond with atopic history and showed a rather inconsistent pattern. However, ECP levels on day +28 showed significant correlation with subsequent acute skin GvHD, so that one might speculate that “non-atopic ECP” exists. Additionally, day +28 and day +100 were time points when ECP did not

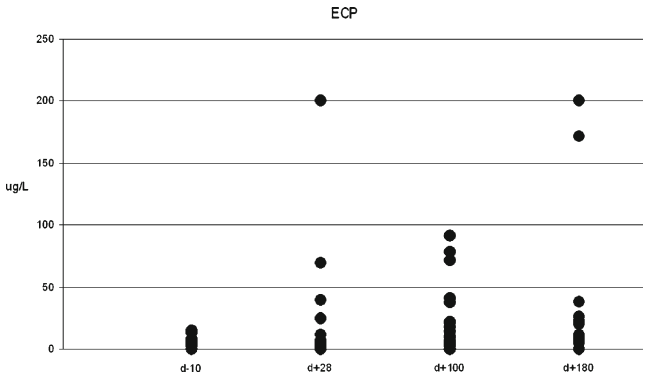

Fig. 2 Total ECP (eosinophil cationic protein) serial measurements. *d-10*: 10 days before HSCT, *d +28*: 28 days after HSCT, *d +100*: 100 days after HSCT, *d +180*: 180 days after HSCT

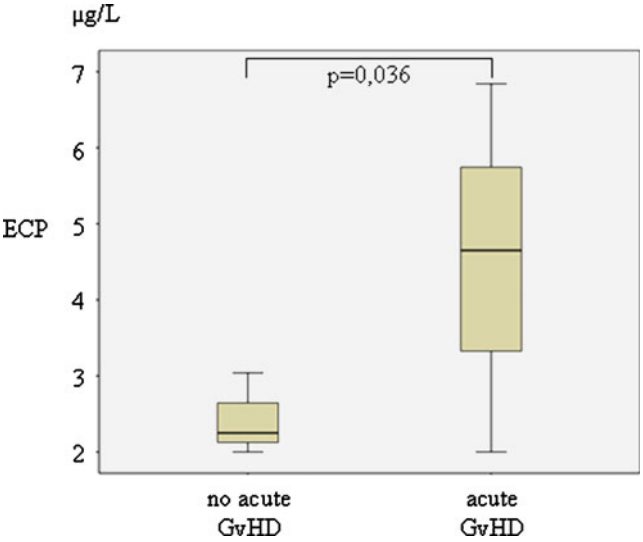

Fig. 3 ECP levels in children with and without acute GvHD after HSCT

correlate with eosinophil granulocytes, which may be due to some hematopoietic dysregulation during engraftment. Eosinophil count correlated with ECP in measurements on days −10 and +180. Yet, it has not proven useful in predicting complications or atopy in our patients after prolonged chemotherapy. Of note, eosinophil granulocytes are mostly undetectable in peripheral blood from transplant until engraftment and hematologic regeneration. In those children with elevated ECP at day +28, the onset of acute GvHD was before day +40, hence the increase of ECP seems to precede instantly the immunologic response of GvHD. Therefore, ECP might be a more sensitive marker during hematopoietic engraftment than eosinophilia, even if sensitivity and specificity could not be calculated due to the small cohort.

Of interest, none of the eight patients suffering from infectious pulmonary complications after day +100 showed signs of chronic GvHD, whereas in the three patients with non-infectious pulmonary complications, only one was diagnosed with chronic GvHD. Due to the immunosuppressive effect of GvHD, there is usually also a higher predisposition to infections in these patients. The three patients receiving ex vivo T cell depletion have been excluded from analysis of GVHD, which had no influence on the results. However, the lack of correlation of LONIPC with GvHD or T cell depletion may be due to the low case number in our cohort.

The second finding of our study were IgE peaks of non-atopic nature on day +28 after transplant, different from the course of ECP, which did not show any generalized peaks as seen in the serial measurement curves. This kind of peak phenomenon has been described in previous studies. Ambivalent opinion existed whether IgE peaks indicated

an onset of GvHD or occurred due to conditioning regimen [1, 16]. We hypothesize that in these children, elevated IgE may be rather a phenomenon of immunologic dysregulation or upregulation. The finding of an atopy-independent increase of IgE in non-T-cell-depleted children with GvHD may demonstrate that a Th2 response is still preserved also in the presence of severe immunosuppression in pediatric patients after HSCT without correlating with signs and symptoms of atopy. In contrast to total IgE, specific IgE levels decreased after transplant, most likely due to a loss of host antibodies after allogeneic HSCT. Thereby, one always has to consider that these patients usually receive high doses of steroids, for example in a MUD setting during administration of ATG, or as therapy for GvHD.

The limitations of the study include the disability of evaluating the atopic profile and history of all donors due to anonymity of unrelated donors. None of the family donors had a history of atopy. Gathering more complete information would have been instrumental in considering possible transfer of atopy from donor to recipient. Another factor is the heterogeneity of the study cohort in terms of underlying diseases and different graft types and conditioning regimens. Therefore, no positive or negative predictive ECP values for acute GvHD could be calculated and clinically relevant cut-off values still have to be established.

In conclusion, this first prospective study on ECP after pediatric HSCT could be the basis for further investigations in a multicenter trial, in order to validate ECP and eosinophils as a potential screening tool for acute GvHD in children after HSCT.

Conflict of interest The authors declare no conflict of interest. The first draft of the manuscript was written by Tamás Fazekas. No honorarium, grant, or other form of payment was given to anone to produce the manuscript.

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