Journal of
Cardio - Thoracic Medicine

http://jctm.mums.ac.ir

Lymphocytes TregFoxP3 as an Immunological Response Associated with Secondary Inflammation by Cardiopulmonary Bypass in Cardiac Surgery

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 13 July 2025
Accepted: 25 October 2025

Keywords:
Cardiopulmonary Bypass
Cardiac Surgery
Inflammatory Interleukins
Lymphocytes TregFoxP3

ABSTRACT

Introduction: Cardiopulmonary Bypass (CPB) triggers a robust systemic inflammatory response, marked by elevated serum levels of inflammatory interleukins, which negatively affect endothelial function and target organs. In response to this inflammatory insult, the body activates TregFoxP3 lymphocytes, which regulate inflammation by inhibiting pro-inflammatory interleukins and promoting restorative macrophage activity. Despite this regulatory mechanism, CPB-related inflammation in cardiac surgery is often associated with hemodynamic disturbances. The impact of TregFoxP3 cell levels—whether increased or depleted—on this relationship remains unclear and was therefore evaluated.

Method: This study aimed to assess the effect of CPB on the relationship between TregFoxP3 cells and inflammatory interleukins in patients undergoing cardiac surgery. Blood samples were collected before and after the CPB procedure to measure levels of IL-2, IL-6, IL-8, and TNF-α using chemiluminescence, and TregFoxP3 lymphocytes were quantified via flow cytometry.

Results: A total of 32 patients (mean age 65 ± 5 years), primarily undergoing cardiac valve replacement (80%), were included in the analysis. The average CPB duration was 80 ± 20 minutes, with aortic clamping lasting 60 ± 15 minutes. Following CPB, serum levels of IL-6, IL-8, TNF-α, and TregFoxP3 significantly increased, while IL-2 levels significantly decreased ($p = 0.001$). Baseline TregFoxP3 levels showed no correlation with inflammatory interleukins; however, post-CPB levels demonstrated a strong and significant positive correlation ($r > 0.50$, $p = 0.001$), except for IL-2, which showed a negative association.

Conclusion: The TregFoxP3 lymphocyte response is strongly and significantly correlated with inflammatory interleukin levels following cardiac surgery involving Cardiopulmonary Bypass, highlighting its potential role in modulating postoperative inflammation.

► *Morales, M.R., Quiroz, G.D., Castillo, G.G., Martínez, S.C., Aceves Chimal, J.L. Lymphocytes TregFoxP3 as an Immunological Response Associated with Secondary Inflammation by Cardiopulmonary Bypass in Cardiac Surgery. J Cardiothorac Med. 2025; 13(4): 1604-1607.* ***Doi : 10.22038/jctm.2025.89562.1496***

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Introduction

Advancements in cardiac surgery and Cardiopulmonary Bypass (CPB) techniques have enabled the treatment of complex cardiac diseases. However, these procedures can have detrimental effects on endothelial function and target organs due to the inflammatory reactions induced by CPB. This inflammatory activity is primarily reflected in elevated serum levels of interleukins IL-2, IL-6, IL-8, and Tumor Necrosis Factor (TNF-α), which result from endothelial injury caused by altered blood flow and hypothermia during CPB (1-5).

TregFoxP3 regulatory lymphocytes possess homeostatic immune functions that help modulate inflammatory responses, promote cellular repair, and restore tissue homeostasis through the activation of restorative macrophages. Multiple experimental and clinical studies have demonstrated that, in the face of inflammatory insult, the body responds by activating TregFoxP3 lymphocytes. These cells exert regulatory control over inflammation by inhibiting pro-inflammatory interleukins and stimulating macrophage-mediated tissue repair (6-10).

However, despite this regulatory mechanism, cardiac surgery involving CPB is frequently associated with hemodynamic disturbances and postoperative complications. This suggests a potential interaction between TregFoxP3 cells and inflammatory interleukins, which may be either beneficial or detrimental depending on whether TregFoxP3 levels increase or decline (12-15). This study aimed to evaluate the impact of CPB on the relationship between TregFoxP3 cells and inflammatory interleukins.

Methods

The study was authorized by the Bioethics and Investigation Institutional committees. Patients selected for cardiac surgery with CPB were part of the cardiac and cardiovascular surgery collegial team. All patients signed informed consent. The cardiac surgery was performed in accordance with international and national standards and guidelines accepted by Health Institutions in México. Before and after CPB blood samples were obtained (5 ml) and placed in a sterile tube with EDTA. The TregFoxP3 cells were quantified in a Flow Cytometer FACS Canto II following the staining guidelines of the BD Biosciences kit, which included anti-CD4 PerCP-Cy5.5, anti-CD25 APC, and anti-FOXP3 PE. The inflammatory interleukins IL2, IL6, IL8 and TNF-α were determined by chemiluminescence in the Immulite 1000 Siemens analyzer.

