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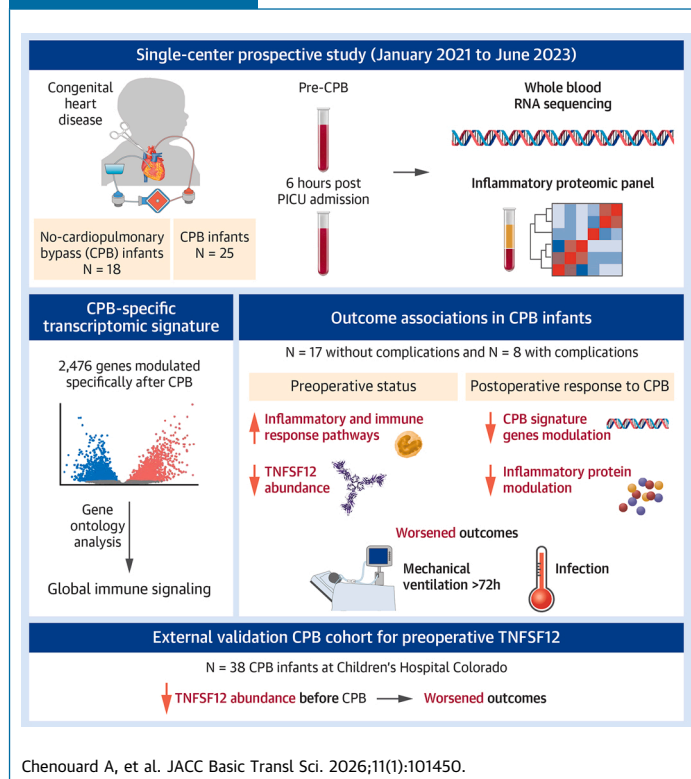
ORIGINAL RESEARCH - CLINICAL

Immune Unresponsiveness to Cardiopulmonary Bypass Is Associated With Unfavorable Outcome in Infants Requiring Cardiac Surgery



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VISUAL ABSTRACT



HIGHLIGHTS

- The inflammatory host response induced by CPB remains a key factor to be considered to improve postoperative outcomes in children.
- We hypothesized that the whole blood transcriptomic response after CPB in children would provide new insights into the pathophysiological mechanisms of CPB-related postoperative complications.
- We identified a CPB-related transcriptomic signature of 2,476 genes, primarily involving immune signaling pathways.
- A global immune unresponsiveness to CPB was observed in infants who will develop postoperative complications.
- Plasma abundance of TNFSF12 may serve as a potential predictor of CPB-related postoperative complications.

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**ABBREVIATIONS
AND ACRONYMS****CPB** = cardiopulmonary bypass**DEG** = differentially expressed genes**HAI** = health care-associated infections**MV** = mechanical ventilation**PCA** = principal component analysis**PICU** = pediatric intensive care unit**VIS** = vasoactive inotropic score**SUMMARY**

Cardiopulmonary bypass (CPB) induces a complex immune response which can lead to complications after pediatric cardiac surgery. We hypothesized that analyzing the whole blood transcriptomic response after cardiac surgery with or without CPB in children would provide new insights into the pathophysiological mechanisms of CPB-related postoperative complications. In this study focusing on infants ≤ 3 months of age requiring CPB who are more likely to develop postoperative complications, we defined an immune transcriptomic signature related to CPB. Among the genes included in the CPB signature, we identified only 18 genes that were differentially expressed after surgery in patients with a complicated outcome. In contrast, more than 2,000 genes were differentially expressed before and after the surgery in the uncomplicated group. We demonstrated the predictive potential of the preoperative plasma TNFSF12 abundance to identify patients at risk of CPB-induced complications, which was confirmed in an external cohort. (JACC Basic Transl Sci. 2026;11:101450) © 2026 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Cardiopulmonary bypass (CPB) is a critical technology in cardiac surgery, enabling the temporary replacement of heart and lung functions during surgical procedures. The initiation of CPB triggers an immediate and complex inflammatory host response, involving various humoral and cellular components.^{1,2} The advanced stage of the CPB-induced inflammatory response can lead to hyperinflammation and immune dysfunction, resulting in complications such as organ failure and increased susceptibility to infections.¹⁻⁴ Limiting immune dysregulation following CPB has been proposed as a strategy to improve patient outcomes and reduce health care costs.⁵ Numerous approaches have been evaluated in pediatric cardiac surgery with varying degrees of efficacy, including pharmacological agents (eg, glucocorticoids,⁶ aprotinin,⁷ and nitric oxide⁸) and nonpharmacological procedures (eg, ultrafiltration,⁹ nonpulsatile perfusion,¹⁰ and controlled reoxygenation¹¹). The underlying heterogeneity of patients, who may respond differently to CPB, could explain the ineffectiveness of specific therapeutic trials. Therefore, a deeper understanding of the molecular mechanisms driving CPB-induced inflammation is essential to identify new pathophysiological mechanisms, define subgroups of patients at high risk, and ultimately to guide the clinical use of targeted immunomodulatory interventions. The emergence of multiomics technologies has enabled deciphering biological changes related to critical care diseases and the identification of novel disease subgroups in adults.^{12,13} To our knowledge, CPB-related inflammatory response has not been thoroughly investigated in children.^{14,15}

We hypothesized that comparing the whole blood transcriptomic response after cardiac surgery with or

without CPB in children would provide new insights into the pathophysiological mechanisms of CPB-related postoperative complications. We identified a specific CPB-related transcriptomic signature involving immune signaling pathways and studied the association between this immune signature and unfavorable clinical outcome.

METHODS

A detailed description of the methods can be found in the [Supplemental Methods](#).

STUDY POPULATIONS. The study protocol was approved by the Ethics Committee in March 2020 (France, Sud-Ouest et Outre-mer IV, reference number AM2021-114/01/CP2020-03-021) and registered in May 2020 (IMMUNOPED2 [Plasma Levels of Danger-Associated Molecular Patterns in Young Children After Cardiac Surgery Under Cardiopulmonary Bypass]; [NCT04423523](#)). This study was conducted according to the Declaration of Helsinki. Parents provided written consent for participation of infants.

Included were infants ≤ 3 months of age who required cardiac surgery with or without CPB at Nantes Hospital between January 2021 and June 2023. In cases of cardiac reoperation within the first 3 months of life, only the initial surgery was considered. Patients requiring postoperative extracorporeal membrane oxygenation, which induces significant immune dysfunctions,² were excluded from the analysis.

