Pediatric RESEARCH

www.nature.com/pr

CLINICAL RESEARCH ARTICLE

Perioperative levels of total IgE correlate with outcomes of prolonged mechanical ventilation after cardiopulmonary bypass in pediatric patients

Youjin Li[1], Limin Zhu[2], Jie Chen[1], Ma Therese G. Singson[3], Xiaoqing Rui[1], Niu Li[4], Lin Zhou[5] and Jinfen Liu[6,7]

BACKGROUND: Although cardiopulmonary bypass (CPB) has been previously studied as risking infection and inflammatory responses, few studies evaluate the relationship of preoperative high total immunoglobulin E (tIgE) to outcomes in pediatric patients predisposed to atopy undergoing cardiac surgery with CPB.

METHODS: Serum tIgE, tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), IL-4, interferon-γ (IFN-γ), and T-helper type 1/2 (Th1/Th2) ratio were quantified in 104 pediatric patients who underwent surgical repair with CPB. Blood samples were obtained: before operation (T1), at the beginning (T2), and before the completion of CPB (T3), after protamine administration (T4), 4 h after CPB (T5), and on postoperative days 1 and 2 (T6, T7). Data on clinical outcomes were collected prospectively.

RESULTS: Compared to 50 cases with normal tIgE, 54 cases with high tIgE were found to have higher TNF-α, IL-10, and IL-4 affected by CPB on the specific timepoints ($p_{TNF\text{-}\alpha} < 0.001$; $p_{IL\text{-}10} = 0.035$; $p_{IL\text{-}4} = 0.001$). TIgE levels shifted transiently towards Th2, which may be caused by high tIgE specific to T4. This resulted in the correlation between prolonged duration of mechanical ventilation (IL-4: $r = 0.426$, $p = 0.015$; Th1/Th2: $r = -0.272$, $p = 0.043$) in patients with high tIgE.

CONCLUSIONS: A high preoperative tIgE level predisposes patients to an aggravated Th2 shift after protamine administration during CPB in association with increased risk of prolonged mechanical ventilation and medical intervention.

Pediatric Research _####################_ ; https://doi.org/10.1038/s41390-018-0048-2

INTRODUCTION

Pediatric patients with congenital heart disease who require cardiopulmonary bypass (CPB) are at high risk of infection and systemic inflammatory responses. Mechanisms underlying the activation of the immune system during CPB include: young age, ischemia–reperfusion (I/R) injury, prolonged duration of operation and CPB, and activation of leukocytes by exposure to artificial surfaces.[1,2] A preoperative predisposition to allergy and exaggerated inflammatory responses may result in untoward responses to CPB and surgical trauma.[3] The synthesis of T-helper type 2 (Th2) immunoglobulins (Igs) has been shown to increase after cardiac surgery with CPB.[4,5] Proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), as well as anti-inflammatory cytokines, such as interleukin-10 (IL-10), are produced rapidly in response to cardiac surgery and are frequently used to monitor inflammatory activation.[6]

The total IgE (tIgE) level is generally considered a marker for higher genetic risk for allergic asthma and atopy. Allergic sensitization (IgE antibodies) to foreign proteins in the environment occurs in up to 40% of the population.[7] In one study, fish allergy or a history of medication allergies increased the adverse events following protamine administration after CPB.[8] There were some case reports which reported that allergic reactions should be the risk factor of bronchospasm during CPB.[9]

There have been a lack of studies that focus on whether the Th2 response attributed to the preoperative status of elevated IgE in correlation with the clinical outcomes of heart surgery by CPB. Thus, the present study is aimed: (1) to identify when Th2 dominance and high tIgE is present in children with congenital heart disease; (2) to determine if such patients might be at risk of poor outcomes of CPB surgery; (3) to potentially inform clinicians regarding the management approach for these children.

MATERIALS AND METHODS

The present prospective observational cohort study was conducted at the Shanghai Congenital Heart Disease Institute. The study protocol was approved by the Institutional Review Board of Shanghai Children's Medical Center affiliated with Shanghai Jiao Tong University School of Medicine. Informed consents were obtained from the parents or guardians of all pediatric patients

[1]Department of Otorhinolaryngology-Head & Neck Surgery, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China; [2]Department of Pediatric Critical Intensive Care, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China; [3]College of Medicine, Our Lady of Fatima University, Manila, Philippines; [4]Department of Medical Genetics & Molecular Diagnostic Laboratory, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China; [5]Research Center, Shanghai Chest Hospital, Shanghai Jiao Ttong University, Shanghai 200030, China; [6]Department of Thoracic and Cardiovascular Surgery, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China and [7]Present address: Department of Pediatrics, Shanghai Children's Medical Center, Shanghai Jiao Tong University Pediatric Institute, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China

Correspondence: Jinfen Liu (liujinfen2002@126.com)

These authors contributed equally: Li Youjin, Zhu Limin

Received: 8 January 2018 Revised: 24 April 2018 Accepted: 27 April 2018
Published online: 24 August 2018

© International Pediatric Research Foundation, Inc. 2018

before collecting the samples. Pediatric patients with congenital heart disease that were hospitalized between January 2014 and December 2014 were screened for eligibility. The enrolled patients were infants (3–24 months) undergoing surgical repair with CPB for heart disease, consisting of ventricular septal defect (VSD) ($n = 40$), atrial septal defect (ASD) ($n = 34$), or atrioventricular septal defect (AVSD) ($n = 30$). The conducted closure procedure was dependent on the patient's specific condition and experience of the institution. Patients were excluded if they had any previous surgical repair, preoperative symptoms of pneumonia or other systemic infection, immunosuppression, immunodeficiency, or corticosteroid usage within the previous 2 weeks. Furthermore, because of the risk of developing cardiogenic edema, patients were excluded if left ventricular ejection fraction was <30%.[10] In addition, patients presenting with hyper IgE syndrome[11] (recurrent eczema, skin abscesses, lung infections, eosinophilia, and extremely high serum IgE levels) were also excluded. Data on clinical outcomes were collected prospectively.

