Original Article

Myocardial tissue expression of mRNA and preoperative neutrophil-lymphocyte ratio in children undergoing congenital heart surgery

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Background: The neutrophil-lymphocyte ratio (NLR) is an easily accessible and inexpensive biomarker that has been shown to predict morbidity and mortality in congenital cardiac surgery. However, its regulatory mechanism remains unclear. This study aims to compare and correlate the tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-10 messenger RNAs (mRNAs) with the NLR in patients with tetralogy of Fallot (ToF) and ventricular septal defect (VSD).

Methods: A prospective translational study was conducted on 10 children with ToF and 10 with VSD, aged between 1 and 24 months. The NLR was calculated from the blood count taken 24 hours before surgery. The expression of these mRNAs was analyzed in the myocardial tissue of the right atrium prior to cardiopulmonary bypass.

Results: Patients with ToF exhibited a higher NLR [ToF 0.46 (interquartile range; IQR) 0.90; VSD 0.28 (IQR 0.17); P=0.02], longer mechanical ventilation time [ToF 24 h (IQR 93); VSD 5.5 h (IQR 8); P<0.001], increased use of vasoactive drugs [ToF 2 days (IQR 1.75); VSD 0 (IQR 1); P=0.01], and longer ICU [ToF 5.5 (IQR 1); VSD 2 (IQR 0.75); P=0.02] and hospital length of stays [ToF 18 days (IQR 17.5); VSD 8.5 days (IQR 2.5); P<0.001]. A negative correlation was found between NLR and oxygen saturation (SaO_2) (r=–0.44; P=0.002). In terms of mRNA expression, the ToF group showed a lower expression of IL-10 mRNA (P=0.03). A positive correlation was observed between IL-10-mRNA and SaO_2 (r=0.40; P=0.07), and a negative correlation with NLR (r=–0.27; P=0.14).

Conclusions: Patients with ToF demonstrated a higher preoperative NLR and lower IL-10 mRNA expression by what appears to be a pro-inflammatory phenotype of cyanotic patients.

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Keywords: Neutrophil-lymphocyte ratio (NLR); messenger RNA (mRNA); interleukin (IL); IL-10; tetralogy of Fallot (ToF)

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Introduction

The inflammatory process is a physiological response to trauma and is inherent in all surgical procedures (1-5). However, in major surgeries, such as those involving cardiopulmonary bypass (CPB), the inflammatory response can become pathological and lead to postoperative complications due to the blood's contact with a non-endothelial surface (6-9). Several risk factors for this inflammatory response exacerbation are well-established, including hypoxia, extended CPB duration, lengthy myocardial ischemia time, preoperative liver dysfunction, lower weight, and preoperative inflammatory conditions (leukocytosis exceeding 15,000). This heightened inflammatory response can have detrimental effects on various organs (9,10). It can result in negative inotropism in the heart, respiratory failure in the lungs, acute kidney failure or injury, liver failure, and endothelial dysfunction in the vessels. This can progress to multiple organ dysfunction, extending the duration of mechanical ventilation (MV) time, increasing the use of renal replacement therapy and vasoactive drugs (VAD), and prolonging the length of stay (LOS) in the ICU and the hospital. Consequently, this can impact mortality rates and the cost of surgery. Therefore, the early identification of patients at a higher risk of an exacerbated inflammatory response is undoubtedly of significant value.

Several studies have established a link between inflammatory mediators and heightened morbidity and mortality rates in cyanotic patients compared to those with acyanotic congenital heart disease (CHD). Highly specific biomarkers such as cytokines, microRNA (miRNA), messenger RNA (mRNA), amino-terminal type III pro-collagen peptide (PIIIP), and N-terminal B-type natriuretic peptide (BNP/NT-proBNP) have been measured in the pre- and/or postoperative periods of CHD. These biomarkers have been associated with poorer outcomes (11-19). However, these biomarkers are costly and not universally available. In recent years, several research groups have demonstrated the prognostic value of the pre- and postoperative neutrophil-lymphocyte ratio (NLR) in children undergoing congenital heart surgery. It has been observed that patients with elevated NLR have a poorer prognosis, particularly cyanotic patients. While this biomarker appears promising, its regulatory or pathogenic mechanism remains unclear (14,15,19-22). Unlike the aforementioned biomarkers, NLR is inexpensive and widely available.

No studies have yet elucidated the regulatory mechanism of preoperative NLR in CHD patients. Consequently, we aimed to investigate this biomarker's behavior in two distinct groups: patients with tetralogy of Fallot (ToF) and those with ventricular septal defect (VSD), both of whom underwent surgical correction. We compared the preoperative NLR levels and the expression of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-10 mRNA in the myocardial tissue (the most frequently studied cytokines in the myocardium) of these patients. This research represents an initial step towards understanding a mechanism that is widely acknowledged as complex. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-23-513/rc).