Statistical Analysis

Descriptive analysis was performed using mean and standard deviation. Inferential statistics included Student's t-test and Pearson correlation analysis. A p-value of < 0.05 was considered statistically significant. All analyses were conducted using IBM SPSS Statistics for Windows, version 20.0.

Results

Thirty-two patients of 65 ± 5 years were analyzed. All were carriers of at least 2 risk factors for cardiovascular disease and most underwent heart valve replacement (80%). The CPB time was 80 ± 20 minutes, and aortic clamping was 60±15 minutes. Upon completion of CPB, the serum levels of inflammatory interleukins 6, 8, TNF-α, and TregFoxP3 showed a significant increase, but only IL2 showed a significant decrease ($p=0.001$). The basal levels of TregFoxP3 did not show an association with inflammatory interleukins but upon completion of CPB, the association was strongly positive and significant ($r > 0.50$, $p = 0.001$), except for IL2, which showed a negative association (Table 1-3).

Discussion

Cardiac surgery with Cardiopulmonary Bypass involves immunological and inflammatory challenges associated with an inflammatory storm triggered by endothelial damage secondary to hemodynamic blood flow changes and hypothermia. These changes activate an immune response from the organism primarily through the buffering capacity of TregFoxP3 lymphocytes (6-10).

Table 1. Sex, Comorbidities and surgical procedure distribution patients.

	n	%
Sex		
Male	16	50
Female	16	50
Comorbidities		
Hypertension	22	68
Diabetes Mellitus II	14	43
Smoking	17	53
Obesity	17	53
Surgical procedure		
Revascularization	7	20
Valvular substitution	25	80

Table 2. Inflammatory interleukins and Lymphocytes TregFoxP3 pre and post Cardiopulmonary Bypass comparation.

	Cardiopulmonar Bypass		P-value*
	Pre	Post	
Interleukin 2 ng/ml	467 ± 98	222 ± 38	0.001
Interleukin 6 ng/ml	18.2 ± 2	79.1 ± 15	0.001
Interleukin 8 ng/ml	18.9 ± 5	173.2 ± 17	0.001
TNFα ng/ml	18.4 ± 7	109.5 ± 14	0.001
TregFoxP3 %	6.3 ± 1.9	19.4 ± 2	0.001

* P-value was calculated with t student test.
TNF: Tumor Necrosis Factor.

Table 3. Lymphocytes TregFOXp3 with inflammatory interleukins association.

Basal pre Cardiopulmonar Bypass		
	r	P-value
Interleukin 2 ng/ml	0.30	0.06
Interleukin 6 ng/ml	0.07	0.68
Interleukin 8 ng/ml	0.33	0.06
TNFα ng/ml	0.23	0.19
Post Cardiopulmonar Bypass		
	r	P-value
Interleukin 2 ng/ml	-0.57	0.041
Interleukin 6 ng/ml	0.52	0.001
Interleukin 8 ng/ml	0.59	0.001
TNFα ng/ml	0.47	0.001

The association was calculated with Correlation Pearson test.
TNF: Tumor Necrosis Factor.

This response was confirmed in our findings, showing a positive correlation of TregFoxP3 with inflammatory interleukins 6, 8 and TNFα, as well as a negative correlation with IL2 in a strong and significant manner upon completion of CPB.

In an inflammatory scenario, lymphocytes are activated by oligomerization of the FoxP3 protein to form dimers and oligomers essential for their transcription with regulatory T cells, forming Treg-FoxP3 lymphocytes.This configuration allows distant regions of the genome to come closer together, facilitating the formation of chromatin loops essential for effective large-scale genetic regulation. This allows for an adaptive response to changes in the cellular environment, optimizing the regulation of critical genes to maintain immunological homeostasis by regulating genes that

produce pro-inflammatory responses while maintaining an adequate balance between activation, immune suppression, and cellular repair (11-15).

The buffering activities of TregFoxP3 cells are thought to involve interleukin 2 inhibition, preventing immunologic cell activation responsible for eliminating organic tissue that is injured, which promotes increased inflammation as identified in surgical trauma experimental models. In this context, TregFoxP3 cells represent a significant opportunity for specific therapeutic anti-inflammatory approaches in complex cardiovascular surgery (6-10). This property has been associated with negative associations between interleukin 2 and TregFoxP3 in patients who underwent cardiac surgery with CPB.

In the field of human cardiology, for extensive surgical interventions with prolonged CPB where there is inflammation and injuries secondary to the myocardial ischemia-reperfusion phenomenon, TregFoxP3 lymphocytes represent a good possibility for therapeutic intervention that promotes the reduction of the inflammatory phenomenon through the development of clinical research of stimulation of TregFoxP3 lymphocytes in patients with heart disease candidates for surgical repair with the use of CPB.

Conclusion

The immune response of TregfoxP3 lymphocytes is strongly and significantly associated with inflammatory interleukins in cardiac surgery involving Cardiopulmonary Bypass.

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