Anesthesia and perfusion techniques and ICU managements

Endotracheal intubation and central venous and arterial catheterizations were performed. Conventional general anesthetics including midazolam, fentanyl, propofol, and rocuronium were administered intravenously. Standardized CPB techniques were performed. The CPB circuit incorporated a roller pump and a Dideco membrane oxygenator. The CPB circuit was primed with human albumin (20 mL/kg) in all patients. It also contained ulinastatin (10,000 U/kg, a serine protease inhibitor), dexamethasone (5 mg/kg), and 5% $NaHCO_3$. Antibiotic prophylaxis was administered before the surgical procedure or during anesthesia. A uniform regimen of continuous ultrafiltration was employed. In all patients, heparinization was achieved with heparin sulfate; the effect was reversed using protamine sulfate. To maintain the hematocrit and pH, the alpha-stat strategy was utilized. All patients received standard care in a dedicated pediatric cardiac intensive care unit (CICU) in the postoperative period.

On return to the CICU, patients were mechanically ventilated with a Servo i ventilator (Maquet Critical Care, Solna, Sweden) capable of delivering pressure control ventilation and pressure support ventilation (PSV). The FiO_2 was 0.30–0.50 in all patients. The target VT was 7–8 mL/kg. Rectal temperature was maintained at 36–37.5 °C using a cooling or warming blanket. Hemoglobin concentration was maintained at or above 120 g/L. Dopamine (5.0–7.5 μg/kg/min) and milrinone (0.5–0.75 μg/kg/min) were infused to maintain hemodynamic stability depending on the procedure the physician performed on each case. Furosemide (1 mg/kg per dose) was injected every 8 h for 24 h postoperation in all the patients. Before extubation, all the patients were ventilated with: PSV (4–6 cmH_2O); pressure support above 3–4 cmH_2O positive end expiratory pressure, and spontaneous breath test (SBT) for 30 min using manual adjustment of PSV by physicians. Additional immunomodulator drugs were not utilized in the patients postoperatively, with the exception of treatment with nebulized budesonide (1 mg per dose), which was performed within 30 min after extubation.

Assessment of total serum IgE

tIgE was determined quantitatively in all the patients using the BN II and BN ProSpec® (N Latex IgE mono, Siemens, Germany), after calibration using commercial standard (also Siemens), and expressed in IU/mL, where 1 IU/mL is equal to 2.4 ng/mL.[12] The reference values for serum IgE (U/mL) with aging were as follows: neonates <1.5 years, infants in the first year of life <15 years, and children (1–2 years) <60 years. Patients with high tIgE (H-tIgE group) or with normal tIgE (N-tIgE group) were categorized based on the results of tIgE test. In addition, atopic history was assessed using the Chinese version of the International Study of Asthma and Allergies in Childhood questionnaire.

Blood sampling and serological cytokines' analysis

All the subjects underwent cardiac surgery at 9:00 a.m. to minimize any time-sensitive variation of cytokine synthesis. Peripheral venous blood samples were obtained: before operation (T1, −1 day), at the beginning of CPB (T2, CPB-I), before CPB ended during reperfusion and rewarming (T3, CPB-II), after protamine administration (T4, protamine), 4 h after CPB (T5, +4 h), and on postoperative days (POD1–2) (T6, +1 day; T7, +2 days). The blood samples were collected in siliconized tubes for serum cytokines and IgE, and potassium ethylenediaminetetraacetic acid preparation tubes for flow cytometry. Clotting was allowed at room temperature. The serum was separated from whole blood by centrifugation at 3000 rpm for 10 min and was preserved at −80 °C until assayed.

Serum Th2 response (as reflected by IL-4), Th1 response (IFN-γ), TNF-α, and IL-10 were measured using high-sensitivity enzyme-linked immunosorbent assay (Thermo-Fisher Scientific, kits BMS225HS, BMS228HS, BMS223HS, BMS215HS). The minimum detectable levels of human IL-4, IFN-γ, TNF-α, and IL-10 were <0.1, 0.06, 0.13, and 0.05 pg/mL, respectively.

Flow cytometric analysis of $CD3^+CD8^-$ T lymphocytes and intracellular cytokines

Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on a Ficoll gradient and resuspended in RPMI medium supplemented with 10% heat-inactivated fetal calf serum and 2% penicillin–streptomycin (Sigma, St. Louis, MO, USA). After 5 h of stimulation (Cell Stimulation, eBioscience, San Diego, CA, USA), PBMCs were mixed with the appropriate volume of fluorescence dye-conjugated monoclonal antibodies (5 μL: 1×10^6 cells/mL) and stained for 15 min at room temperature in the dark. Then the reaction mixtures were centrifuged at 1500 rpm, followed by the addition of 100 μL fixation and permeabilization reagent (Intraprep; Beckman Coulter, Danvers, MA, USA). Next, cells were stained with anti-human CD3-PerCP (eBioscience), anti-human CD8-fluorescein isothiocyanate (eBioscience), anti-human IL-4-APC (eBioscience, 0.2 μg/10^6 cells), and anti-human IFN-γ-phycoerythrin (eBioscience, 0.5 μg/10^6 cells). IgG isotype controls were utilized for every antibody and patient. The cell pellets were resuspended in 400 μL phosphate-buffered saline containing 0.2% (w/v) paraformaldehyde (Sigma, St. Louis, MO, USA) for flow cytometric analysis after washing and centrifugation.

Acquisition and analysis of flow cytometric data

The stained and fixed samples were injected into a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA), and the data were analyzed using the FlowJo software (Tree Star Inc., Ashland, OR, USA) according to the manufacturer's instructions. Ten thousand $CD3^+CD8^-$ lymphocyte events were typically acquired for analysis of the frequency of intracellular cytokine staining. Cytokine production was confirmed based on the staining of isotype control. The results were expressed as the percentage of cytokine-positive lymphocytes in stimulated culture. The percentage of cytokine-producing Th cells (IL-4^+CD4^+ and IFN-γ^+CD4^+) was calculated within the total $CD3^+CD8^-$ subpopulation. (Fig. 1a–d). There was no difference in mean fluorescence intensity for each sample or across the different timepoints.