Highlight box

Key findings

- Patients with tetralogy of Fallot (ToF) exhibited lower myocardial tissue expression of IL-10 messenger RNA compared to those with ventricular septal defect. This difference is associated with O_2 saturation levels and the neutrophil-lymphocyte ratio (NLR).

What is known and what is new?

- The NLR serves as an affordable and readily accessible inflammatory biomarker, enabling the prediction of morbidity in pediatric cardiac surgery.
- Patients suffering from ToF exhibited a higher preoperative NLR, correlating with poorer outcomes.

What is the implication, and what should change now?

- This is the first study that seeks to understand the probable regulatory mechanism of NLR. In this way, we can start thinking of a way to change the inflammation in these patients.

Methods

This observational prospective translational study included cyanotic patients with ToF and acyanotic patients with VSD who underwent surgical correction. The study was conducted at the Children's Cardiovascular Surgery Service of the Division of Cardiovascular Surgery and the Laboratório de Genética e Cardiologia Molecular (LGCM; Laboratory of Molecular and Genetics Cardiology), both located at the Instituto do Coração (Heart Institute), Hospital das Clínicas, School of Medicine, University of São Paulo (InCor HCFMUSP, São Paulo, SP, Brazil, from June 2021 to February 2023. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). The Scientific Committee and the Ethics Committee of the Instituto do Coração (Heart Institute), Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil, approved the study (SDC: 5188/20/217). Written informed consent was secured from the children's guardians.

Sample size

The sample size and statistical power were determined according to the method outlined in "Quick calculation for sample size while controlling the false discovery rate with application to microarray analysis" (23). This method stipulates that to detect a minimum 2-fold difference in gene expression between two groups with a 95% confidence level and a desired statistical power of 80% (Cohen's d of 0.8), and an alpha error of 0.05, a total of nine patients would be required in each group.

Inclusion and exclusion criteria

All patients, irrespective of gender or race, aged between 1 to 24 months diagnosed with ToF or VSD and who underwent surgery were included in the study. Conversely, the exclusion criteria encompassed patients diagnosed with ToF or VSD who were either less than 30 days old or older than 24 months, those requiring reoperation, those with ToF but without documented or reported episodes of cyanosis and with saturation above 90%, and those with ToF or VSD and VSD with fixed pulmonary hypertension. Additionally, patients with suspected or confirmed infections, those on antibiotics, those in preoperative extracorporeal membrane oxygenation (ECMO), those with preoperative oral or nasotracheal intubation, those with immunological conditions, those on immunosuppressants, and those with genetic syndromes were also excluded.

Study variables

The preoperative variables included age (in months), gender, weight (in kg) at the time of surgery, oxygen saturation (SaO_2, %), and total neutrophil and lymphocyte counts. These counts were obtained from a blood test conducted 24 hours prior to surgery for the purpose of calculating the NLR.

The intraoperative variables included the duration of CPB, the length of myocardial ischemia, lactate levels in the operating room (OR), and the use of VAD, as determined by the vasoactive inotropic score (VIS).

During the postoperative period, we monitored the duration of MV, the number of days VAD was used, the occurrence of acute kidney injury (AKI), and the incidence of low cardiac output syndrome (LCOS). We also tracked the LOS in the ICU, the total hospital LOS, and followed up until discharge or within a 30-day period. We analyzed the tissue gene expression of TNF-α, IL-1β, IL-6 mRNAs, and IL-10 mRNA.

Definition of variables

The NLR was determined by dividing the total count of neutrophils by the total count of lymphocytes from the 24-hour preoperative blood analysis.

Hypoxia: defined as SaO_2 measured with pulse oximetry ≤90%.

AKI: was defined according to the Acute Kidney Injury Network definition. An increase in serum creatinine level from baseline can be used to classify AKI according to the grade of increasing.

LCOS: was considered as a clinical condition that was caused by a transient decrease in systemic perfusion secondary to myocardial dysfunction.

MV time was defined as the time (hours) between the orotracheal intubation and extubating. The criteria for extubating depended on the patient's clinical condition.

Operative mortality was characterized as any death, irrespective of cause, that occurred during hospitalization or within 30 days post-surgery, should the discharge occur prior to this timeframe.

The primary outcome involved comparing and correlating inflammatory activity, as indicated by myocardial tissue mRNA and NLR in peripheral blood. This study represents an initial effort to comprehend a mechanism acknowledged as complex. Secondary outcomes included

MV duration, VIS score, AKI incidence, ICU and overall hospital LOS, and mortality rates.