STANDARD OF CARE. Anesthesia protocols and surgical procedures in our center have been detailed previously.¹⁶⁻¹⁸ CPB use or not was decided based on the type of cardiopathy (ie, intracardiac repair or not)

and the comorbidity of infants (ie, low weight, prematurity). For infants requiring CPB, the size of the oxygenator and cannulas was selected based on the patient's body surface area. CPB priming included red blood cells to maintain a hematocrit of 30% to 32% during CPB. Surgery was conducted under stable pH conditions, with partial pressure of carbon dioxide maintained at 35 to 40 mm Hg, and partial pressure of oxygen at 120 to 150 mm Hg. The target pump flow rate was set at 2.4 to 3 L/m²/min at 37 °C, with the temperature potentially lowered to a minimum of 28 °C depending on the surgical procedure. No steroid was given during CPB. However, hydrocortisone (2 mg/kg every 6 hours) was routinely administered in the first 48 hours after surgery in case of low cardiac output syndrome. The decision regarding sternal closure was made intraoperatively, based on hemodynamic and respiratory conditions. Ventilator weaning and extubation practices were at the discretion of the treating clinician. The use of vasoactive inotropic medications postoperatively was summarized through a vasoactive inotropic score (VIS), which combines the doses of dopamine, dobutamine, epinephrine, norepinephrine, milrinone, and vasopressin in a single score, with a higher score indicating a greater level of cardiovascular support.¹⁹

DEFINITIONS. The primary criterion was complicated outcome defined as the occurrence of health care-associated infections (HAIs) within 30 postoperative days and/or a mechanical ventilation (MV) duration longer than 72 hours. Duration of MV corresponded to the time from arrival in pediatric intensive care unit (PICU) to successful extubation. HAIs were prospectively recorded within 30 days and defined as sepsis, pneumonia, wound infection, or mediastinitis according to criteria reported in the European Congenital Heart Surgeons Association (ECHSA) registry (see the [Supplemental Appendix](#) for definitions). HAI is a common complication following CPB and MV duration is a key patient-related outcome used to assess postoperative morbidity and recovery.⁸ Prolonged MV was defined as 72 hours or more based on a previous study²⁰ and because this threshold represented the third quartile for postoperative MV duration in the ECHSA database (2014–2020) ([Supplemental Figure 1](#), [Supplemental Table 1](#)).

BLOOD SAMPLING. For the flow cytometry analyses (n = 8), EDTA-anticoagulated blood samples (4 mL) were collected on the day of surgery before the initiation of CPB (Pre) and immediately after weaning from CPB (Post). In case of prolonged CPB duration or a delay in the start of surgery, the second sample was

collected before the weaning from CPB due to logistical constraints. For transcriptomic and proteomic analyses (n = 43), EDTA-anticoagulated blood samples (2 mL) were collected preoperatively (ie, after anesthetic induction but before skin incision) (pre) and 6 hours after PICU admission (H6). Plasma was separated by centrifugation and stored at –80 °C until proteomic analysis. PAXgene Blood RNA tube samples (0.5 mL) were also collected (pre and H6), and stored at –80 °C until RNA sequencing.

FLOW CYTOMETRY DATA ANALYSIS. Samples (pre- and post-CBP) were acquired the day following surgery on a 3-laser LSR-II flow cytometer (Becton Dickinson). Instrument settings were standardized throughout the study using 8-peak Rainbow Calibration Particles (BD Biosciences, lot number 2020173). Data were analyzed using FlowJo v10.8.1 software (BD Life Sciences). Fluorescence-minus-one controls were used to accurately distinguish between positive and negative populations. Absolute numbers of lymphoid and myeloid cell populations were estimated using automated complete blood counts. Statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software). Data are presented using median with 25th–75th percentiles or count (percentage). The Wilcoxon signed-rank test was used to compare data from pre- and post-CBP samples. Correlations between time under CPB and cytokine production were evaluated using Spearman correlation coefficients (r). Significance was defined as P value <0.05. Analysts were not blinded to CPB duration and clinical outcome.

STATISTICAL AND BIOINFORMATIC ANALYSIS. All the following analyses were conducted using the R software, version 4.3.2.²¹ The packages used and their version are listed in [Supplemental Table 2](#).

Principal component analysis. A principal component analysis (PCA) was performed on transcriptomic data from whole blood collected before (pre) or after (H6) cardiac surgery. Because of the high number of very low-expressed genes, these data follow a negative binomial distribution. Therefore, the matrix of gene counts was first transformed to ensure a constant variance along the range of mean values (variance stabilizing transformation). Then the PCA was conducted on the normalized data to obtain principal components (PCs). The PC values were presented with boxplots and statistically compared with Wilcoxon rank-sum tests.

Differential analyses. The DESeq2 package contains a built-in normalization method which accounts for differences in sequencing depth and RNA composition between samples. The comparisons of

the samples' expression profiles using this package were thus performed on the un-normalized matrix of gene counts as input. In the cases of paired comparisons (H6 vs pre), the patient identification was included as the first predictor in the design formula. For the differential analysis on proteomic data, Wilcoxon signed-rank or rank-sum test was performed depending on if the comparison was paired or unpaired, respectively. *P* values were adjusted with the Benjamini-Hochberg false discovery rate. Proteomic data being already normalized in a logarithmic scale, the log fold change value of each protein was estimated by subtracting its average abundance in one group from its average abundance in the second group. For both omics, the adjusted *P* values significance threshold was set to 0.05.

Prediction of postoperative complications among CPB patients.

The prediction of postoperative complications was performed using the preoperative plasma abundance of tumor necrosis factor superfamily member 12 (TNFSF12). Given the small sample size ($n = 25$ patients, including 17 uncomplicated and 8 complicated patients), Leave One Out Cross-Validation (LOOCV) was used for model training and evaluation. Its principle is to train a model using every sample, except one being used to test the trained model. Therefore, 25 models could be trained and tested, corresponding to the sample size. First, logistic regression models were built with the outcome as the variable to predict (uncomplicated/complicated) and the predictor as the preoperative abundance of TNFSF12 (expressed as normalized protein expression). The best probability threshold was determined by maximizing the Youden index. Therefore, the patients with a predicted probability of complications >0.35 were classified into the complicated group. Then, the receiver operating characteristic (ROC) curve and the associated area under the ROC curve (AUC) was calculated. Univariable linear regressions were performed to model the MV duration or the maximal VIS score at 72 hours as a function of the preoperative abundance of TNFSF12 in the plasma. The linearity was verified by representing the response variable (MV duration or VIS score) as a function of the TNFSF12 abundance with scatter plots, whereas the normality assumption of the model was assessed with quantile-quantile (Q-Q) plots. Linear regression model results are presented as regression coefficient (β) with 95% CI. The correlation between the predicted probability of complicated CPB and the preoperative abundance of TNFSF12 in the plasma was evaluated with Spearman correlation coefficients.