Clinical data collection

Data on clinical outcomes were collected prospectively (Table 2). The intraoperative data included the duration of CPB, aortic cross-clamp and circulatory arrest times, blood products administered, hypothermia, and reperfusion and rewarming. Heart rate, arterial blood pressure, respiratory rate, airway pressure (such as positive inspiratory pressure (PIP) and mean airway pressure (MAP)), tidal volumes (VTs) delivered by the ventilator, and rectal temperature were monitored continuously. The PIP and MAP 30 min and 3 h

after ICU admission were recorded as PIP-30 min, PIP-3 h, MAP-30 min, MAP-3 h, respectively. The PIP and MAP during SBT were recorded as PIP-SBT and MAP-SBT. Arterial blood gas was analyzed during every recording time. Mixed venous oxygen saturation (%) of blood samples obtained from the internal jugular venous was determined by standard blood gas analysis. All the patients were assessed for weaning time, defined as the time from assessing the ability of the patient for spontaneous breathing to the time of the last extubation when the patient did not require noninvasive ventilator support (NIV) or reintubation within 48 h of extubation.[13] The weaning outcome in patients who failed SBT at first attempt and required up to three trials was evaluated. Using mechanical ventilator support noninvasively within 48 h of extubation or reintubation were recorded. Any application of inotropics (such as dopamine, epinephrine, milrinone), vasodilators (such as sodium nitroprusside), and bronchodilator therapies (such as salbutamol, terbutaline, and ipratropium bromide) were evaluated during the postoperative peroid. The inotropic score was calculated as described by Wernovsky et al.[14] listed in Table 2. Postoperative outcomes were recorded as well.

Statistical analysis

In this study, there is a 80% statistical power for the interaction with H-IgE/N-IgE groups for a total of 60 participants in the setting of $\alpha = 0.05$, $1-\beta = 0.2$, partial $\eta^2 = 0.02$, and nonsphericity correction $e = 0.75$ using the G*Power 3.1.9.2 software (http://www.gpower.hhu.de/).

The normally distributed continuous data was expressed as mean ± standard deviation (SD). The differences between groups for baseline characteristics and intraoperative and postoperative parameters were measured with χ^2 test for categorical variables or t test for continuous variables. We used repeated-measures analysis of variance (ANOVA) to assess the data from time courses and between groups perioperatively at a group level or an individual level. Within-group analysis of the data at different timepoints were performed using Dunnett's t test, wherein the samples were compared with the preoperative baseline (T1). The strength of association between cytokines, Th1/Th2 ratio, and selected clinical measures were assessed by Spearman's correlation coefficient. A p value <0.05 was considered statistically significant. All the statistical analyses were conducted using SPSS 17.0 (Statistical Package for the Social Sciences Inc., Chicago, IL, USA), and data were plotted using the GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Baseline characteristics

Hundred and four patients were enrolled in the study, 54 of which were categorized into the H-tIgE group (H-tIgE) according