Technical details

Anesthetic management was executed in accordance with the standard procedures outlined in the InCor Manual of Conduct Routines in Pediatric Cardiac Anesthesia-HCFMUSP (24).

CPB was conducted in accordance with the standards and guidelines for perfusion practice, as outlined by the Brazilian Society for Cardiovascular Surgery (SBCCV) and the Brazilian Society for Extracorporeal Circulation (SBCEC). This procedure is a routine practice in our service (25).

All surgical procedures were conducted by the most seasoned surgeons in the department, utilizing a median sternotomy and under the conditions of CPB.

In all patients, myocardial protection was achieved anterogradely using Del Nido's solution at 4 ℃, administered in the aortic root.

Biopsies were conducted on patients who satisfied the inclusion criteria. Following general anesthesia and orotracheal intubation, asepsis, antisepsis, and the application of sterile drapes were carried out. An incision and sternotomy were performed with meticulous attention to hemostasis. Depending on the need to access the heart, a partial or total thymectomy was performed. Pericardiotomy was routinely performed, and the pericardium was fixed with separate stitches in the surgical field for enhanced exposure. A purse-string suture was placed in the right atrium's (RA) auricle, and the patient was heparinized based on weight (1 mg/kg) via the central catheter, without puncturing the RA. Lateral clamping of the auricle was then carried out, followed by a biopsy at the purse-string suture's boundaries. Bleeding was mitigated by tightening the tourniquet on the purse-string suture. The biopsy fragment, measuring 3 mm × 5 mm, was isolated, placed in a bottle with RNAlater solution, and dispatched to the laboratory (LGCM). While in the OR, the fragment was maintained at room temperature (18–21 ℃); in the laboratory, it was stored at 4 ℃ for 24 hours, then transferred to an ultra-freezer at –80 ℃ for long-term storage. To rule out any potential confounding effects of an inflammatory reaction related to CPB and/or ischemia brought on by aortic cross-clamping, the biopsy was performed before CPB. This approach ensured that the patient's status was comparable to that during blood count collection.

Fragments of RA biopsies were collected and stored in RNAlater (Thermo Fisher Scientific, MA, USA) at –80 ℃ in an ultrafreezer until RNA extraction. Total RNA was subsequently extracted using Trizol (Thermo Fisher Scientific), and reverse transcribed into cDNA with a cDNA synthesis kit (Thermo Fisher Scientific) as per the manufacturer's guidelines. The expression levels of target mRNA were quantified through quantitative reverse transcription polymerase chain reaction (qRT-PCR), utilizing SYBR Green reagents (Thermo Fisher Scientific) and specific primers for IL-1β, IL-10, IL-6, TNF, and cyclophilin, which served to normalize the results. Each sample was analyzed in triplicate.

Statistical analysis

Standard descriptive statistics were utilized. Continuous numerical variables were represented as either mean ± standard deviation (SD) or median and interquartile range (IQR), based on normality tests. The Mann-Whitney or Student's *t*-test was employed to analyze differences between groups, as applicable. Linear regression was conducted to examine the behavior of the NLR in relation to SaO_2 and age using Spearman correlation. The linear regression model was also applied to analyze the correlation between NLR and SaO_2, as well as the tissue expression of IL-1β, IL-10, IL-6, and TNF-α mRNAs, using Spearman correlation. For risk analysis, a logistic regression model was utilized to determine if NLR is an independent predictor of morbidity and mortality. Data analysis was performed using IBM SPSS 23.0 software (IBM Corp., Armonk, NY, USA). The gene expression levels were calculated using the ΔΔCT method, a commonly used method for relative quantification in qPCR experiments.

Results

A total of 30 patients were initially recruited, with 20 (10 ToF and 10 VSD) ultimately selected for the final analysis (*Figure 1*). *Table 1* presents the baseline characteristics of these 20 patients. Patients with ToF exhibited a higher NLR [ToF 0.46 (IQR 0.90); VSD 0.28 (IQR 0.17); P=0.02] and a lower SaO_2 [ToF 86.2% (±6.4%); VSD 97.7% (±1.5%); P<0.001] compared to those with VSD. No significant differences were observed between the groups in terms of other variables.

