Cardiology in the Young

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Analysis of inflammatory cytokines in the chest tube drainage of post-operative superior cavopulmonary connection patients

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Abstract

Introduction: Prolonged pleural effusions are common post Fontan operation and are associated with morbidity. Fontan pleural effusions have elevated proinflammatory cytokines. Little is known about the chest tube drainage after a superior cavopulmonary connection. We examined the chest tube drainage and the inflammatory profiles in post-operative superior cavopulmonary connection patients. *Methods:* This prospective cohort study enrolled 25 patients undergoing superior cavopulmonary connection and 10 age-similar controls. Data are also compared to 25 previously published Fontan patients and their 15 age-similar controls. Chest tube samples were analysed with a 17-cytokine BioPlex Assay. Descriptive statistics and univariate comparisons were made between groups. *Results:* Duration of chest tube drainage was significantly shorter in superior cavopulmonary connection patients (median 4 days, [interquartile range 3–5 days]) versus Fontan patients (10 days, [7–11 days], $p < 0.0001$). Cytokine concentrations were higher on post-operative day 1 in superior cavopulmonary connection patients versus Fontan patients (all $p \leq 0.01$), however levels were comparable to age-similar controls. While proinflammatory IL 8, MIP-1β, and TNF-α concentrations increased in chest tube drainage of Fontan patients from post-operative day 1 to last chest tube day (all $p < 0.0001$), there was no change in these biomarkers in superior cavopulmonary connection patients, their controls, or Fontan controls. *Conclusions:* Our study demonstrates that after superior cavopulmonary connection, proinflammatory cytokines in the chest tube drainage remain similar to biventricular controls of both age groups, unlike the significant rise over time observed in Fontan patients. Inflammation within the chest tube drainage is likely not innate to single ventricle patients.

Mortality and morbidity after single ventricle palliation have improved significantly in the modern era, particularly once the palliation was staged to include the bidirectional Glenn or hemi-Fontan procedure which involves superior cavopulmonary connection.[1,2] One such improvement was a decrease in post-operative pleural effusions after the Fontan procedure in patients who had undergone a prior superior cavopulmonary connection.[2,3] The duration of chest tube drainage is shorter in superior cavopulmonary connection patients (2–5 days) as compared to Fontan patients (7–14 days). Prolonged chest tube drainage remains a dominant morbidity after Fontan procedure in 12–45% of patients.[4]

While there is an abundance of literature surrounding post-operative chest tube drainage in Fontan patients, there are very few studies investigating post-operative chest tube drainage in patients after the superior cavopulmonary connection. It remains unclear what the dominant aetiology for prolonged chest tube drainage is in Fontan patients, though hypotheses are often multifactorial and include elevated central venous pressure, inadvertent surgical disruption of lymphatic channels during the Fontan operation, abnormalities of the lymphatic channels themselves, or exaggerated inflammation both systemically and within the pleural space.[5–10] Risk factors for a longer duration of drainage in superior cavopulmonary connection patients also include elevated central venous pressure and transpulmonary gradient, and no one has yet investigated inflammation within the chest tube drainage in this population.[11,12] Current treatment strategies aimed at the above aetiologies are lacking in efficacy or not widely available, thus further delineating mechanisms for prolonged chest tube drainage in the single ventricle population is warranted.[5,13,14]

This study aims to investigate the inflammatory profile in the chest tube drainage after superior cavopulmonary connection for single ventricle heart disease. We also compared localised inflammation within the chest tube drainage after the superior cavopulmonary connection to a

previously published Fontan cohort, in an effort to understand the characteristics of single ventricle chest tube drainage.

Materials and method

All consecutive patients undergoing a superior cavopulmonary connection (either hemi-Fontan or bidirectional Glenn) operation performed at the University of Michigan between October 2017 and October 2019 were approached for consent. Similarly, to serve as control patients, all bi-ventricular patients undergoing cardiopulmonary bypass surgery within the same age range (3–10 months of age) at the same institution and time frame were approached for consent. Patients were consecutively approached until enrolled patients reached 25 superior cavopulmonary connection patients and 10 control patients. The data were also compared to a historical cohort of 25 patients undergoing the Fontan operation and 15 biventricular patients undergoing cardiopulmonary bypass surgery within the same age range (2–5 years of age – who acted as Fontan controls). These patients underwent identical sampling protocol in 2016.[10] This study received institutional review board approval. There were no exclusion criteria.