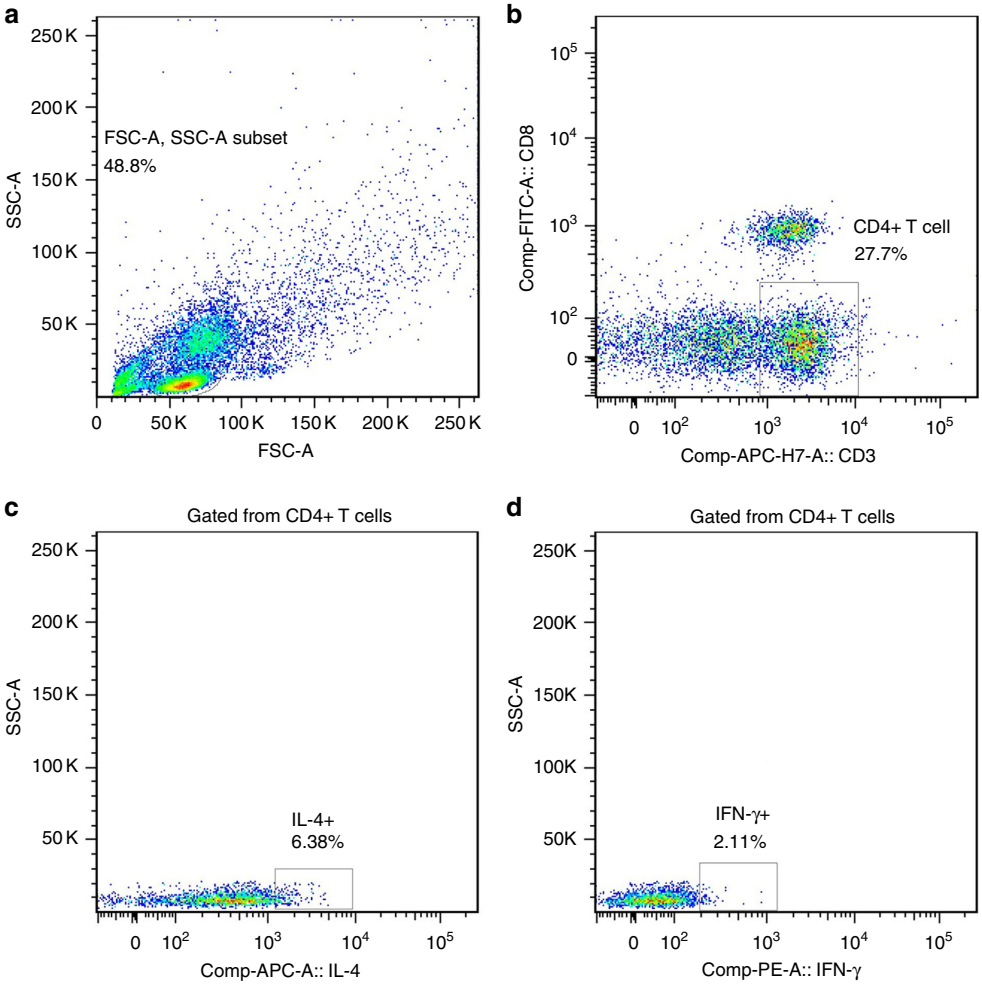

Fig. 1 IL-4$^+$CD4 and IFN-γ$^+$CD4$^+$ were expressed in peripheral blood samples by flow cytometry after intracellular stimuli. **a** FACS gating was used in the analysis of PBMCs in all the patients. **b** Representative FACS data and statistical analysis showed the expression of CD4$^+$(CD3$^+$CD8$^-$) in PBMCs. **c** FACS data and statistical analysis showed the percent of IL-4$^+$CD4$^+$ in the CD4$^+$ T cells. **d** FACS data and statistical analysis showed the percent of IFN-γ$^+$CD4$^+$ in the CD4$^+$ T cells

to serum tIgE levels. Fifty patients with normal tIgE were assigned to the N-tIgE group (N-tIgE). The preoperative demographic data was similar in both groups (Table 1). Thirty-four patients in the H-IgE group and six patients in the N-IgE group were found with a family history of atopy, respectively. Symptoms related to atopy were reported more frequently by parents in the H-tIgE group than that in the N-tIgE group ($p = 0.002$).

Clinical data

Intraoperative variables are described in Table 2. No significant differences were observed with respect to intraoperative variables between the H-tIgE and N-tIgE groups undergoing CPB. However, the duration of mechanical ventilation was essentially twice as long in patients with H-tIgE than those with normal IgE ($p = 0.02$). Higher peak inspiratory pressure and higher MAP were noticed in the H-tIgE groups, associated with lower PaO_2/FiO_2 ratio. Similarly, weaning time in the H-tIgE group was longer than that in the N-tIgE group ($p = 0.038$), which could be relevant to more patients with high IgE level who failed SBT at first attempt. As outcomes of extubation, 19 patients (35.19%) required NIV within 48 h of extubation in the H-tIgE group, whereas 5 patients (10%) the N-tIgE group required NIV ($p = 0.005$). No patients required reintubation. The phenomenon could be explained by the tendency of longer ICU stay in the H-IgE group; however, no significance was found in the comparison of the H-IgE and N-IgE group. In addition, no difference of mediastinal and pericardial drainage between high and normal IgE subgroups were recorded. Thirty-four (62.7%) patients in the H-tIgE group received a compound albuterol/ipratropium bromide (250 µg per dose) medication as nebulized bronchodilator treatment in general inpatient care after CICU, which was significantly more than that in the N-tIgE group ($p < 0.001$). No vasodilator therapies differed significantly between two groups during the post-op period ($p > 0.05$, data not shown).

The differences of cytokine release and Th2 shift during CPB in the H-IgE vs N-IgE group

We found that the effect of CPB differs across H-tIgE/N-tIgE groups. Significant changes in the concentration of TNF-α, IL-10, IL-4, tIgE, and Th1/Th2 were seen perioperatively with time in H-tIgE vs. N-tIgE groups, of which IL-4, tIgE, Th2, and Th1/Th2 were also affected by CPB on the specific timepoints (Table 3).

Significant changes in the concentration of TNF-α, IL-10, IL-4, and tIgE were observed postoperatively in comparison with the baseline in H-tIgE patients undergoing CPB over time at T4 (Fig. 2a–c, e), while IFN-γ levels were not significantly different at any certain timepoint (Fig. 2d). The H-tIgE group demonstrated rising IgE levels through the perioperative period to a maximum at T5 (Fig. 2e) and a stronger Th2 response, especially at the time for protamine injection (T4) (Fig. 2f).

Table 1. Baseline characteristics of H-IgE and N-IgE group undergoing CPB

Characteristics	H-tIgE (54)	N-tIgE (50)	*p* value
Age (months)	10.8 ± 5.1	11.2 ± 4.4	NS
Gender (male/female), *n*	30/24	24/26	NS
Weight (kg)	9.6 ± 4.6	8.3 ± 3.8	NS
Ejection fraction (%)	70.6 ± 6.8	71.7 ± 6.26	NS
Disease, *n* (%)			NS
VSD	22 (40.7)	18 (36)	
ASD	18 (33.3)	16 (32.0)	
AVSD	14 (25.9)	16 (32.0)	
Questionnaire for atopy, *n* (%)			0.002
Family history of atopy	34 (62.96)	6 (12.0)	
AR-like symptoms	26 (48.15)	0 (0)	
Allergy to egg yolk	22 (40.7)	0 (0)	
Eczema	38 (70.4)	8 (16.0)	
Dry cough apart from a cold	36 (66.7)	7 (14.0)	
Wheezing	22 (40.7)	5 (10.0)	
Allergy to fish	0 (0)	0 (0)	

Data: mean ± SD
NS: $p > 0.05$

Table 2. Characteristics and intraoperative and postoperative parameters in 104 patients with CPB