Intraoperative observations revealed significant differences between the groups in terms of mean CPB [ToF 152.8 (±41.3); VSD 116.6 (±30.1); P=0.04]; as shown in *Table 2*. Postoperative evaluations indicated that patients with ToF required more VAD [ToF 2 (IQR 1.75); VSD 0 (IQR 1); P=0.01], experienced longer MV durations [ToF 24 h (IQR 93); VSD 5.5 h (IQR 8); P<0.001], and had extended ICU LOS [ToF 5.5 (IQR 1); 2 (IQR 0.75); P=0.02] as well as total hospital LOS [ToF 18 days (IQR 17.5); VSD 8.5 days (IQR 2.5); P<0.001] (*Table 3*). However, NLR was not identified as an independent risk factor for MV duration (P=0.453), use of VAD (P=0.68), AKI (P=0.69), ICU (P=0.81), or hospital LOS (P=0.81) (*Table 4*).

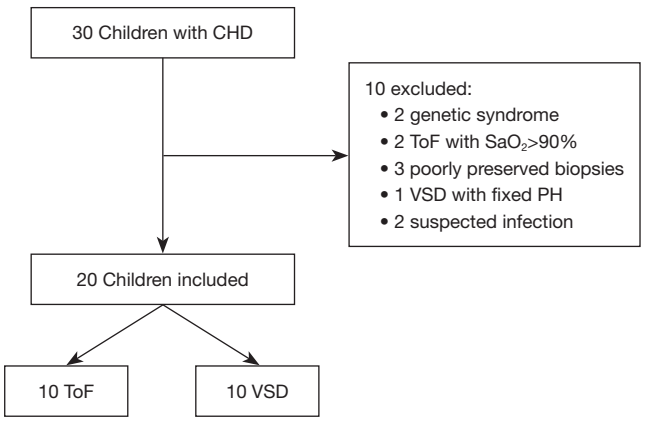

Figure 1 Flowchart for selecting the 20 patients included in the final analysis. CHD, congenital heart disease; ToF, tetralogy of Fallot; SaO_2, oxygen saturation; VSD, ventricular septal defect; PH, pulmonary hypertension.

Upon conducting a linear regression analysis with SaO_2 as the dependent variable and NLR as the independent variable, a negative correlation was observed between SaO_2 and NLR (r=–0.40; P=0.002). This correlation persisted even after adjusting for age (r=–0.44; P=0.002) (*Figure 2*).

Myocardial tissue expression of TNF-α, IL-1β, IL-6 and IL-10 mRNAs

In all patients with ToF, TNF-α, IL-1β, IL-6, and IL-10 mRNAs were identified. Conversely, in VSDs, IL-1β

Table 1 Baseline characteristics of ToF patients and VSD patients who underwent surgical correction

Variable	ToF (n=10)	VSD (n=10)	P value
Age, months	8.3 (±4.4)	11.8 (±7.3)	0.22
Gender, female	4 (40%)	8 (80%)	0.17
Weight, kg	6.8 (±2.0)	6.8 (±1.9)	0.95
O_2 saturation, %	86.2 (±6.4)	97.7 (±1.5)	<0.001§
Total of neutrophils/mL	3,239.2 (±1,732.5)	2,268.2 (±1,452.6)	0.19
Total of lymphocytes/mL	5,592.6 (±2,575.0)	7,977.8 (±3,452.0)	0.10
NLR	0.46 [0.90]	0.28 [0.17]	0.02*

Variables exhibiting a normal distribution are depicted as the mean and standard deviation. Variables exhibiting an asymmetrical distribution are represented by the median [interquartile range]. §, Student's *t*-test. *, Mann-Whitney *U* test. ToF, tetralogy of Fallot; VSD, ventricular septal defect; NLR, neutrophil-lymphocytes ratio.

Table 2 Intraoperative data of ToF patients and VSD patients who underwent surgical correction

Variable	ToF (n=10)	VSD (n=10)	P value
Lactate in OR, mg/dL	14.9 (±8.3)	11.1 (±5.6)	0.25
VIS in OR	13.11 (±8.8)	7.6 (±4.3)	0.10
CPB time, min	152.8 (±41.3)	116.6 (±30.1)	0.04§
Ischemia time, min	100.6 (±39.7)	79.6 (±28.4)	0.19

§, Student's *t*-test. Variables exhibiting a normal distribution are represented by the mean and standard deviation. ToF, tetralogy of Fallot; VSD, ventricular septal defect; OR, operating room; VIS, vasoactive-inotropic score; CPB, cardiopulmonary bypass.