Chest tube fluid samples were taken in a sterile fashion on post-operative days 1–4, 7, 10, 14 and weekly thereafter until chest tubes were removed. Chest tube removal was based on a decision by the clinical team; standard removal criteria at this institution were output at ≤1 ml/kg/8 hours for two consecutive 8-h shifts. The institution changed its practice to align with the Pediatric Acute Care Cardiology Collaborative (PAC3) chest tube initiative,[15] and thus the last three patients enrolled in the study had chest tubes removed at 48 hours as long as their chest tubes had < 10 ml/kg/day. Samples were not collected after additional procedures in the chest. Blood samples were collected prior to bypass and on post-operative days 1 and 8. Inflammatory cytokines and lipid profiles were obtained from both pleural drainage and blood.

Pleural fluid was assayed using the Bio-Rad Bio-Plex ProHuman Cytokine 17-plex Assay (#M5000031YV) on the Bio-Rad MAGPIX Multiplex Reader for the following inflammatory cytokines: IL-1β, IL-2, IL-4 through 8, IL-10, IL-12, IL-13, IL-17A, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), interferon-gamma (IFN-γ), monocyte chemoattractant protein (MCP-1), and macrophage inflammatory protein (MIP-1β).[16,17] Assay values below the limit of detection for a given cytokine were set to 0.01 pg/ml below the limit of detection value of that cytokine.

Clinical information gathered included patient demographics, prior cardiac operations, prolonged effusion or chylothorax after any prior operation, pre-operative heart catheterisation data, and pre-operative echocardiogram findings including atrioventricular valve regurgitation and ventricular function via myocardial performance index and qualitative grading. Intraoperative data collected included the surgical procedure, bypass, cross clamp, deep hypothermic circulatory arrest times, and use of intra- or peri-operative steroids. Post-operative data included total daily pleural fluid output, total chest tube days, and presence of chyle. Chyle was defined by an institutional consensus: an increase in chest tube output with milky appearance after initiation of post-operative feeds. It was further assessed by pleural triglyceride level which aided in the diagnosis of chylothorax when pleural triglycerides > serum triglycerides. Lymphocytes were not used in the definition of chyle. Post-operative mechanical ventilation time, length of stay, and daily maximum vasoactive-inotropic score within 2 hours and 24 hours of the operation were recorded.[18] Complications were recorded including the need for extracorporeal membrane oxygenation, renal replacement therapy, infection, unplanned surgical procedure or catheterisation, cardiopulmonary resuscitation, and death. Treatment for pleural drainage or chylothorax was recorded including periods of nil per os status, total parenteral nutrition, octreotide infusion, thoracic duct ligation, and pleurodesis.

Statistical design

Data are reported using mean ± SD for normally distributed continuous variables, median, and interquartile range for non-normally distributed continuous variables, and frequency (%) for categorical variables. Univariate comparisons were made in patient characteristics, peri-operative characteristics, and cytokine concentrations between superior cavopulmonary connection patients, superior cavopulmonary controls, Fontans, and Fontan controls, using Student's t-test, Wilcoxon rank sum test, Chi-square test, or Fisher's exact test, as appropriate. Wilcoxon signed rank test was used to compare cytokine concentration within each patient for post-operative day 1 compared to the last chest tube day for which cytokine assay data were collected. Absolute change was calculated as the difference in cytokine concentration from post-operative day 1 to last chest tube day for each patient, and this was compared for patients following superior cavopulmonary connection versus superior cavopulmonary controls, superior cavopulmonary connection versus Fontan, and Fontan versus Fontan controls using Wilcoxon rank sum test. All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC). A p value < 0.05 was considered statistically significant. Only the cytokines that showed significant differences (p-value <0.05) in comparisons of concentrations between cohorts are reported.