Parameters	H-tIgE (54)	N-tIgE (50)	*p* value
Intraoperative variables			
CPB time (min)	51.5 ± 19.2	49.7 ± 22.0	NS
Cross-clamp time (min)	29.2 ± 14.0	30.6 ± 17.7	NS
Hypothermia (°C)	33.7 ± 1.5	33.8 ± 1.2	NS
Reperfusion and rewarming (min)	14.5 ± 7.9	12.1 ± 4.2	NS
ICU parameters			
PIP-30 min	13.9 ± 3.9	11.3 ± 3.7	<0.001
PIP-3 h	11.5 ± 3.5	9.7 ± 5.2	0.017
PIP-SBT	8.9 ± 3.1	8.5 ± 3.0	NS
MAP-30 min	6.4 ± 2.1	4.5 ± 1.7	<0.001
MAP-3 h	5.1 ± 2.1	4.0 ± 1.8	0.03
MAP-SBT	4.5 ± 1.6	3.7 ± 1.6	0.002
PaO_2/FiO_2-30 min	308.1 ± 90.2	347.3 ± 83.8	0.04
PaO_2/FiO_2-3 h	364.9 ± 87.0	384.5 ± 79.4	0.03
PaO_2/FiO_2-SBT	372.5 ± 85.7	378.7 ± 81.6	NS
Weaning time (h)	5.18 ± 1.58	4.23 ± 1.97	0.038
Failed SBT at first attempt, *n* (%)	19 (35.19)	10 (20)	NS
NIV within 48 h of extubation, *n* (%)	18 (33.3)	5 (10)	0.005
Inotropic score (cumulative POD1–2)	13.1 ± 4.8	14.9 ± 3.6	NS
MV_{sat} (%) (POD1)	71.7 ± 14.0	72.6 ± 13.1	NS
CVP (mmHg) (POD1)	9.9 ± 2.3	9.5 ± 2.6	NS
CVP (mmHg) (POD2)	10.3 ± 3.6	10 ± 2.76	NS
Lactate (mmol/L) (POD 1)	1.3 ± 0.2	1.0 ± 0.3	NS
Mediastinal and pericardial drainage (POD1) (mL/kg)	9.6 ± 5.7	12.5 ± 1.8	NS
Mediastinal and pericardial drainage (POD2) (mL/kg)	6.2 ± 4.9	5.8 ± 1.6	NS
Postoperative outcomes			
Intensive care unit stay (days)	3 ± 1.9	2.9 ± 1.3	NS
Mechanical ventilation (h)	14.7 ± 4.4	6.3 ± 2.4	0.02
Hospital stay (days)	9.4 ± 3.6	9.5 ± 3.2	NS
Utilization of nebulized bronchodilators after CICU, *n* (%)	34 (62.7)	16 (32)	<0.001

Data: mean ± SD
ICU intensive care unit, *PIP* positive inspiratory pressure, *SBT* spontaneous breath test, *MAP* mean airway pressure, *NIV* noninvasive ventilation, MV_{sat} mixed venous oxygen saturation, *CVP* central venous pressure, *CICU* cardiac intensive care unit
NS: $p > 0.05$

Table 3. Overviews of the results of a repeated-measures ANOVA test for the H-tIgE /N-tIgE group at T1–T7

	Main effects of time			Interaction effects of group × time			Main effects of group		
	F	df	*p* value	*F*	df	*p* value	*F*	df	*p* value
TNF-α	**33.50**	**(4.82, 492.55)**	**<0.001**	**36.9**	**(4.82, 492.55)**	**<0.001**	3.09	(1, 102)	0.095
IL-10	**58.89**	**(3.44, 651.64)**	**<0.001**	**2.76**	**(3.44, 651.64)**	**0.035**	3.356	(1, 102)	0.065
IL-4	**4.95**	**(4.84, 498.17)**	**<0.001**	**4.47**	**(4.84, 498.17)**	**0.001**	**29.52**	**(1, 102)**	**<0.001**
IFN-γ	1.68	(3.94, 402.28)	0.165	0.92	(3.94, 402.28)	0.435	0.34	(1, 102)	0.559
tIgE	**5.66**	**(2.51, 255.52)**	**0.005**	1.34	(2.51, 255.52)	2.73	**12.53**	**(1, 102)**	**0.001**
Th1	2.30	(3.44, 351.37)	0.7	2.50	(3.44, 351.37)	0.099	7.07	(1, 102)	0.101
Th2	1.21	(4.34, 396.04)	0.299	1.08	(4.34, 396.04)	0.341	**12.20**	**(1, 102)**	**0.001**
Th1/Th2	**5.53**	**(3.38, 344.28)**	**0.001**	**3.52**	**(3.38, 344.28)**	**0.018**	**4.63**	**(1, 102)**	**0.041**

Notes: Degrees of freedom for this study are Greenhouse–Geisser corrected
Significant effects ($p < 0.05$) as shown in bold text