Table 3 Postoperative data of ToF patients and VSD patients who underwent surgical correction

Variable	ToF (n=10)	VSD (n=10)	P value
VIS in 24 h	7.25 [14]	4.45 [7]	0.06
Time of vasoactive drugs, days	2 [1.75]	0 [1]	0.01*
AKI	3 (30%)	0	0.21
LCOS	3 (30%)	0	0.21
MV time, hours	24 [93]	5.5 [8]	<0.001*
ICU time, days	5.5 [1]	2 [0.75]	0.02*
Hospital LOS, days	18 [17.5]	8.5 [2.5]	<0.001*
Mortality	1 (10%)	0	1.0

*, Mann-Whitney *U* test. Variables with an asymmetrical distribution are depicted as the median [interquartile range]. ToF, tetralogy of Fallot; VSD, ventricular septal defect; VIS, vasoactive-inotropic score; AKI, acute kidney injury; LCOS, low cardiac output syndrome; MV, mechanical ventilation; ICU, intensive care unit; LOS, length of stay.

Table 4 Linear regression models evaluating the influence of the NLR on different outcomes

Univariate	Coefficient	Standard error	*t* value	P value
MV time, hours	19.57	25.52	0.767	0.453
Time of vasoactive drugs, days	0.5098	1.2020	0.424	0.68
AKI	0.5475	3.0072	0.422	0.69
ICU time, days	−0.4972	2.0111	−0.247	0.81
Hospital LOS, days	−1.323	5.373	−0.246	0.81

The logistic regression model was used for analysis. NLR, neutrophil-lymphocytes ratio; MV, mechanical ventilation; AKI, acute kidney injury; ICU, intensive care unit; LOS, length of stay.

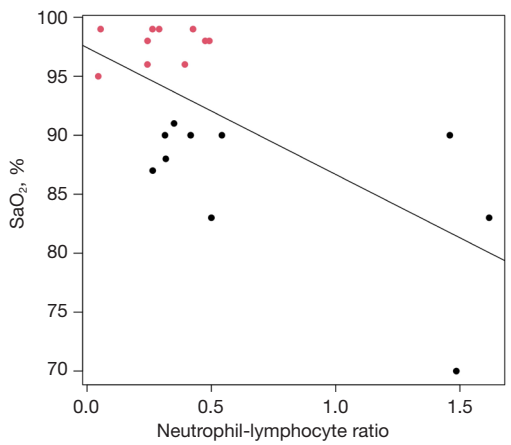

Figure 2 Linear regression plot adjusted for NLR and SaO_2 with r=−0.44 (P=0.002). Black dots: represent patients with tetralogy of Fallot. Red dots: represent patients with ventricular septal defects. NLR, neutrophil-lymphocyte ratio; SaO_2, oxygen saturation.

mRNA was found in 8 out of 10 patients, TNF-α mRNA in 9 out of 10, IL-6 mRNA in 9 out of 10, and IL-10 mRNA in all patients. The tissue expression of TNF-α, IL-1β, and IL-6 mRNAs did not significantly differ between the two groups (P=0.79, P=0.76, P=0.21, respectively) (*Figure 3*). However, the expression of IL-10 mRNA was significantly lower in patients with ToF (P=0.03).

A negative correlation was observed between IL-10 mRNA and NLR (r=−0.27) as depicted in *Figure 4*. Upon correlating SaO_2 levels with tissue mRNAs, a positive correlation emerged between SaO_2 and IL-10 mRNA (r=0.40; P=0.07) (*Figure 5*). Conversely, no correlation was found between SaO_2 and either TNF-α mRNA (r=0.02, P=0.90) or IL-1β mRNA (r=0.03, P=0.96). These findings are summarized in a schematic representation of the likely regulatory mechanism in *Figure 6*.

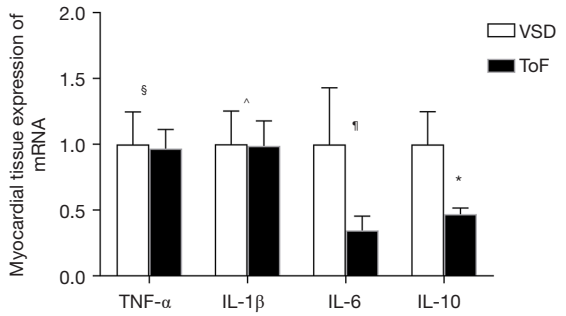

Figure 3 Myocardial tissue expression of TNF-α mRNA, IL-1β mRNA, IL-6 mRNA and IL-10 mRNA analyzed in the myocardium of the right atrium before cardiopulmonary bypass in children with ToF (black bar) and ventricular septal defect (white bar). The bars express the intensity of the mRNA. §, P=0.79; ^, P=0.76; ¶, P=0.21; *, P=0.03. mRNA, messenger RNA; VSD, ventricular septal defect; ToF, tetralogy of Fallot.