Results

Demographics

Superior cavopulmonary connection patients and their controls

In the 25 superior cavopulmonary connection patients and 10 superior cavopulmonary control patients, there were no group differences in gender, race, or other organ abnormalities (Table 1). The superior cavopulmonary control patients had a higher proportion of chromosomal abnormalities as compared to superior cavopulmonary connection patients ($p = 0.001$, Table 1). The superior cavopulmonary connection patients were slightly older than their controls. Of the 25 superior cavopulmonary connection patients, 92% had at least one prior operation, whereas only 20% of the superior cavopulmonary controls had at least one prior operation – a reflection of single ventricle heart disease typically requiring a neonatal operation.

Fontan patients and their controls

As previously published, there were no differences between the 25 Fontan and 15 Fontan control patients in gender, race, chromosomal abnormalities, or other organ abnormalities (Table 1).[10] The Fontan patients were slightly younger than their controls. Similarly to above, all 25 Fontan patients had at least one prior operation, whereas 31% of Fontan control patients had a prior operation, again as a reflection of the staged palliation of single ventricle heart disease.

The single ventricle cohorts were made up of predominantly hemi-Fontan operations (84%) and fenestrated lateral tunnel Fontan operations (84%), which was the standard institutional

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Table 1. Demographics, pre-, and intra-operative characteristics

	Superior cavo-pulmonary connection (n = 25)	Superior cavo-pulmonary control (n = 10)	p-value	Fontan (n = 25)	Fontan control (n = 15)	p-value	p-value Superior cavo-pulmonary connetion versus Fontan
Demographics							
Male	13 (52.0)	6 (60.0)	0.72	18 (72.0)	7 (46.7)	0.11	0.15
Caucasian	19 (76.0)	9 (90.0)	1.00	22 (88.0)	11 (73.3)	0.39	0.70
Chromosomal abnormality	0 (0.0)	5 (50.0)	**0.001**	1 (4.0)	3 (20.0)	0.14	1.00
Other organ abnormality	11 (44.0)	5 (50.0)	1.00	15 (60.0)	8 (53.3)	0.68	0.26
Age at surgery, years	0.4 (0.4–0.5)	0.4 (0.3–0.4)	**0.02**	2.5 (2.1–2.9)	3.7 (2.8–3.9)	**0.02**	<**0.0001**
Primary diagnosis			**0.01**			0.0003	0.78
HLHS	13 (52.0)	0 (0.0)	–	14 (56.0)	0 (0.0)	–	–
Not HLHS	12 (48.0)	10 (100.0)	–	11 (44.0)	15 (100.0)	–	–
Pre-operative characteristics							
MPI	0.47 ± 0.15	0.34 ± 0.11	**0.02**	0.42 ± 0.11	0.30 ± 0.13	**0.003**	0.14
Ventricular EDP; mmHg							
LVEDP	8 (6–10) n = 7	N/A	–	8 (5–8) n = 3	7 (6–15) n = 3	–	0.58
RVEDP	8 (5–9) n = 13	9	–	6 (5–8) n = 21	9 (6–10) n = 3	–	0.32
SVC pressure; mmHg	6 (5–7) n = 17	6	–	11 (10–12) n = 24	Range 2–7 n = 2	–	<**0.0001**
TP gradient; mmHg	7.1 ± 2.5 n = 16	N/A	–	4.7 ± 1.0 n = 24	7.33 ± 1.15 n = 3	–	**0.002**
Intra-operative characteristics							
CPB time, minutes	89 (80–118)	75.5 (56–112)	0.19	57 (53–73)	61 (43–110)	0.92	<**0.0001**
Cross-clamp time, minutes	29 (17–37)	37 (25–72)	0.10	25 (19–29)	26 (16–56)	0.17	0.20
Peri-operative steroids	3 (12.0)	2 (20.0)	0.61	1 (4.0)	1 (6.7)	1.0	0.61

Values are presented as n (%) for categorical variables and median (interquartile range) or mean ± SD for continuous variables.
p-values are from Chi-square test or Fisher's exact test for categorical variables and Wilcoxon rank sum test or Student's t-test for continuous variables.
CPB = cardiopulmonary bypass; LVEDP = left ventricular end diastolic pressure; RVEDP = right ventricular end diastolic pressure; HLHS = hypoplastic left heart syndrome; MPI = myocardial performance index; SVC = superior vena cava.
Bold indicates p-value < 0.05.

approach. The majority of single ventricles had a diagnosis of hypoplastic left heart syndrome (52% superior cavopulmonary connection patients, 56% Fontan patients, Table 1).