Sensitivity analysis. To explore the robustness of the results, we performed a sensitivity analysis using VIS score threshold. The CPB patients having a maximal VIS score at 72 hours higher or equal to 26, corresponding to the 75th percentile in our cohort, were classified into the complicated CPB group. For each new group, the preoperative and postoperative expression of the genes belonging to the CPB signature was compared. The relation between the preoperative abundance of TNFSF12 in the plasma and the probability of complication was assessed by logistic regression. These analyses were performed using the methods previously described for the initial grouping.

RESULTS

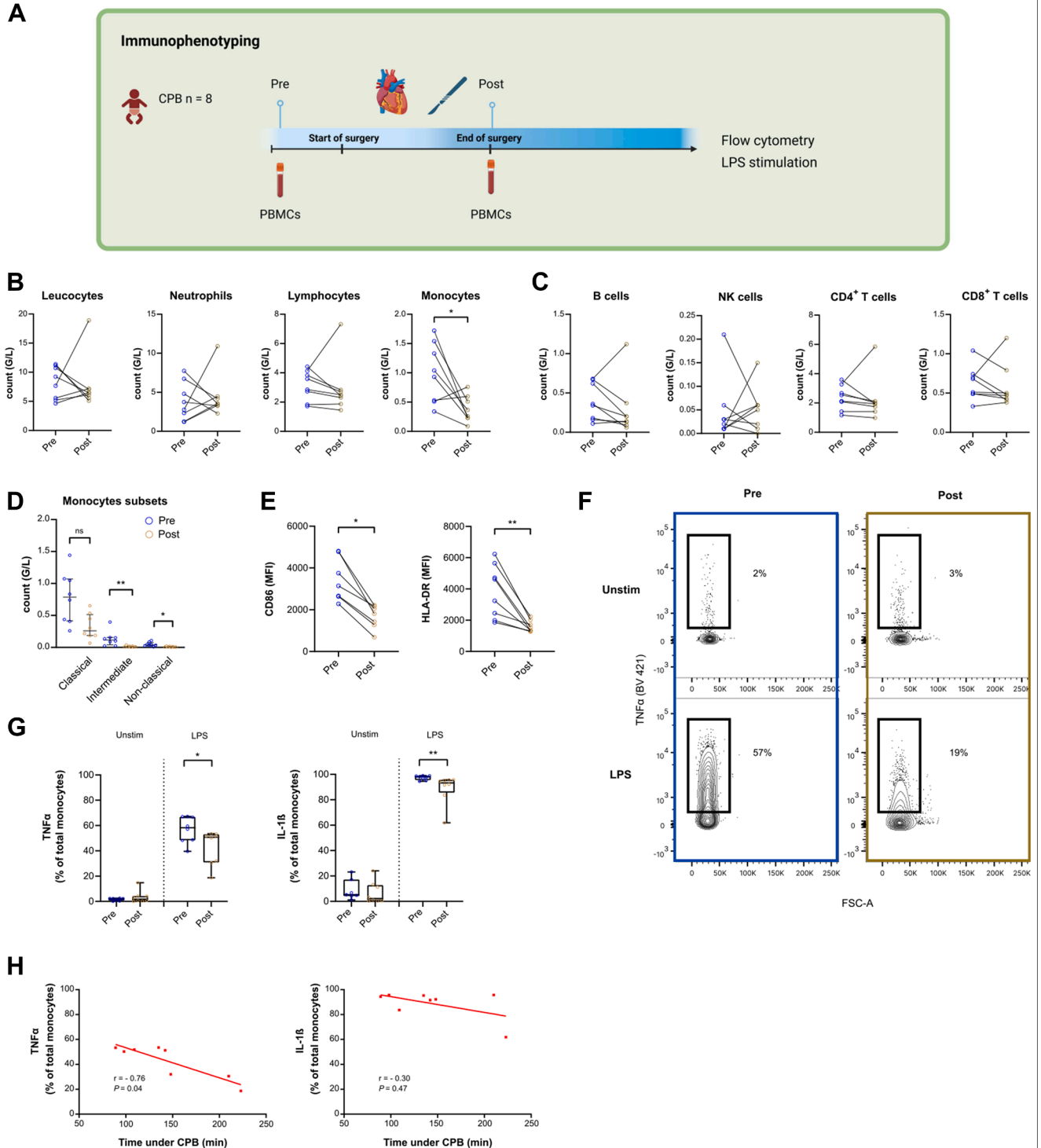
CARDIAC SURGERY WITH CPB INDUCES IMMEDIATE PHENOTYPIC AND FUNCTIONAL ALTERATIONS IN CIRCULATING MONOCYTES.

Age and CPB are known to influence the clinical outcome in children after surgery.²² From a local ECHSA database, we determined a relevant threshold on age ≤ 3 months to decipher the mechanisms underlying the CPB-induced inflammatory response in young infants (Supplemental Figure 1, Supplemental Table 1).

We first examined the cellular inflammatory immune landscape using flow cytometry on fresh isolated peripheral blood mononuclear cells (PBMCs) collected before and immediately after CPB in 8 infants ≤ 3 months of age (Figure 1A, Supplemental Tables 3 and 4). Automated complete blood cell counts revealed a significant reduction in absolute monocyte counts following CPB, from 0.99 (Q1-Q3: 0.52-1.49) G/L to 0.32 (Q1-Q3: 0.23-0.58) G/L ($P = 0.047$). In contrast, the absolute counts of total leukocytes, neutrophils and lymphocytes remained unchanged after cardiac surgery with CPB (Figure 1B). Overall, 10.5 (Q1-Q3: 7.2-13.3) million viable PBMCs were obtained at each time point for phenotyping and in vitro stimulation. Although the numbers of B cells, NK cells, CD4⁺ T cells, and CD8⁺ T cells were unaffected (Figure 1C), the absolute numbers of classical (CD14⁺ CD16⁻), nonclassical (CD14^{dim} CD16⁺), and intermediate (CD14⁺ CD16⁺) monocytes decreased significantly after the surgery with CPB (Figure 1D, Supplemental Figures 2A to 2C).