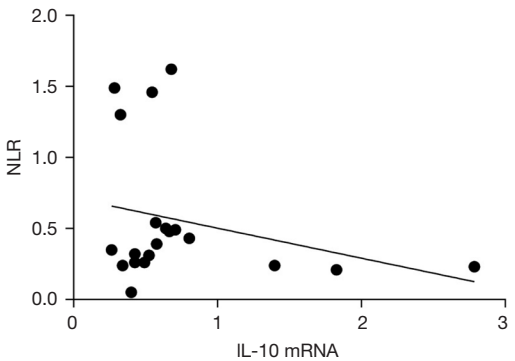

Figure 4 A negative correlation between IL-10 mRNA and NLR (r=–0.27; P=0.14). mRNA, messenger RNA; NLR, neutrophil-lymphocyte ratio.

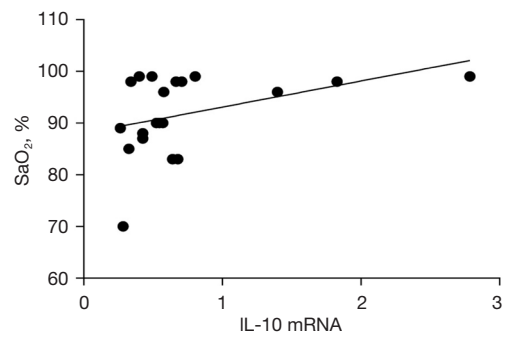

Figure 5 A positive correlation between SaO_2 and the IL-10 mRNA (r=0.40; P=0.07). SaO_2, oxygen saturation; mRNA, messenger RNA.

Discussion

The current study revealed that patients with ToF exhibited decreased myocardial tissue expression of IL-10 mRNA, elevated NLR, and a poorer prognosis. These patients required more vasoactive medications, experienced extended MV durations, longer ICU stays, and an increased hospital LOS.

The NLR, an easily calculable biomarker, reflects a patient's preoperative inflammatory status. Its effectiveness in predicting morbidity and mortality has been demonstrated, offering an inexpensive and readily accessible option, with numerous studies corroborating these findings (21,22,26-29). The NLR gained widespread use as a prognostic biomarker during the COVID-19 pandemic, with studies even illustrating its correlation with IL-10 in this demographic (30-34). Given its susceptibility to change, understanding its underlying mechanism is crucial. However, several questions remain unresolved, such as the regulatory mechanism of the NLR, its potential for change, and the reason for varying NLR levels in patients with the same diagnosis in the absence of an infectious process. It is noteworthy that the majority of the studies published to date are observational and retrospective, failing to provide answers to these questions (26,27).

This translational study serves as an initial exploration into a clearly complex mechanism. It aims to determine if observations made in the preoperative period in peripheral blood mirror events occurring within myocardial tissue, thereby tracing the likely regulatory pathway of the NLR balance. The findings of this study suggest that myocardial and endothelial stress, induced by cyanosis or ischemia (SaO_2 ≤90%), triggers the expression of TNF-α, IL-1β, and IL-6 mRNAs in patients with ToF. However, it was also noted that the expression levels of these mRNAs did not differ in the VSD group. The disparity between the two groups was primarily due to the reduced expression of IL-10 mRNA in ToF patients, a finding that aligns with a previous study (13). This imbalance is reflected in the subtypes of leukocytes, specifically neutrophils and lymphocytes (NLR), as measured by the blood count.

Patients with cyanosis often experience chronic inflammation due to persistent myocardial and endothelial stress (13,35). Under normal circumstances, endogenous IL-10 inhibits the production of pro-inflammatory cytokines, thereby protecting the myocardium in ischemic situations (13). In these patients, the presence of IL-10