Clinical characteristics

Pre-operative myocardial performance index was higher in both superior cavopulmonary connection patients and Fontan patients compared to their respective controls ($p = 0.02$ and $p = 0.003$[10] respectively), but similar when compared with each other ($p = 0.14$); i.e., ventricular function was significantly worse in the single ventricle patients (Table 1). Superior cavopulmonary connection patients had lower pre-operative superior vena cava pressures as compared to Fontan patients ($p < 0.0001$, Table 1). Superior cavopulmonary connection patients had longer bypass times than Fontan patients ($p < 0.0001$, Table 1). There was no difference in the use of peri-operative steroids among any of the groups. Of note, steroids are not standard of care in peri- or post-operative bypass patients at this institution. Median chest tube duration in the Fontan group was significantly longer as compared to superior cavopulmonary connection patients ($p < 0.0001$), as well as compared to their controls[10] (Table 2). Hospital length of stay in the Fontan patients was similar when compared with the superior cavopulmonary connection patients ($p = 0.91$, Table 2). Fontan patients had higher proportion of high-output effusions as compared to superior cavopulmonary connection patients ($p = 0.01$) or Fontan controls ($p = 0.02$,[10] Table 2). The Fontan group had 3 (12%) patients develop prolonged chest tube drainage (>14 days), and there were none in the other three groups.

Pleural inflammatory cytokines

Of the 25 superior cavopulmonary connection patients and 10 superior cavopulmonary control patients, one superior cavopulmonary connection patient, and 2 superior cavopulmonary control patients were excluded from cytokine analysis due to the absence of a sample on post-operative day 1. Chest tube cytokines were assayed in all Fontan patients and 11 of the 15 Fontan controls. Cytokine concentrations were higher in superior cavopulmonary connection patients compared to Fontan patients on post-operative day 1 (Fig 1a and b; $p \leq 0.01$ for IL-1β, IL-2, IL-4, IL-5, and IFN-γ). Cytokine concentrations in superior cavopulmonary connection patients are similar to their age-similar controls on post-operative day 1 (Fig 1c and d). IL-6, a variable pro- and

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Table 2. Post-operative characteristics

	Superior cavopulmonary connection (n = 25)	Superior cavopulmonary control (n = 10)	p-value	Fontan (n = 25)	Fontan control (n = 15)	p-value	p-value Superior cavo-pulmonary connection versus Fontan
Chest tube characteristics							
Chest tube duration, days	4 (3–5)	4 (2–4)	0.40	10 (7–11)	3 (3–3)	**<0.0001**	**<0.0001**
Chest tube output, ml/kg/day	12.4 (8.8–16.4)	12.9 (10.4–15.1)	0.83	13.4 (10.3–16.36)	6.2 (4.9–10.5)	**0.0002**	0.84
High output pleural effusion (>20 ml/kg/day on any POD after POD 2)	3 (12.0)	1 (10.0)	1.00	11 (44.0)	1 (6.7)	**0.02**	**0.01**
Presence of chyle	4 (16.0)	0 (0.0)	0.3	3 (12.0)	0 (0.0)	**0.28**	1.00
Pleural TG on POD1	38.5 (28.5–55)	38 (31–52)	0.94	38 (34–43)	39 (32–48)	**0.95**	0.88
Treatment for pleural drainage							
Low-fat diet	4 (16.0)	0 (0.0)	0.30	1 (4.0)	0 (0.0)	1.0	0.35
NPO	2 (8.0)	0 (0.0)	1.00	1 (4.0)	0 (0.0)	1.0	1.00
Hospital characteristics							
Hospital LOS	9 (7–27)	8 (6–17)	0.47	11 (9–13)	4 (4–5)	**<0.0001**	0.91
Unplanned surgical procedure or catheterisation	8 (32.0)	0 (0.0)	0.07	4 (16.0)	1 (6.7)	0.63	0.19
Readmission for intervention/re-accumulation of pleural fluid	2 (8.0)	2 (20.0)	0.56	2 (8.0)	1 (6.7)	1.0	1.00
Mechanical ventilation required	12 (48.0)	8 (80.0)	0.13	4 (16.0)	1 (6.7)	0.63	**0.02**
Diaphragm plication	1 (4.0)	0 (0.0)	1.00	3 (12.0)	0 (0.0)	0.28	0.61
Highest VIS in 1st 24 hours	5 (3–9)	10 (5–11)	0.34	8 (5–17)	5 (3–5)	**0.08**	0.14

