VILNIUS UNIVERSITY

Marija Svetikienė

# Effects of postoperative immunonutrition on cellular and systemic immune responses and outcomes in low-risk cardiac surgery

**SUMMARY OF DOCTORAL DISSERTATION**
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VILNIAUS UNIVERSITETAS

Marija Svetikienė

# Pooperacinės imunomitybos poveikis ląsteliniam ir sisteminiam uždegiminiam atsakui bei išeitims mažos operacinės rizikos ligoniams po širdies operacijų

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CONTENT

# LIST OF ABBREVIATIONS

WHO – World Health Organization
CPB – Cardiopulmonary bypass
PCI – Percutaneous coronary intervention
STS - Society of Thoracic Surgeons
ESC - European Society of Cardiology
EACTS - European Association for Cardio-Thoracic Surgery
CABG – Coronary artery bypass graft surgery
AVR – Aortic valve replacement
MVR – Mitral valve replacement
TVR – Tricuspid valve repair
NYHA – New York Heart Association, classification for chronic heart failure
ASA – American Society of Anesthesiologists, physical status classification system
CCI – Charlson comorbidity index
USA – United States of America
UK – United Kingdom
SIRS – Systemic inflammatory response syndrome
DM – Diabetes mellitus
IAH – Idiopathic arterial hypertension
LVEF – Left ventricular ejection fraction
BIA – Bioelectrical impedance analysis
PhA – Phase angle
BMI – Body mass index
CRP – C-reactive protein
IL – Interleukin
TNF – Tumour necrosis factor
PCT – Procalcitonin
PLT - Platelets
ESPEN – European Society of Enteral and Parenteral Nutrition
IN – Immunonutrition
C – Control

ICU – Intensive care unit
GFR – Glomerular filtration rate
CC – Creatinine clearance
MV – Mechanical ventilation
KDIGO – Kidney Disease: Improving Global Outcomes
RIFLE - Risk, Injury, Failure, Loss of kidney function, End-stage kidney disease classification

# 1. INTRODUCTION

## 1.1. Research problem and relevance

According to the World Health Organization (WHO), cardiovascular diseases have remained the leading cause of mortality worldwide for more than three decades. According to the 2019 WHO registry, deaths from ischemic heart disease alone accounted for 16% of all causes (1). Figures from the Health Information Centre of the Institute of Hygiene indicate that cardiovascular diseases accounted for 54.6% (2) of all deaths in Lithuania in 2019. Therefore, the problem remains highly relevant, despite the many ongoing research efforts in the area of prevention, diagnosis and treatment.

With the introduction of cardiopulmonary bypass (CPB) into clinical practice in the 1960s, cardiac surgery has become an essential treatment method for patients with ischemic heart disease (IHD) and those with valvular disorders (3). Later, the emergence and proliferation of percutaneous coronary intervention (PCI) and other interventional cardiology techniques have reduced the popularity and number of cardiac surgeries worldwide. The accelerating progress of new technologies and the desire to provide quality healthcare services while minimising surgical risks have led to the view that open heart surgeries should eventually be replaced by minimally invasive techniques. However, multicentre studies (4, 5, 6, 7, 8, 9) comparing outcomes of PCI and coronary artery bypass grafting (CABG) in patients with CHD have clearly shown that, in the presence of more advanced disease, CABG patients have better long-term outcomes than PCI patients in terms of survival (10). Surgical intervention also remains the “gold standard” for the treatment of progressive valvular heart diseases (11). Thus, it is clear that surgery remains an essential and indispensable part of the comprehensive management of cardiovascular diseases. In 2019, a total of 6,903,706 heart surgeries were performed in the United States of America (USA) (12), 32,295

in the United Kingdom (UK) (13), and approximately 2,000 in Lithuania (14). Thus, the number of heart surgeries, although decreasing slightly every year, remains high and relevant (13).

Over the last 30 years, post-operative mortality has fallen dramatically as a result of technological developments, enhanced diagnostic capabilities, better access to healthcare, and better quality of services. It was estimated that from 1995 to 2010 mortality at 30 days after cardiac surgery fell by almost 26% to 3.9% (15). According to the 2019 annual reports, the overall postoperative mortality rate in 2018 was 1.8% for elective surgeries and 2.74% for all surgeries in the UK (13) and, depending on the type and complexity of the surgery, between 2.2% and 5.4% in the USA (12). However, mortality is not the only outcome variable used to measure the quality or cost-effectiveness of services. In particular, late mortality rarely correlates directly with complication rates or length of hospital stay after surgery (16). Postoperative complications such as cardiac, respiratory, neurological diseases, renal failure and various infectious complications, as well as prolonged stay in the intensive care unit, not only characterise the quality of healthcare services, but also directly correlate with the patients' quality of life after cardiac surgery and their capability for work, and even their long-term survival (17, 18). On deeper analysis, the rates of postoperative morbidity or complications in cardiac patients range up to 42%, depending on the type of surgery and patients' preoperative status (12). This can be explained by the fact that improvements in surgical techniques and implementation of new ones, new possibilities for the processing and use of blood and blood products, as well as significant improvements in intensive care techniques and the overall equipment, have led to older and more complex patients receiving surgeries (16, 18, 19). In Denmark, the age of cardiac surgery patients is estimated to have increased by 4 years between 1999 and 2012 (19), and it is steadily increasing worldwide. In this study, it has been estimated that although 30-day survival improved by 40% to 2.44%, the one-year