To further characterize the monocyte response to CPB, we measured the expression of surface markers CD86 (a monocyte costimulatory molecule) and HLA-DR (antigen presentation molecule) on total monocytes as well as the production of tumor necrosis factor (TNF)- α and interleukin (IL)-1 β following

FIGURE 1 Phenotypic and Functional Alterations in Circulating Monocytes After CPB



(A) Fresh peripheral blood mononuclear cells (PBMCs) were isolated from 8 infants (≤ 3 months of age) before (pre) and after (post) cardiopulmonary bypass (CPB). (B) Absolute counts of total leukocytes and different white blood cell types before and after CPB. (C) Absolute counts of B cells, NK cells, CD4⁺ T cells, and CD8⁺ T cells before and after CPB were determined using flow cytometry. (D) Monocytes' subsets were characterized using flow cytometry into classical (CD14⁺ CD16⁻), nonclassical (CD14^{dim} CD16⁺), and intermediate (CD14⁺ CD16⁺) monocytes. (E) Monocyte CD86 and HLA-DR expression were measured by mean fluorescence intensity (MFI) before and after CPB. (F) Representative plots gated of tumor necrosis factor (TNF)- α production by unstimulated (Unstim) and lipopolysaccharide-stimulated (LPS) monocytes before and after CPB. (G) TNF- α and interleukin (IL)-1 β production by monocytes in unstimulated and stimulated monocytes before and after CPB. *P* values were calculated using Wilcoxon matched-pairs signed rank test. (H) Spearman's correlation between the percentage of TNF- α and IL-1 β positive monocytes upon LPS stimulation observed after CPB and the time under CPB. **P* < 0.05, ***P* < 0.01.

TABLE 1 Clinical and Biological Features of 43 Infants Enrolled in the Study

	CPB (n = 25)	No CPB (n = 18)	P Value
Male	18 (72.0)	9 (50.0)	0.20
Age, days	13 (8-30)	26 (21-37)	0.040
Weight, kg	3.6 (3.3-4.0)	3.3 (2.4-4.0)	0.26
Down syndrome	4 (16.0)	0 (0.0)	0.13
RACHS-1 score ^a			<0.001
Categories 1-2-3	8 (32.0)	18 (100.0)	–
Categories 4-5-6	17 (68.0)	0 (0.0)	–
Cyanotic cardiopathy	8 (32.0)	1 (5.6)	0.060
Prostaglandin E1 infusion	13 (52.0)	4 (22.2)	0.064
Preoperative blood values, g/L			
Total leucocytes	9.1 (7.0-11.9)	9.4 (8.4-10.6)	0.96
Lymphocytes	4.0 (3.1-5.1)	5.1 (4.1-5.9)	0.068
Neutrophils	3.2 (2.5-4.1)	2.5 (2.2-3.0)	0.20
Monocytes	0.9 (0.6-1.6)	1.0 (0.8-1.2)	0.63
IgG	6.4 (5.3-7.3)	4.9 (3.4-6.4)	0.039
CPB duration, minutes	147 (123-190)	–	–
Sternum left open	9 (36.0)	0 (0.0)	0.006
Postoperative steroids ^b	20 (80.0)	2 (11.1)	<0.001
MV duration, hours	37 (22-96)	23 (11-25)	0.011
MV duration >72 h	8 (32.0)	1 (5.6)	0.057
HAI	5 (20.0)	2 (11.1)	0.68
PICU stay (days)	7 (5-10)	4 (3-6)	0.001
30-d mortality	0 (0.0)	1 (5.6)	0.42

Values are median (Q1-Q3) or n (%). The nonparametric Wilcoxon rank-sum and chi square tests were used to analyze continuous and categorical variables, respectively. ^aRACHS-1: Risk Adjustment for Congenital Heart Surgery-1, a higher RACHS-1 score indicating the severity of cardiopathy. ^bSteroids (hydrocortisone, 2 mg/kg every 6 hours) were administered in the first 48 hours after surgery.

CPB = cardiopulmonary bypass; HAI = health care-associated infection; IgG = immunoglobulin G; MV = mechanical ventilation; PICU = pediatric intensive care unit.

lipopolysaccharide (LPS) stimulation of PBMCs. Immediately after CPB, we observed decreased expressions in CD86 and HLA-DR, and in TNF- α and IL-1 β productions by monocytes upon LPS stimulation (Figures 1E to 1G). Interestingly, we observed a negative correlation between the duration of CPB and the percentage of TNF- α -positive monocytes upon LPS stimulation, demonstrating the rapid endotoxin tolerance in children undergoing cardiac surgery. We observed no correlation between CPB duration and IL-1 β production (Figure 1H), suggesting that the inflammasome activation pathway was not altered during cardiac surgery with CPB. Together, these results reveal that young infants undergoing CPB display severe immune dysregulation characterized by phenotypic and functional impairments particularly within the monocyte compartment.