Values are presented as n (%) for categorical variables and median (interquartile range) for continuous variables.
p-values are from Chi-square test or Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables.
LOS, length of stay; NPO, nil per os; POD, post-operative day; TG, triglyceride; VIS vasoactive inotropic support.
Bold indicates p-value < 0.05.

anti-inflammatory cytokine, had higher concentrations in superior cavopulmonary connection patients and their age-similar controls as compared to both Fontan patients and Fontan controls. Pro-inflammatory cytokines IL-8, MIP-1β, and TNF-α were not significantly different on the last chest tube day compared to post-operative day 1 in superior cavopulmonary connection patients or either control patients, but were significantly higher in Fontan patients (Fig 2, all $p < 0.0001$ for Fontan patients). When comparing superior cavopulmonary connection patients to Fontan patients, the change over time was significantly different in MIP-1β and TNF-α ($p < 0.01$, Fig 2). Trends over time are similar in superior cavopulmonary connection patients to both control groups (Fig 2). There was one superior cavopulmonary anastomosis patient who drained longer than 7 days (drained for 13 days), and this patient had persistently elevated TNF-α concentrations.

Serum versus pleural cytokine levels

Superior cavopulmonary connection patients had significantly higher concentrations of pleural cytokines (IL-6, IL-8, and MIP-1β) as compared to serum on post-operative day 1, similar to Fontan patients and Fontan controls (Supplementary Table). Levels of TNF-α were higher but did not reach significance in superior cavopulmonary connection patients. The superior cavopulmonary control patients had no significant difference (Supplementary Table).

Discussion

As long as 40 years ago, it has been reported that the addition of staged palliation (hemi-Fontan or bidirectional Glenn) prior to completion of a Fontan is beneficial in decreasing post-operative pleural effusions after the Fontan operation.[2,3] However, to date, there is limited data on describing superior cavopulmonary connection chest tube drainage, and no data on the inflammatory cytokines. Our study highlights a gap in the current knowledge regarding inflammation in post-operative paediatric cardiac patients, as this is the first to describe the cytokine profile within the chest tube drainage in patients after superior cavopulmonary connection, the staged palliation for single ventricle heart disease. We have three main findings. First, patients after a superior