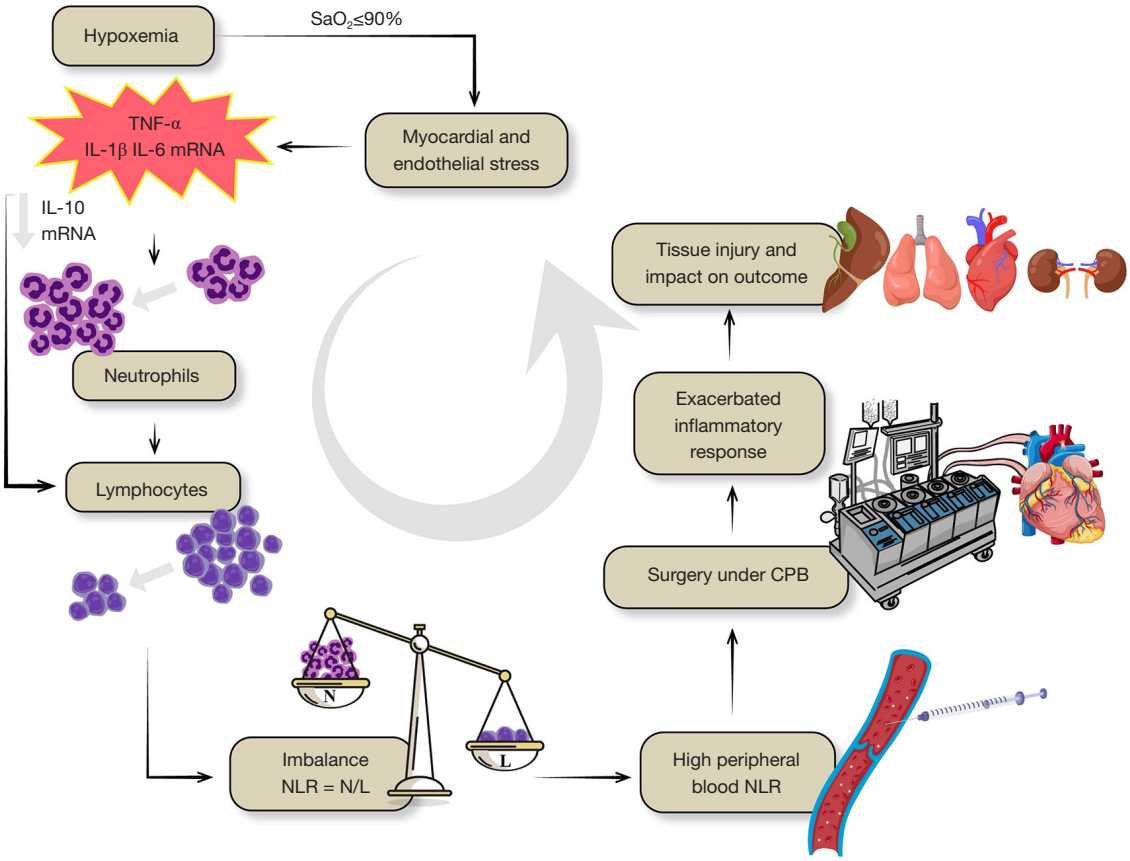

Figure 6 Schematic representation of the probable mechanism of preoperative NLR regulation in ischemic patients undergoing surgical repair. We used a model patient with tetralogy of Fallot and the results of the present study. Ischemia induces a systemic stress involving the myocardium and endothelium of these patients. This stress, in turn, activates the inflammation cascade that was possible to perceive by the expression of TNF-α, IL-1β, IL-6 mRNAs, which in turn stimulates the synthesis of neutrophils, increasing their concentration. On the other hand, there is a low expression of IL-10 mRNA, and this inhibits the synthesis of lymphocytes, decreasing their concentration. All of this leads to an imbalance in the neutrophil-lymphocyte ratio, leaving the patient in a condition of chronic inflammation, which can be easily seen in a simple preoperative blood count. This patient, when subjected to surgical stress associated with the use of CPB, contact with a non-endothelial surface, generates a physiological inflammatory response, but which in these patients can be exacerbated. The exacerbated inflammatory response causes tissue injury in the heart (negative inotropism), lung (respiratory failure), kidney (acute kidney failure or injury), liver (liver failure), and vessels (endothelial dysfunction) and may evolve with multiple organ dysfunction, increasing the duration of mechanical ventilation time, the use of renal replacement therapy, the use of vasoactive drugs, the length of stay in the ICU, and hospital, consequently impacting mortality and surgery cost. SaO_2, oxygen saturation; mRNA, messenger RNA; CPB, cardiopulmonary bypass; NLR, neutrophil-lymphocyte ratio; ICU, intensive care unit.

suppresses neutrophil recruitment, which otherwise causes tissue damage. Conversely, the absence of IL-10 results in an increased number of neutrophils leading to an imbalance in the NLR level (*Figure 6*) (36-39). It is established that cytokines such as TNF-α, IL-1β, IL-6, IL-8, and IL-10 are produced in the myocardium. However, the specific cells responsible for this synthesis-whether they are cardiomyocytes, fibroblasts, or other infiltrated cells in the myocardium-remain unclear in existing literature (40-42). Our study found myocardial tissue expression of TNF-α, IL-1β, IL-6, and IL-10 mRNAs, as they were present in nearly all samples.

Over two decades ago, a study involving 16 mice—8 with an IL-10 deficit and 8 with normal IL-10 synthesis—

was conducted (43). Each mouse was subjected to cardiac ischemia by occluding the anterior interventricular coronary artery for a duration of 30 minutes, followed by a 24-hour reperfusion. The outcome revealed that 6 out of the 8 mice with an IL-10 deficit did not survive, whereas all the mice with normal IL-10 synthesis survived the 24-hour reperfusion (P<0.001). This study underscores the regulatory role of IL-10 in the inflammatory response to myocardial ischemia or cyanosis, a finding that aligns with other studies conducted on mice and dogs (43-45).