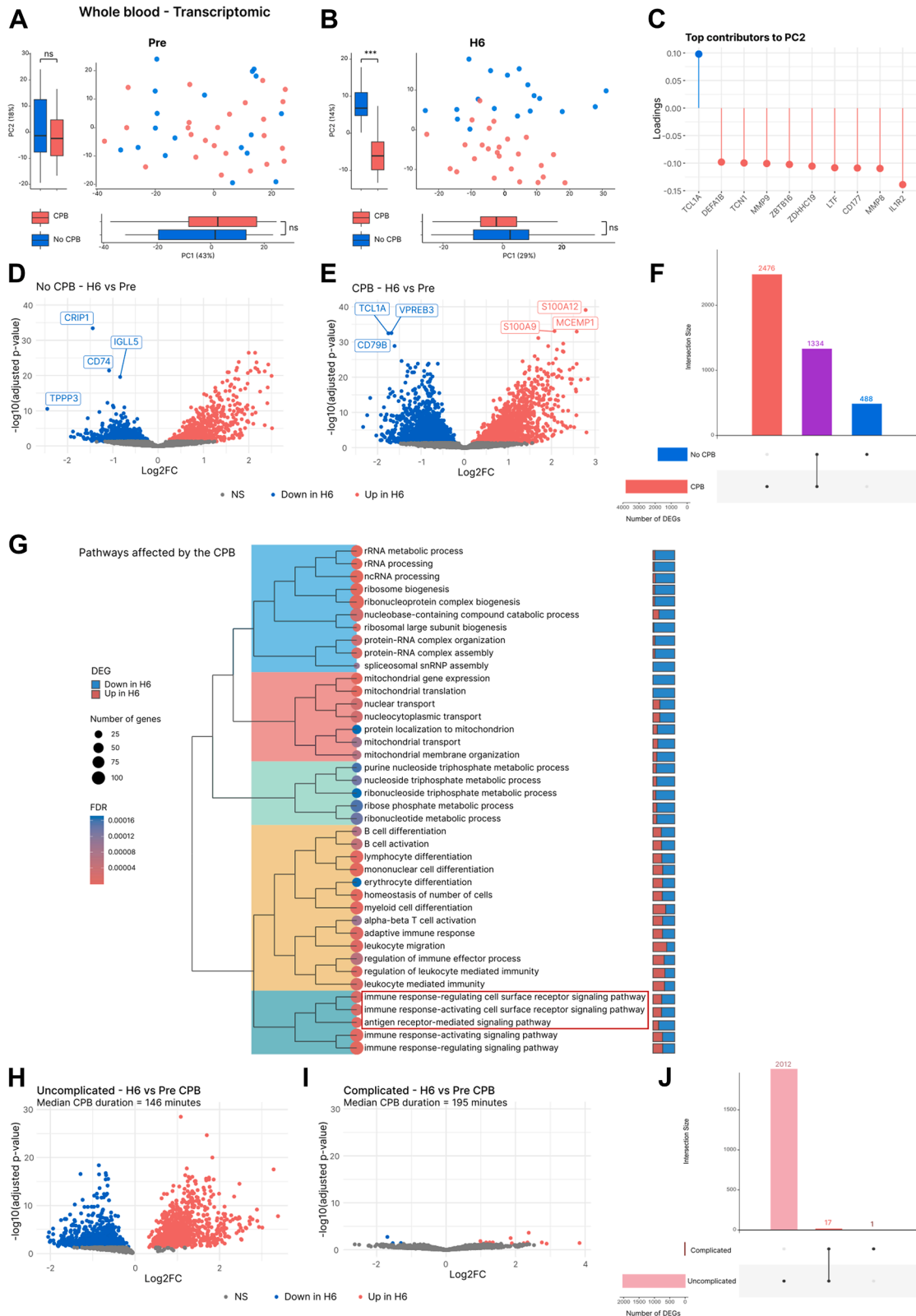
ABSENCE OF TRANSCRIPTOMIC IMMUNE RESPONSE TO CPB IS ASSOCIATED WITH CLINICAL OUTCOMES. We next investigated whether the dysregulation of immune signaling pathways was specifically related to CPB and associated with clinical outcomes in young infants. For this purpose, we performed

transcriptomic analysis of whole blood collected preoperatively (pre) and 6 hours after PICU admission (H6) in infants undergoing CPB. Although the monocyte compartment was the most impacted by the CPB, we performed the study from whole blood due to logistical constraints (ie, limited volume of blood sample). Moreover, to define the transcriptomic signature of the response to CPB, independently of the cardiac surgical procedure, infants requiring cardiac surgery without CPB were also included. Among the 43 patients analyzed, 25 required CPB with a median CPB duration of 147 (Q1-Q3: 123-190) minutes. The overall median age at surgery was 21 (Q1-Q3: 10-37) days. Four infants had an identified genetic disorder (Down syndrome), but none of them displayed infections before surgery. Only 1 patient in the no-CPB group died during the 30-days follow-up (Table 1).

We first assessed whether whole blood gene expression profiles differed before and after surgery according to the use of CPB. PCA, a common dimensionality reduction method, was used to condense the information contained in a large dataset into fewer variables while preserving the main data patterns. The newly generated variables (PCs) enable the unsupervised visualization of the samples which are similar (clusters) or distant to each other. PCA revealed no difference in preoperative transcriptomic profiles between children with or without CPB (Figure 2A). However, CPB emerged as a major determinant of the transcriptomic variability 6 hours after the surgical procedure (Figure 2B). Principal component 2 values were significantly different between CPB and no-CPB patients at H6 ($P < 0.001$), with several inflammation-related genes contributing mostly to that difference (eg, *IL1R2*, *MMP8*, *MMP9*, and *CD177* being increased in patients with CPB) (Figure 2C). Then, we aimed to define the transcriptomic signature of the response to CPB independently of the cardiac surgical procedure. We thus identified genes whose expression was modulated following surgery without CPB (Figure 2D) and with CPB (Figure 2E). Although the transcriptomic response to cardiac surgery without CPB included 1,822 genes (1,334 in common with CPB), 2,476 genes were significantly modulated in the CPB group only (1,597 and 879 genes down- and upregulated after CPB compared to the presurgical timing, respectively) (Figure 2F). These 2,476 genes were designated as the CPB-specific transcriptomic signature.

To gain insight into the biological pathways underlying this signature, Gene Ontology enrichment analysis was conducted. This analysis revealed that the genes belonging to the CPB-specific signature

FIGURE 2 Identification of a CPB-Specific Signature by Whole Blood Transcriptomic Analysis



Continued on the next page

were involved in main immune functions, notably the regulation of antigen receptor-mediated signaling pathway (Figure 2G). This reinforces the functional alteration results which notably demonstrated a reduced expression of HLA-DR and CD86 on monocytes, both being key elements in antigen presentation to T cells.

To determine whether the CPB-related transcriptomic signature was associated with an unfavorable outcome, we compared the expression of the 2,476 differential expression genes (DEGs) previously identified, before and after surgery, in CPB patients with (n = 8) and without (n = 17) complicated outcomes. Among the 8 patients in the complicated group, all had a MV duration exceeding 72 h and 4 developed HAI (2 sepsis and 2 wound infections). Patients' characteristics according to the outcome are reported in Supplemental Table 5. Surprisingly, among the genes included in the CPB signature, we identified only 18 genes that were differentially expressed after surgery in patients with a complicated outcome. In contrast, more than 2,000 genes were differentially expressed before and after the surgery in the uncomplicated group (Figures 2H to 2J). This result suggests that, although CPB induces a genomic storm with thousands of DEGs within a 6-hour timeframe, unfavorable clinical outcomes are characterized by an absence of transcriptomic immune response to CPB.