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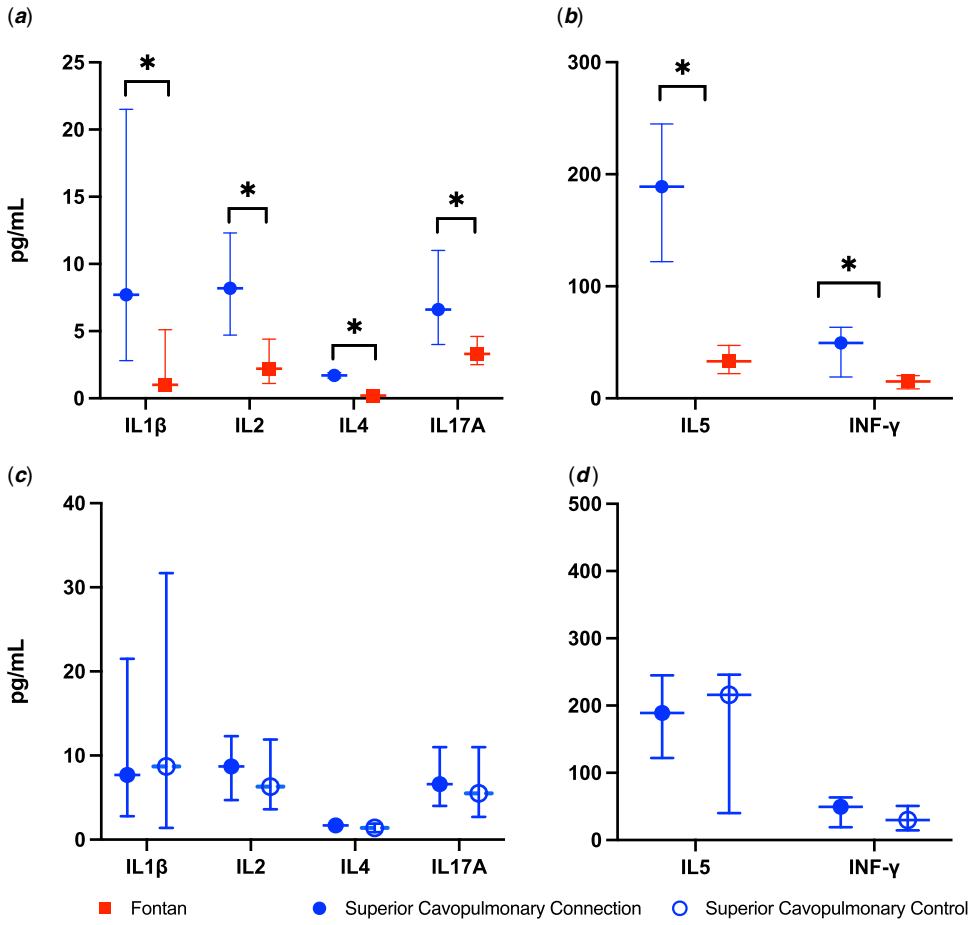

Figure 1. Comparison of cytokine concentrations on postoperative day one. (***a*** and ***b***) Concentration of cytokines IL-1β, IL-2, IL-4, IL-5, IL-17A, and INF-γ are higher in superior cavopulmonary connection patients (N = 24) as compared to Fontan patients (N = 25). (***c*** and ***d***) Concentration of cytokines IL-1b, IL-2, IL-4, IL-5, IL-17A, and INF-γ have no difference between superior cavopulmonary connection patients and superior cavopulmonary control patients (N = 8). Data represents median with interquartile range (IQR). The symbol * represents $p \leq 0.01$. IL = interleukin; INF-γ = interferon gamma.

cavopulmonary connection have inflammatory chest tube drainage characteristics similar to patients with two ventricles undergoing cardiopulmonary bypass. Superior cavopulmonary connection patients have a similarly short duration of chest tube drainage that is characterised by cytokines that downtrend over time, comparable to patients with two ventricles. This is in stark contrast to post-operative Fontan patients, who have a longer duration of chest tube drainage and rising pro-inflammatory cytokines over time.[10] Second, the high initial inflammatory cytokine profile within the chest tube drainage in superior cavopulmonary connection patients is also comparable to their age-similar controls. Thus, this elevation on post-operative day 1 is likely a result of their younger age. Our third main finding demonstrated a higher concentration of proinflammatory cytokines within the pleural space as compared to serum, indicating a localised inflammatory process.

The superior cavopulmonary connection patients described here had a similarly short duration of chest tube drainage (4 days) when compared with their age-similar controls (4 days) and the older Fontan-aged controls (3 days). It is well known that Fontan patients have prolonged chest tube drainage as compared to other paediatric post-cardiac surgery cohorts, and this was again confirmed here with the median duration of drainage in historical Fontan patients significantly longer than any of the other cohorts at 10 days. Furthermore, there were no patients in our superior cavopulmonary connection cohort or either control cohort who had prolonged pleural effusions (> 14 days), which was seen in 12% of the Fontan patients. One superior cavopulmonary anastomosis patient drained for 13 days.

The focus on chest tube drainage in post-operative patients is due to the effect on prolonged hospital stay, higher cost of hospitalisation, the lack of effective treatment strategies, and the diminished short- and long-term survival seen in Fontan patients with this condition.[19–21] Currently, treatment for prolonged chest tube drainage includes aggressive diuresis, fluid restriction, low fat diet, parenteral nutrition, and time. Octreotide administration, thoracic duct ligation, pleurodesis, and targeted lymphatic therapies are reserved for refractory drainage with variable results, and some may not be widely available.[5,13,21–23] Understanding the mechanism of prolonged effusions could lead to more effective treatment strategies. There have been no studies to date investigating the inflammatory profile after a superior cavopulmonary connection for single ventricle heart disease, and to our knowledge, Goldstein et al remains the only examination of cytokines within the Fontan chest tube drainage.[10] With our finding of increased pro-inflammatory cytokines localised to the chest tube drainage in a population that has prolonged chest tube drainage, this may be a novel target for therapy to reduce the duration of drainage.