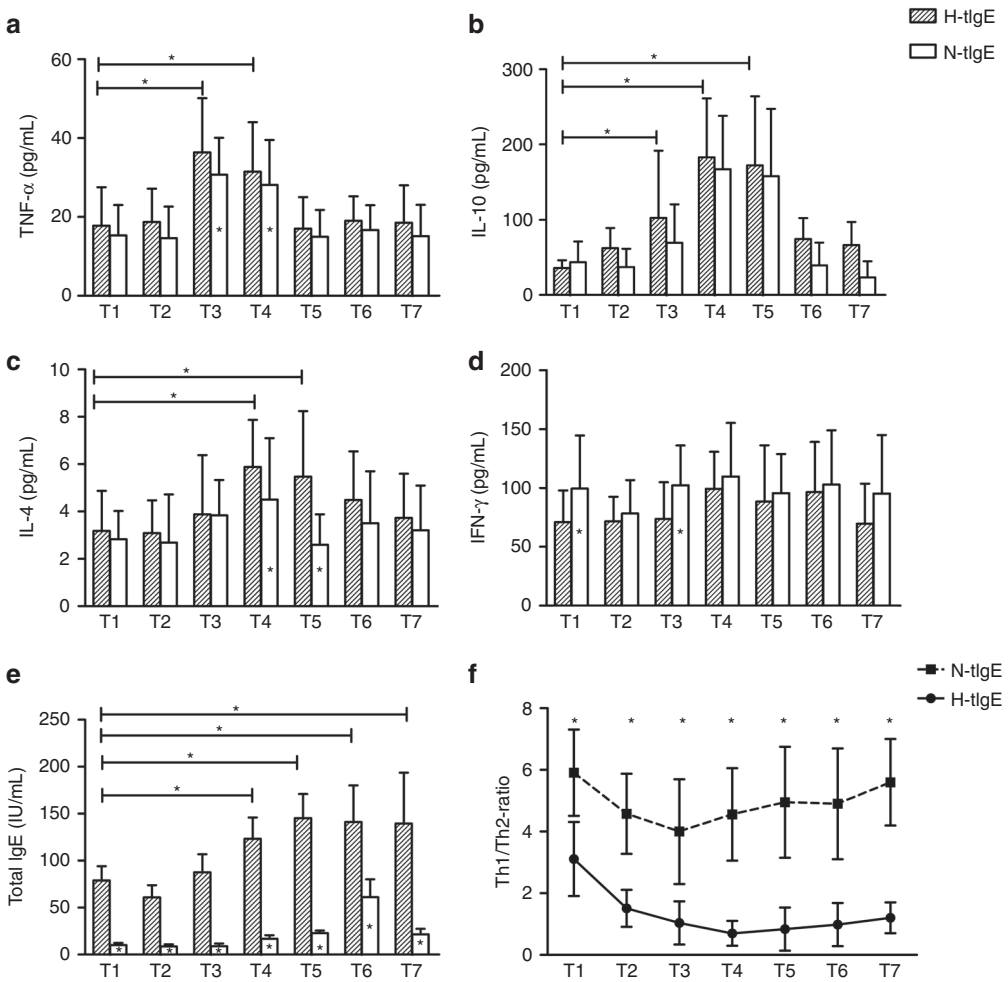

Fig. 2 Time course of the serum level of TNF-α, IL-10, IL-4, IFN-γ, total IgE, and the ratio of Th1/Th2 in H-tIgE vs. N-tIgE group undergoing CPB. **a–e** The lower and upper lines of the box indicate the 25th and 75th percentiles of the data, and the center line in the box indicates the median. The whiskers extend the range of the 10th and 90th percentiles, and the black circles denote the 5th and 95th percentiles, *$p < 0.05$, asterisks within the white box indicate statistical comparison of the groups at the same timepoint (repeated-measures ANOVA). *$p < 0.05$, asterisks outside the box indicate statistical comparison of baseline in pre-CPB (−1 day) (Dunnett's *t* test). **f** *$p < 0.05$, asterisks indicate statistical comparison of the groups at the same timepoint (repeated-measures ANOVA). Error bars denote SDs. **a** TNF-α; **b** IL-10B; **c** IL-4; **d** IFN-γ; **e** total IgE; **f** the ratio of Th1/Th2

The levels of TNF-α, IL-10, IL-4, and Th1/Th2 perioperatively were affected by the interaction of group and time in the H-IgE vs. N-IgE group ($p_{TNF\text{-}\alpha} < 0.001$; $p_{IL\text{-}10} = 0.035$; $p_{IL\text{-}4} = 0.001$; $p_{Th1/Th2} = 0.018$).

Flow cytometry revealed that in patients who underwent surgery involving CPB, the mean fluorescence intensity of peripheral Th lymphocytes producing IL-4 ($IL\text{-}4^{+}CD4^{+}$) were significantly higher at T4 and T5 in the H-tIgE group, compared

to the N-tIgE group (T4: $p = 0.025$; T5: $p = 0.043$) (Fig. 3a). IFN-γ (IFN-γ$^+$CD4$^+$) did not vary significantly over time as compared to the baseline in either group, although there were modest differences between groups (Fig. 3b), with IFN-γ$^+$CD4$^+$ tending to be higher overall in the N-tIgE group.

Correlations of clinical outcomes with cytokine responses in H-tIgE patients with CPB

A significant correlation was found only between the maximum cytokine (at T4) response, the measurement of the Th1/Th2 ratio (at T4), and the duration of mechanical ventilation specifically in the H-tIgE group. Within the H-tIgE group, higher IL-4 levels (T4) correlated with prolonged mechanical ventilation, whereas Th1/Th2 ratio (T4) negatively correlated with mechanical ventilation ($r = 0.426$, $p = 0.015$; $r = -0.272$, $p = 0.043$, respectively) (Fig. 4a, b).

DISCUSSION

In this prospective trial, we have demonstrated that CPB surgery may strengthen the Th1/Th2 imbalance in H-tIgE patients moreso than that in the N-tIgE patients. The Th2 shift correlated with the requirement for increased duration of mechanical ventilation in H-tIgE patients after CPB surgery. These results, for the first time, show an aggravated Th2 shift in children with H-tIgE during open-heart surgery.

It is well known that CPB contributes to such an inflammatory response through tissue injury as well as its clinical consequences, which is the cytokine-mediated activation of inflammatory cells. The fact that the release of TNF-α, IL-4, and IL-10 significantly increased (peaked T3 and T4) in both H-tIgE and N-tIgE groups, which is consistent with the previously reported studies in cardiac surgery following CPB.[4,6] However, IFN-γ were not detected to be suppressed significantly as expected.[15] Interestingly, we found that IgE increased significantly postoperatively and transient Th1/Th2 disbalance during CPB, which was similar to another study.[4] Significant histamine released in association to CPB was also reported.[16]Histamine (a marker of both basophil and mast cell degranulation) release plays an important role in allergic reactions by IgE-mediated signaling transduction.[17]

The increase in the Th1 and Th2 signatory cytokines (IFN-γ and IL-4) highlight this biased immune response.[18] In our study, patients with H-tIgE had a significantly increased IL-4 at the protamine timepoint and 4 h after CPB. The low IFN-γ detected in the H-tIgE patients is associated with a predisposition towards a preoperative Th2 dominance. Flow cytometry revealed a CPB response with Th1/Th2 imbalance, which was previously reported after open-heart surgery,[19] CPB,[4] and red blood cell transfusion.[20] Moreover, fish allergy and history of non-protamine medication allergy might be major risk factors for adverse events following protamine administration after CPB,[8] which could be attributed to Th1/Th2 imbalance. However, fish allergy, non-protamine medication, and adverse events after protamine administration following CPB were all not observed, even in patients with high IgE in our study. An allergic predisposition in adults leading to risk of cardiovascular death has been described.[21] However, tIgE, independent of specific IgE increasing with age beginning at the birth,[22] can simply express allergy to untested environmental allergens.[23] Therefore, we conclude that this aggravated Th2 shift during and after CPB, especially after protamine administration in children with congenital heart disease, relates to the underlying Th2 immunologic phenotype. This was contributed by the high tIgE levels and amplified by exposure to CPB antigens (e.g., foreign