THE PREOPERATIVE INFLAMMATORY STATUS AFFECTS THE CLINICAL OUTCOME IN CPB PATIENTS. We then questioned whether this abnormal response to CPB (ie, the absence of transcriptomic changes associated with unfavorable outcome) was caused by pre-existing factors or by factors applied during the CPB procedure. To investigate this, we compared the

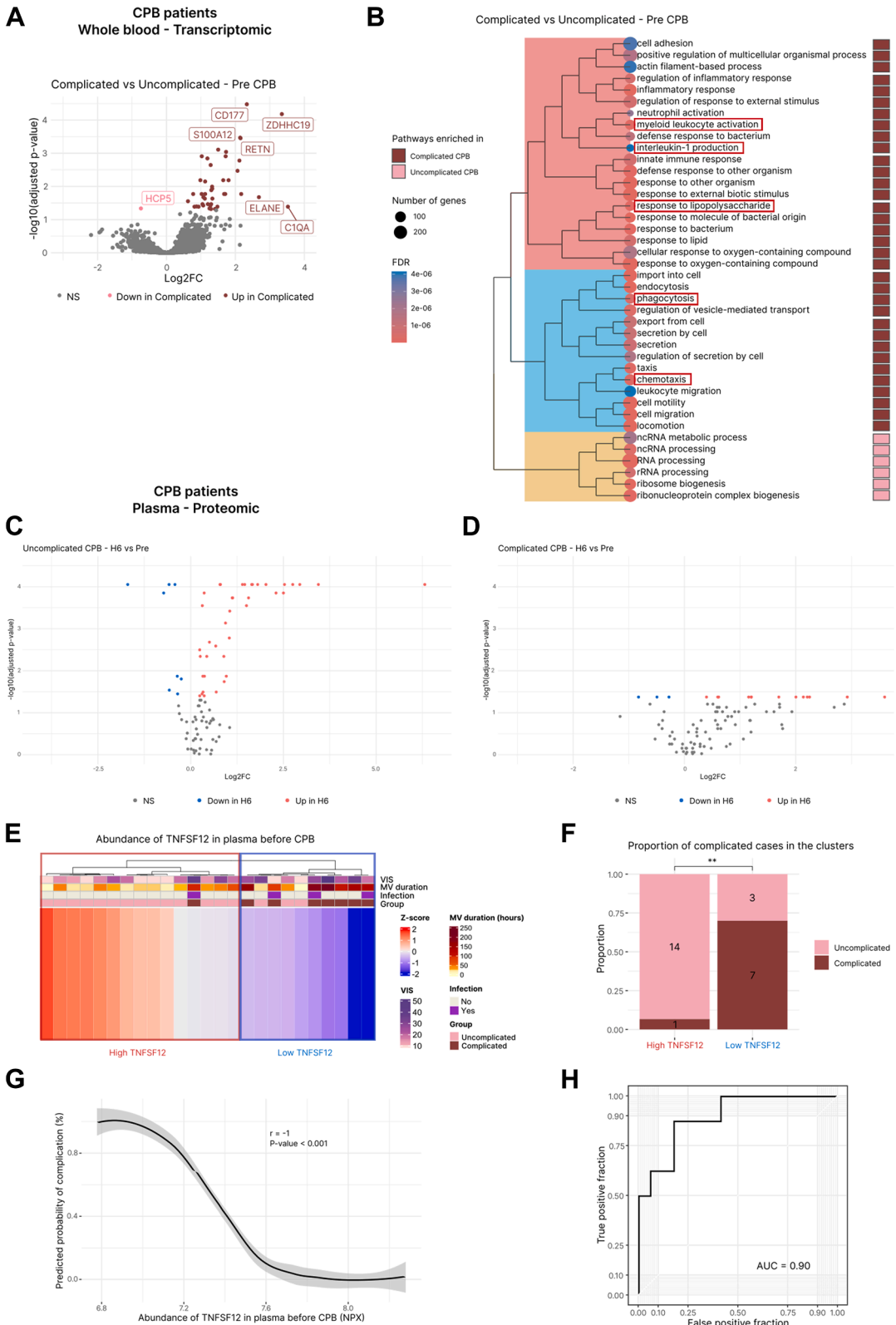
preoperative transcriptomic profiles of patients with or without complicated outcomes. Overall, we identified 44 DEGs before surgery, most of them being upregulated in patients who will develop complications compared to those who will not (Figure 3A, Supplemental Table 6). Gene Set Enrichment Analysis revealed that inflammatory and immune response pathways were enriched preoperatively in patients from the complicated group. The enriched pathways notably included processes such as activation of myeloid leukocytes, phagocytosis, chemotaxis, the response to LPS, and the production of IL-1 (Figure 3B).

To reinforce this series of transcriptomic analyses, we measured the abundance of 92 circulating plasma inflammatory-related proteins from CPB patients before (pre) and after surgery (H6) using the Olink Target 96 Inflammation assay. Differential analyses show that patients who develop complications exhibited a less marked inflammatory protein response after CPB (17 of 92 proteins significantly modulated after CPB in complicated vs 47 of 92 in uncomplicated patients) (Figures 3C and 3D), aligning with the conclusion that post-CPB complications are associated with the absence of immune response during the surgical procedure. Moreover, when comparing preoperative plasma protein abundance between complicated and uncomplicated patients, TNFSF12 was the single protein differentially abundant before CPB (adjusted $P = 0.030$, $\text{Log}_2\text{FC} = -0.6$) (Supplemental Figure 3). Hierarchical clustering of samples based on scaled TNFSF12 preoperative abundance identified 2 distinct clusters, notably 10 and 15 samples with low and high TNFSF12 abundance, respectively (Figure 3E). A Fisher exact test showed that the proportion of complicated cases was significantly higher in the low TNFSF12 cluster

FIGURE 2 Continued

(A and B) Principal component analysis (PCA) plots of the whole blood transcriptome from 25 CPB patients (red dots) and 18 no-CPB patients (blue dots) before surgery (pre) and 6 hours after pediatric intensive care unit (PICU) admission (H6). The principal component (PC) values are presented with boxplots and were compared between CPB and no-CPB patients using Wilcoxon rank-sum tests. (C) Top genes contributing to the PC2 of the PCA performed at H6. (D and E) Comparisons of the whole blood transcriptomic profiles before and after surgery in infants from the non-CPB group and from the CPB group. Grey dots indicate genes with no significant difference in expression at H6 compared to preoperative time (adjusted $P > 0.05$), red and blue dots indicate genes with significantly increased and decreased expression at H6, respectively (adjusted $P < 0.05$). (F) UpSet plot of the number of differentially expressed genes (DEGs) at H6 compared to preoperative time, in CPB and no-CPB patients (horizontal bars), and the number of DEGs exclusively observed in CPB, no-CPB patients or shared by the whole cohort (vertical bars). (G) Gene Ontology (GO) pathway enrichment analysis of the CPB-specific signature (2,476 genes). The 40 most significant pathways are represented, as well as the proportion of the mapped genes which are down- or upregulated after CPB (blue and red, respectively). (H and I) Comparisons of the CPB-specific signature gene expression in whole blood, before and after CPB, in uncomplicated and complicated patients. Grey dots indicate genes with no significant difference in expression at H6 compared to preoperative time (adjusted $P > 0.05$), red and blue dots indicate genes with significantly increased and decreased expression at H6, respectively (adjusted $P < 0.05$). (J) UpSet plot of the DEGs identified after CPB, in uncomplicated and complicated CPB patients (horizontal bars), and the number of DEGs exclusively observed in uncomplicated, complicated patients or shared by the whole CPB cohort (vertical bars). FDR = false discovery rate; NS = not significant; other abbreviations as in Figure 1.