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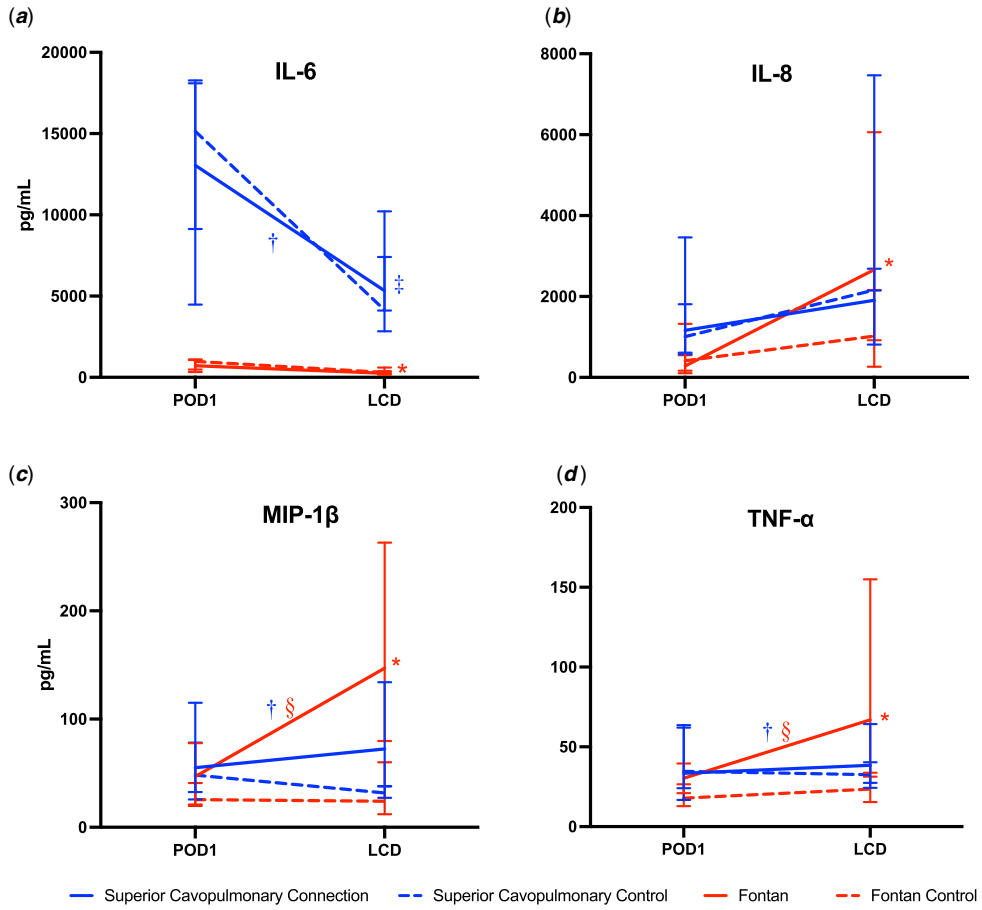

Figure 2. Change in pleural cytokine concentration from postoperative day 1 to last chest tube day. IL-8 (***b***), MIP-1β (***c***), and TNF-α (***d***) increase significantly over time in patients following Fontan (*p < 0.0001). IL-6 (***a***), a variable pro- and anti-inflammatory cytokine, decreases significantly over time in Fontan (*p < 0.001) and superior cavopulmonary connection patients (‡ p < 0.0001). The trend approaches significance in superior cavopulmonary control patients (p = 0.06). None of the above pro-inflammatory cytokines (***b, c or d***) show a significant change over time among controls. Change over time (via Wilcoxon signed rank test) is significantly greater for Fontan vs. superior cavopulmonary connection patients († p ≤ 0.01). Change over time (via Wilcoxon signed rank test) is significantly greater for Fontan vs. Fontan controls (§ p < 0.004). Data represents median with interquartile range (IQR). LCD = last chest tube day; IL = interleukin; MIP-1β = macrophage inflammatory protein beta; POD = postoperative day; TNF-α = tumor necrosis factor alpha.