Lymphocytes that produce IL-10 play a crucial role in maintaining the delicate equilibrium between inflammation and immunoregulation, thus earning the designation of a "regulatory cell" (44,45). The balance between the total count of neutrophils and lymphocytes is indicative of reduced inflammation. In comparison to patients with VSD, those with ToF exhibit a lower lymphocyte concentration (46).

Our findings contrast with another study that examined myocardial biopsies from the RA and right ventricle. This study compared cytokine concentrations in seven patients with ToF and eight with VSD. The results showed increased levels of TNF-α (P<0.02), IL-1β (P<0.05), and IL-6 (P<0.01) in patients with ToF, with no significant difference in IL-10 concentration (12). Both studies analyzed myocardial biopsy samples from two patient groups, those with ToF and those with VSD, undergoing total correction. However, the methodologies differed. Our study focused on the qRT-PCR analysis (mRNA expression) of RA myocardial tissue before CPB, comparing and correlating it with the NLR. In contrast, the study by Qing *et al.* (12) based its analysis on enzyme-linked immunosorbent assay (ELISA) results (cytokine concentration) from a right ventricle biopsy performed during CPB and deep hypothermia. This procedure can induce and increase an inflammatory response due to contact with the non-endothelial surface of the CPB tubes, potentially explaining the difference in IL-10 concentration or mRNA expression. Therefore, we avoided performing the biopsy during CPB. In a related study by the same research group (13), conducted a year earlier with 20 patients (10 with ToF and 10 acyanotic with different diagnoses), pro-inflammatory and anti-inflammatory cytokine concentrations in venous blood were compared using the ELISA method. The authors noted a lower concentration of IL-10 (P=0.02) and a higher concentration of IL-6, which correlated with the SaO_2 level (r=–0.74; P=0.02), in the ToF group. These results align with our findings. Our data suggest that a lower expression of IL-10 mRNA by myocardial tissue can disrupt the inflammatory response balance, leading to an exaggerated inflammatory response to surgical trauma and prolonged CPB time. This imbalance can result in cardiac and pulmonary damage, potentially increasing mortality risk. As anticipated, CPB time was statistically significant for the ToF group in our study and likely influenced the outcomes in this group, particularly in patients who were already more inflamed pre-surgery and exhibited low expression of IL-10 mRNA.

A lower SaO_2 level corresponds to a greater imbalance, resulting in a higher preoperative NLR level, indicating increased inflammation in the patient. This correlation is evident in three patients depicted in *Figure 2*, who exhibited lower saturation levels and higher NLR levels (SaO_2/NLR patient 1: 70%/1.49; SaO_2/NLR patient 2: 83%/1.62; SaO_2/NLR patient 3: 90%/1.46). Conversely, the study also found that a higher SaO_2 level corresponds to a lower imbalance, resulting in a lower preoperative NLR level. This suggests that the patient is less inflamed and likely to experience fewer complications, as illustrated in *Figure 6*.

While the correlation was not robust, this can be attributed to the difference not exceeding twice the value indicated in the sample calculation or to anesthesia induction, arterial line insertion, central venous catheter insertion and sternotomy, procedures performed before RA biopsy, make two otherwise different groups more similar. Low expression of IL-10 mRNA was related to higher NLR, the IL-10 mRNA behaving as an immunoregulator of the NLR. We may be facing a potential pro-inflammatory phenotype in cyanotic patients. We may be facing a potential pro-inflammatory phenotype in cyanotic patients. This finding aligns with a previous study involving 20 patients, half of whom were cyanotic and the other half acyanotic. The study found a correlation between SaO_2 and IL-10 levels, with lower cytokine concentrations observed in more cyanotic patients (13).

Limitations

The limitations of this study include the sample size, which should have been calculated based on the NLR rather than tissue gene expression for more robust conclusions. Additionally, a comparison and correlation with the same cytokines in peripheral blood would have been beneficial.

Conclusions

Patients with ToF exhibited a higher preoperative NLR and lower expression of IL-10 mRNA which is associated with O_2 saturation. No significant difference was observed in the expression of TNF-α, IL-1β, and IL-6 mRNAs between patients with ToF and those with VSD.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-23-513/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). The Scientific Committee and the Ethics Committee of the Instituto do Coração (Heart Institute), Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil, approved the study (SDC: 5188/20/217). Written informed consent was secured from the children's guardians.

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