FIGURE 3 The Preoperative Inflammatory Status Affects the Outcome in CPB Patients



Continued on the next page

($P = 0.002$) (Figure 3F). We next aimed to evaluate the predictive power of preoperative TNFSF12 abundance using logistic regression models. Given the limited population size, we trained and evaluated models with the LOOCV method. The predicted probability of postoperative complications was negatively correlated with the plasma abundance of TNFSF12 ($r = -1$, $P < 0.001$) (Figure 3G). The best probability threshold was identified by maximizing the Youden index. Therefore, the patients with a predicted probability greater than 0.35 were classified in the complicated group. The prediction had an overall accuracy of 0.84 (95% CI: 0.64-0.95), a sensitivity and specificity of 0.88 (95% CI: 0.53-0.99) and 0.82 (95% CI: 0.60-0.96), respectively, as well as an AUC of 0.90 (95% CI: 0.70-1.00) (Figure 3H).

To explore the robustness of the results, we performed a sensitivity analysis using VIS score threshold, reflecting the severity of postoperative cardiovascular failure. Patients with a VIS score higher or equal to the 75th percentile (ie, higher or equal to 26) were classified in the complicated CPB group. Therefore, the uncomplicated and complicated CPB groups were composed of 18 and 7 patients, respectively. As previously, the expression of the 2,476 genes forming the CPB signature was compared before and after surgery for each group, and a modulation of the genes' expression was mostly observed in the uncomplicated patients (Supplemental Figures 4A to 4C). The association between the preoperative abundance of TNFSF12 in the plasma and this binary outcome was also confirmed (Supplemental Figures 4D to 4F). Additionally, we found a significant negative correlation between plasma abundance of TNFSF12 and VIS score ($\beta = -12.6$; 95% CI: -22.3 to -2.9 ; $P = 0.013$) (Supplemental Figure 5A), and we noted a significant negative correlation between plasma abundance of

TNFSF12 and MV duration ($\beta = -96.1$; 95% CI: -143.6 to -48.5 ; $P < 0.001$), demonstrating that our results did not depend on the cut-off of 72 hours for MV duration (Supplemental Figure 5B).

Finally, we aimed to validate this association between preoperative TNFSF12 abundance and clinical outcomes using an external dataset from Children's Hospital Colorado ($n = 38$ neonates and infants undergoing CPB).²³ Patient demographics for this cohort include the following: age 7 (Q1-Q3: 6-70.2) days, weight 3.52 (Q1-Q3: 3.23-4.0) kg, 68% male, CPB time 150 (Q1-Q3: 134-187) minutes. As HAI data were not collected in this cohort, we tested the association of preoperative TNFSF12 blood level with MV duration and VIS score at 48 hours. We found a significant negative correlation between the preoperative amount of TNFSF12 and the VIS score at 48 hours ($\beta = -13.97$; 95% CI: -27.7 to -0.24 ; $P = 0.046$) as well as MV duration ($\beta = -265.2$; 95% CI: -502.1 to -28.3 ; $P = 0.029$) (Supplemental Figures 5C and 5D). This series of results validate the global unresponsiveness inflammatory response of complicated patients after CPB and show the predictive potential of the preoperative plasma TNFSF12 abundance to identify patients at risk of CPB-induced complications.

DISCUSSION

The transcriptomic analysis of whole blood samples from young infants less than 3 months old undergoing cardiac surgery provided new insights into the pathophysiology of the CPB-induced inflammatory response, defining specific transcriptomic and proteomic features associated with postoperative complications.

Dysregulation of the immune system is a well-documented phenomenon occurring after CPB, involving both humoral and cellular components.^{1,2}

FIGURE 3 Continued

(A) Comparison of the whole blood transcriptomic profiles before CPB, between uncomplicated and complicated patients. Grey dots indicate genes with no significant difference in expression between patient groups (adjusted $P > 0.05$), dark red and pink dots indicate genes with significantly increased and decreased expression in complicated CPB patients, respectively (adjusted $P < 0.05$). (B) Gene Set Enrichment Analysis showing the biological processes significantly depleted or enriched before surgery between the complicated and uncomplicated CPB patients (adjusted $P < 0.05$). Only the 40 most significant pathways are represented. (C and D) Comparisons of the plasma proteomic data before and after CPB in uncomplicated and complicated CPB patients. Grey dots indicate proteins with no significant difference in abundance (adjusted $P > 0.05$), red and blue dots indicate proteins with significantly increased and decreased abundance at H6, respectively (adjusted $P < 0.05$). (E) Unsupervised hierarchical clustering of the CPB patients based on the scaled (z-score) preoperative abundance of tumor necrosis factor superfamily 12 (TNFSF12) in plasma, defining 2 clusters of patients (high or low TNFSF12, $n = 15$ and 10, respectively). (F) Proportion of complicated cases among CPB patients according to the clusters identified in (E). The P value was calculated with a Fisher exact test. (G) The predicted probabilities of belonging to the complicated group as a function of the preoperative abundance of TNFSF12 in plasma. The probabilities were obtained with logistic regression models trained and evaluated by leave-one-out cross-validation using the preoperative plasma abundance of TNFSF12. The P value was calculated by testing the Spearman correlation coefficient (r). (H) The receiver operating characteristic (ROC) curve and its associated area under the curve (AUC) – evaluating the CPB-induced complication prediction. ** $P < 0.01$. Abbreviations as in Figure 1.

Based on flow cytometry experiments on PBMCs, we highlighted the critical role of circulating monocytes in response to CPB. The phenomenon of monocyte paralysis, demonstrated here by decreased expression of CD86 and HLA-DR, as well as a reduced production of TNF- α and IL-1 β upon LPS stimulation compared to preoperative period, has been widely described for decades in cardiac surgery patients as a marker for identifying patients at risk of complications.^{3,4,24-26} However, clinical implications remain uncertain,²⁷ and a deeper understanding of molecular mechanisms underlying CPB-induced inflammation with high-throughput omics technologies are likely to fill this gap.