Our study refutes the hypothesis that single ventricle disease alone leads to rising inflammation within the post-operative chest tube drainage and supports the hypothesis that inflammation may be related to prolongation of the effusions.

Our study also demonstrates a concentration of inflammatory cytokines within the chest tube drainage, similar to non-cardiac effusions such as malignant pleural effusions after thoracotomy, non-cardiac pleural effusions in children, prenatal chylothorax, and hip effusions after total hip replacement.[24–27] We showed a localised inflammatory response out of proportion to systemic inflammation in nearly all groups of patients, and we suspect the reason for statistical insignificance in the superior cavopulmonary control patients was secondary to inadequate power. The pleural space appears to be a location of concentrated inflammation, regardless of the surgery a child undergoes.

Our study along with the prior publication clearly shows that Fontan patients are an outlier in both duration of chest tube drainage and rising localised proinflammatory cytokines. An altered lymphatic system is one possible explanation for this difference. Raised mean capillary hydrostatic pressure, decreased reabsorption of interstitial fluid, failure of capillary filtration equilibrium, lymphangiectasia, and lymphangiogenesis have been described in Fontan patients.[28–30] The lymph fluid itself is immunomodulatory. Cytokines cause lymphatic endothelial cells to express interleukins, tumour necrosis factors, and adhesion molecules.[31–33] Cytokine blockade (such as interleukin receptor antagonists or TNF-α inhibitors) can decrease lymphatic branching, decrease the formation of abnormal valve-less lymph vessels, and has been shown to improve the drainage of ascites.[34] The Fontan circulation may create a detrimental feedback loop of abnormal lymphatics and increased inflammation. More investigation is required to elucidate this potential mechanism further and to determine if cytokine blockade may be able to break this deleterious cycle.

This study is limited by its small sample size. Control groups, in particular, were small and some controls were excluded from cytokine analyses due to a lack of samples. Due to power limitations, we were not able to perform a multivariable analysis which would have been able to account for potential confounders such as elevated transpulmonary gradient, elevated superior vena cava pressure, prolonged mechanical ventilation, or elevated end diastolic pressure. The majority of our cohorts, however, had normal values for the above. The Fontan cohort, however, did have a higher pre-operative superior vena cava pressure as compared to the other groups, and further studies should account for this difference. Our

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study also is limited in that it is not a mechanistic study. We have been able to show elevated and increasing pro-inflammatory cytokines in post-operative Fontan chest tube drainage, and these Fontan patients also have prolonged chest tube drainage as compared to age-matched controls, superior cavopulmonary connection cohort, and superior cavopulmonary controls. In this study, we are not, however able to mechanistically connect the two. This study also had very few participants with pleural drainage > 14 days. This is both a limitation and a strength. We could not analyse associations between prolonged pleural drainage and clinical modifiers or cytokine levels. However, this allowed us to better describe the baseline cytokine composition. Finally, the last chest tube day was clearly different between the Fontan group and the other groups. The investigators felt that comparing these time points was most representative of the disease state of effusions just prior to chest tube removal, but they are not equal comparisons.

Our study is the first to show that the pro-inflammatory cytokine profiles within the chest tube drainage in superior cavopulmonary connection patients are more similar to two different control cohorts with biventricular physiology, than it is to Fontan patients. Thus, this difference in the inflammatory response within the chest tube drainage after a Fontan procedure is likely not innate to single ventricle patients. Further mechanistic work is needed to delineate the effect of cytokines on post-operative chest tube drainage, and larger studies are needed to determine if cytokines may play a role in prolonged chest tube drainage or chylothorax. Targeting cytokine blockade or inflammatory pathways may be a novel therapeutic approach to decrease the duration of chest tube drainage in Fontan patients.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951122001913.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation (Title 45 of the Code of Federal Regulations) and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees (the University of Michigan Institutional Review Board).

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