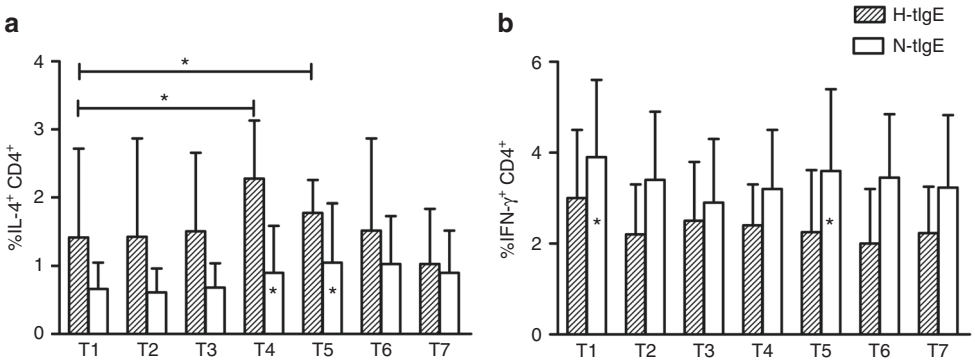

Fig. 3 In the H-tIgE vs. N-tIgE group in CPB, time course of the percentage of IL-4$^+$CD4$^+$ and IFN-γ$^+$CD4$^+$. *$p < 0.05$, asterisks within white box indicate statistical comparison of the groups at the same timepoint (repeated-measures ANOVA). *$p < 0.05$, asterisks outside the box indicate statistical comparison of baseline in pre-CPB (−1 day) (Dunnett's t test). Error bars denote SDs. **a** IL-4$^+$CD4$^+$; **b** IFN-γ$^+$CD4$^+$

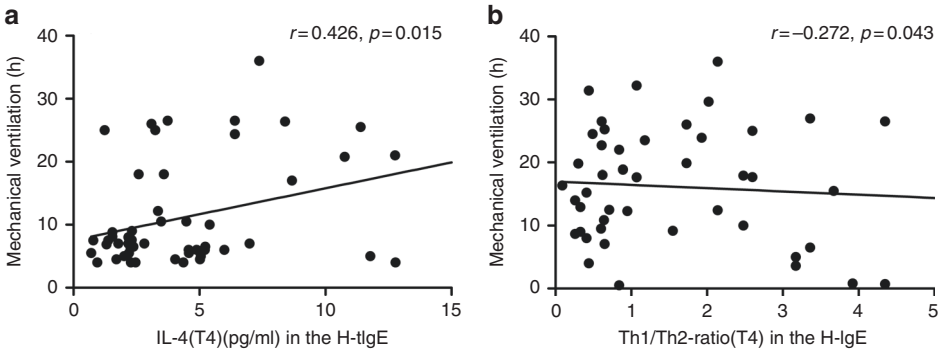

Fig. 4 Correlation between the serum level of IL-4, the ratio of Th1/Th2 at protamine administration, and mechanical ventilation (hours) among the H-tIgE group undergoing CPB. **a** H-tIgE patient with CPB duration of mechanical ventilation show significant positive correlation with serum IL-4 levels (T4) (Spearman's correlation coefficient). **b** H-tIgE patient with CPB Th1/Th2 ratio levels (T4) show a negative correlation with duration of mechanical ventilation (Spearman's correlation coefficient)

surfaces of CPB and protamine). Our study shows that the H-IgE levels mentioned in the patients dramatically increase serum IgE levels after the initiation of CPB.

The duration of mechanical ventilation among the H-IgE group undergoing surgery requiring CPB was significantly positively correlated with the serum concentrations of IL-4 after protamine administration and negatively correlated with the magnitude of the Th2 dominance as estimated by the Th1/Th2 ratio, although the clinical relationship is weak. We speculate that in infants undergoing low complex cardiac surgery or short cross-clamp time, the contribution of high IgE levels to impressive post-operative morbidity is relatively limited. Our data are consistent with a recent study showing CPB as a contributing factor to the length of stay and duration of mechanical ventilation,[24] an important factor for resource allocation and is correlated with postoperative morbidity and mortality.[25] In addition, our study demonstrates that atopic predisposition is disadvantageous for the patient. It has been suggested that when the allergic disease is previously established, CPB (as the inducing factor of severe acute stress) may dysregulate the corticotropin-releasing hormone–mast cell–histamine axis, and thus may facilitate or sustain atopic reactions. Moreover, these effects would not be moderated by the effects of stress hormones on the mast cell, but rather amplify the Th2 shift, potentially initiating a new or exacerbated chronic allergic condition.[26] The enhancement of IgE-mediated histamine release from an aggravated Th2 shift increases the risk of airway hyperresponsiveness and pulmonary hypertension with cardiac surgery,[19] all of which could contribute to acute respiratory distress. In our study, we found more frequent bronchodilator therapies supplied to H-tIgE during the postoperative period. It is implicated that delayed weaning imposes no tolerated SBT at first attempt, implying a possibility that NIV is required and more bronchodilator therapy is needed. This may be associated with airway hyperresponsiveness or high resistance in patients with high IgE levels. High IgE levels perioperatively increased the risk of the need for ongoing mechanical ventilation and higher airway pressure, as well as higher main airway resistance, during mechanical ventilation. Although the restricted oxygenation in our study was acceptable, it renders more attention to the tendency of poor oxygenation in the patient, especially in the patients with complex heart malformation.