From a cohort of 43 young infants undergoing cardiac surgery, we identified a whole-blood transcriptomic signature related to CPB, consisting of 2,476 genes specifically modulated 6 hours after the end of CPB. The control group of cardiac surgery infants without CPB enabled us to remove the nonspecific effects related to surgery or anesthesia, as several factors during surgery most likely influence the transcriptomic response.^{28,29} Pathway enrichment analysis of this CPB-specific transcriptomic signature showed strong enrichments in immune-related signaling pathways, consistent with previous transcriptomic data previously reported in adults²⁹⁻³¹ and in children.^{14,15} This signature, involving both innate and adaptive immune pathways, may reflect a physiological immune adaptation to protect tissues during CPB and counteract an expected ischemia-reperfusion sequence. Interestingly, patients who developed complications showed substantially lower modulation of CPB signature genes, suggesting a state of global immune unresponsiveness to CPB-induced stress. This transcriptomic result was strengthened by a less pronounced circulating inflammatory protein response in complicated patients after CPB. To our knowledge, this significant result has never been described. Jain et al¹⁴ reported an altered transcriptome profile related to inflammatory pathways, with more than 4,000 genes modulated 24 hours after CPB in neonates with complicated course (ie, low cardiac output syndrome). The late sample timing used in this study may explain the difference, as it was demonstrated that the number of DEG increase significantly over time after CPB.¹⁵

When focusing on preoperative status, we identified a distinct inflammatory profile at both the transcriptomic and proteomic levels. Whether the reduced modulation of gene expression is directly linked to this distinct preoperative state remains to be explored. Jones et al³² have reported in a large

cohort of children undergoing cardiac operation that a presence of preoperative neutrophilia (eg, neutrophil count of >7 G/L) was associated with the development of postoperative complications, suggesting that a preoperatively activated immune system may disrupt the inflammatory response induced by the stress of cardiac operation, particularly in patients undergoing CPB, and could contribute to worsened outcomes.³ Here, preoperative neutrophil counts were similar between complicated and uncomplicated patients, but at the proteomic level, we identified TNFSF12 as a potential predictor of postoperative complication after CPB. TNFSF12 (also known as TWEAK) is a member of the TNF superfamily, which acts via the Fn14 cell-surface receptor (also known as TNFSFR12A). The TNFSF12/Fn14 signaling pathway is a pivotal regulator of tissues homeostasis, with a dual role in mediating both repair and pathological inflammation and seems to play a beneficial role in tissue repair following acute injury.³³ However, its role in the pathophysiology of the CPB-induced inflammatory response remains largely unknown.

We found a negative correlation between TNFSF12 blood level and complicated outcomes in 2 independent cohorts of children undergoing CPB, supporting the robustness of this association. However, the robustness of a specific threshold could not be tested because the dosage units of TNFSF12 quantification were different between the discovery and validation cohorts. Further investigations are needed to assess its predictive accuracy and explore potential preventive interventions before clinical implementation can be recommended.

Altogether, we propose that the stress induced by CPB may represent either a physiological host immune adaptation involving several immune signaling pathways, or act as a second hit in patients with a preoperative activated immune system, leading to global immune unresponsiveness after surgery and worsening outcomes. Thus, strategies aiming to enhancing the immune response to overcome the absence of transcriptomic signature might be useful in preventing CPB-related complications. Moreover, our findings provide important insight into the inefficacy of immunomodulatory interventions (eg, steroids, nitric oxide) in unselected patients who underwent CPB.^{6,8} Preoperative immune phenotyping—using blood inflammatory cytokine levels or gene expression—could improve patient selection by identifying those at higher risk of complications after CPB. This approach may enhance the efficiency of research in pediatric cardiac surgery, particularly in large, pragmatic randomized trials. Although

conducting an immune assessment before patient inclusion remains challenging, we hypothesized that administering steroids or nitric oxide during CPB to preselected patients with specific immune phenotypes could be more effective. Such targeted interventions might help partially restore the immune unresponsiveness associated with CPB.

The composite outcome used here (ie, occurrence of either HAI within 30 days and/or a prolonged mechanical ventilation) must be discussed. Duration of MV is subject to significant variations from one site to another and could be related to a complication independently of the inflammatory response (eg, diaphragmatic paralysis,³⁴ residual lesion³⁵). However, it remains an important quality metric in pediatric cardiac surgery due to its strong association with complications.^{20,36} Although no cardiovascular endpoint was included in the composite primary outcome, we observed more severe cardiovascular dysfunction in complicated patients, as evidenced by higher VIS scores and lactate levels, underscoring the clinical relevance of the chosen primary endpoint. Finally, a sensitivity analysis using VIS score threshold provides additional validation of the composite primary outcome. However, assessment of early proxy measures of host response dysfunction may provide a more precise definition of immune-related outcomes in pediatric cardiac surgery.³⁷

STUDY LIMITATIONS. First, the sample size of patients was relatively small and significant hospital variability in morbidity and mortality after congenital heart surgery has been reported,³⁸ limiting the generalization of our results. Second, we noted significant differences between CPB and non-CPB populations (eg, age, severity of the congenital heart disease, use of postoperative steroids), which could explain some of the transcriptomic differences observed. Third, we did not include age-matched infants without congenital heart disease, and it would be interesting to know the level of TNFSF12 biomarker in this group of patients. Fourth, we studied peripheral blood although it does not always reflect processes at the site of tissue injury. Recently, characterizing the compartmentalized inflammatory status in the pericardial space has emerged as a promising approach to better understand CPB-related physiopathology, but this requires further investigation in pediatric populations.³⁹ Finally, as we cannot establish direct causality between the preoperative inflammatory status and the reduced

modulation of gene expression related to CPB, the use of animal model of CPB could be relevant to further address this issue.⁴⁰

CONCLUSIONS

We defined an immune transcriptomic signature related to CPB in pediatric cardiac surgery and observed a global immune unresponsiveness in patients who develop postoperative complications. Because immunomodulatory interventions failed to improve patient outcomes in large randomized controlled trial when administrated in unselected patients,^{6,8} we are convinced that a deeper characterization of preoperative immune status of infants requiring CPB will help to pave the way towards a more personalized approach and that strategies restoring immune functions should be considered for future clinical trials.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Infants younger than 3 months of age exhibited an immune transcriptomic response related to CPB. A global immune unresponsiveness to CPB-induced stress is associated with postoperative complications which could be linked to a distinct preoperative inflammatory state.

TRANSLATIONAL OUTLOOK: A deeper characterization of preoperative immune status of infants requiring CPB may prove beneficial to reducing CPB-related postoperative complications and will help to pave the way towards a more personalized approach, enhancing the efficiency of research in pediatric cardiac surgery.

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APPENDIX For an expanded Methods section, as well as supplemental tables and figures, please see the online version of this paper.