Our study has some limitations. The observational data were assimilated from a single center, which may limit its generalizability. Given the severe comorbidities and complex procedural effect on cytokine balance and clinical outcomes, the demographics, overall severity of illness, and mode of surgery were well matched. Furthermore, the patients enrolled in the study suffered from simple congenital heart disease and follow-up was limited to 2 postoperative days. More complex cases, prolonged postoperative duration, and the process of weaning should be a concern in future studies.

CONCLUSION

The present study shows that the aggravated Th2 shift typical of CPB is associated with the preoperative status of high tIgE level. The excessive level of Th2 response has the potential to be unfavorable for the patients. High tIgE levels are associated with a subsequent requirement for a longer duration of mechanical ventilation.

ACKNOWLEDGEMENTS

All phases of this study were supported by the Chinese National Natural Science Foundation (81502815, 81602818), Science and Technology Commission of Shanghai Municipality (15401933800) and Shanghai Jiao Tong University (YG2016MS29).

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Costello, J. M. et al. Risk factors for surgical site infection after cardiac surgery in children. *Ann. Thorac. Surg.* **89**, 1833–1841 (2010). discussion 1841–1832.
2. Hirai, S. Systemic inflammatory response syndrome after cardiac surgery under cardiopulmonary bypass. *Ann. Thorac. Cardiovasc Surg.* **9**, 365–370 (2003).
3. Tarnok, A. et al. Preoperative prediction of postoperative edema and effusion in pediatric cardiac surgery by altered antigen expression patterns on granulocytes and monocytes. *Cytometry* **46**, 247–253 (2001).
4. Bocsi, J. et al. Transient Th1/Th2 disbalance indicates postoperative effusions and edema after cardiopulmonary bypass in children. *Cytom. A* **69**, 165–168 (2006).
5. Lante, W. et al. Immunoglobulin levels and lymphocyte subsets following cardiac operations: further evidence for a T-helper cell shifting. *Thorac. Cardiovasc. Surg.* **53**, 16–22 (2005).
6. Allan, C. K. et al. The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. *Anesth. Analg.* **111**, 1244–1251 (2010).
7. Ruby Pawankar G. W. C. et al. *WAO White Book on Allergy*, World Allergy Organization, UK, 2011–2012: Executive Summary (2011).
8. Kimmel, S. E. et al. Risk factors for clinically important adverse events after protamine administration following cardiopulmonary bypass. *J. Am. Coll. Cardiol.* **32**, 1916–1922 (1998).
9. Kawahito, S. et al. Bronchospasm induced by cardiopulmonary bypass. *Ann. Thorac. Cardiovasc. Surg.* **7**, 49–51 (2001).
10. Zhang, S. et al. Capillary leak syndrome in children with C4A-deficiency undergoing cardiac surgery with cardiopulmonary bypass: a double-blind, randomised controlled study. *Lancet* **366**, 556–562 (2005).
11. Giberson, M. & Finlayson, L. Hyper IgE syndrome in an infant. *J. Cutan. Med. Surg.* **20**, 340–342 (2016).
12. O'Connor, G. T. et al. Smoking, atopy, and methacholine airway responsiveness among middle-aged and elderly men. The Normative Aging Study. *Am. Rev. Respir. Dis.* **140**, 1520–1526 (1989).
13. Zein, H. et al. Ventilator weaning and spontaneous breathing trials; an educational review. *Emerg. (Tehran)* **4**, 65–71 (2016).
14. Wernovsky, G. et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low-flow cardiopulmonary bypass and circulatory arrest. *Circulation* **92**, 2226–2235 (1995).
15. Justus G. et al. Immunodepression after CPB: cytokine dynamics and clinics after pediatric cardiac surgery—a prospective trial. *Cytokine.* **S1043-4666**, pii S1043-4666(17)30085-6 (2017).
16. Seghaye, M. C. et al. Histamine liberation related to cardiopulmonary bypass in children. Possible relation to transient postoperative arrhythmias. *J. Thorac. Cardiovasc. Surg.* **111**, 971–981 (1996).
17. Wills-Karp, M. Histamine-releasing factor: a promising therapeutic target for food allergy. *J. Clin. Invest.* **127**, 4238–4241 (2017).
18. Magnan, A. O. et al. Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. Increased IFN-gamma-producing CD8(+) T cells in asthma. *Am. J. Respir. Crit. Care Med.* **161**, 1790–1796 (2000).
19. Prondzinsky, R., Knupfer, A. & Loppnow, H. et al. Surgical trauma affects the proinflammatory status after cardiac surgery to a higher degree than cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* **129**, 760–766 (2005).
20. Leal-Noval, S. R., Munoz-Gomez, M. & Arellano, V. et al. Influence of red blood cell transfusion on $CD4^{+}$T-helper cells immune response in patients undergoing cardiac surgery. *J. Surg. Res.* **164**, 43–49 (2010).
21. Hospers, J. J., Rijcken, B. & Schouten, J. P. et al. Eosinophilia and positive skin tests predict cardiovascular mortality in a general population sample followed for 30 years. *Am. J. Epidemiol.* **150**, 482–491 (1999).
22. Nickel, R., Illi, S. & Lau, S. et al. Variability of total serum immunoglobulin E levels from birth to the age of 10 years. A prospective evaluation in a large birth cohort (German Multicenter Allergy Study). *Clin. Exp. Allergy* **35**, 619–623 (2005).
23. Platts-Mills, T. A. E. P. S. Asthma and serum levels of IgE (letter). *N. Engl. J. Med.* **320**, 1696 (1989).
24. Madhok, A. B., Ojamaa, K. & Haridas, V. et al. Cytokine response in children undergoing surgery for congenital heart disease. *Pediatr. Cardiol.* **27**, 408–413 (2006).
25. Shi, S., Zhao, Z. & Liu, X. et al. Perioperative risk factors for prolonged mechanical ventilation following cardiac surgery in neonates and young infants. *Chest* **134**, 768–774 (2008).
26. Elenkov, I. J. Glucocorticoids and the Th1/Th2 balance. *Ann. NY Acad. Sci.* **1024**, 138–146 (2004).