mortality rate remained just slightly above 6% (19). Similar figures have been also reported in other studies from other countries (17, 18). Thus, morbidity rates and quality of life after cardiac surgery remain a major problem requiring attention.

Cardiac surgeries with the use of CPB lead to significant changes in homeostasis and a more or less pronounced immune response (20). Depending on the degree of the response and patient's physiological reserve, this may directly influence postoperative morbidity which, as mentioned above, has a direct impact on the quality of life of the patient (21, 22).

Even uncomplicated cardiac surgeries with CPB may cause a full range of systemic inflammatory response symptoms (SIRS) in low-risk patients - from fever, leukocytosis, tachycardia, hypotension and extracellular fluid accumulation to mild multiple organ dysfunction syndrome, such as mild kidney injury, pulmonary oedema, impaired consciousness or intestinal motility (23, 24).

For most low-risk cardiac surgery patients, this is still a mild disorder due to the availability of a good physiological reserve provided by other organ systems, most of which are functioning well. However, as mentioned above, increasingly older patients are being submitted to cardiac surgeries. Such patients are likely to have had diabetes mellitus (DM), primary arterial hypertension (PAH) or other chronic diseases for many years, i.e. conditions that eventually affect homeostasis and reduce patients' preoperative physiological reserve, which is very difficult to measure with our standardised tests and preoperative scales (24).

### 1.2. Research aim

To determine the immune-modulating effects of early postoperative immunonutrition based on glutamine and antioxidant and

relationships with systemic inflammatory response syndrome (SIRS) and postoperative outcomes in low-risk cardiac surgical patients.

### 1.3. Research objectives

1. To identify the effects of early postoperative oral immunonutrition based on glutamine and antioxidants on cellular immunity in low-risk cardiac surgery patients with reduced cell viability;
2. To identify the effects of early postoperative oral immunonutrition based on glutamine and antioxidants on systemic inflammatory response in low-risk cardiac surgery patients with reduced cell viability;
3. To identify the effects of early postoperative oral immunonutrition based on glutamine and antioxidants on early and late outcomes in low-risk cardiac surgery patients with reduced cell viability.

### 1.4. Research novelty and contribution

The process of participant selection in this study was designed to identify a highly selective and homogeneous cohort of low-risk surgery patients with impaired cell viability and physiological reserve, who underwent cardiac surgery at the Cardiac Surgery Centre of Vilnius University Hospital Santaros Klinikos. In this study, immunonutrition is put forward as a hypothesis, a possibility and perhaps a solution for stabilising the immune response and improving clinical outcomes after cardiac surgery. In order to form a group of low-risk surgery patients, the study included only elective patients with a good left ventricular ejection fraction (LVEF) >40%, without pronounced pulmonary hypertension and not in critical condition, as well as patients with an uneventful course of surgery and early postoperative period. To assess operative risk, EuroScore II, a standard risk stratification model for cardiac surgery, was used,

indicating an estimated low postoperative mortality of less than 2% in the selected cohort (25). To assess cell viability of these patients and thus indirectly measure their physiological reserve, we also performed bioimpedance analysis (BIA) and used the resulting phase angle (PhA) value as a selection criterion. As there is no clear phase angle value reflecting the vulnerability of cardiac surgery patients, we relied on the study carried out by A. Bosy-Westphal et al. (26), the large database they developed, and their calculations where phase angle reference values stratified by race, age, and body mass index (BMI) were generated. Taking into account patients most commonly operated on at the Cardiac Surgery Centre of Vilnius University Hospital Santaros Klinikos, we chose a phase angle value of less than 5.5. Thus, as mentioned above, the study cohort included patients at low operative risk with reduced physiological reserve.

Immunonutrition has been studied for more than 20 years as a therapy that could stabilise the immune response and improve outcome after major surgery and in critically ill patients. Many clinical trials have demonstrated the beneficial effects of immunomodulating nutrients (omega–3 fatty acids, arginine, glutamine, etc.) (27, 28, 29, 31, 32) in postoperative patients, including those with severe trauma and burns.

We have chosen an oral complex of glutamine, vitamins and antioxidants for immunonutrition. Glutamine is a relatively essential amino acid, playing an important role in the development of nonspecific and in particular specific cellular immune responses and in the maintenance of intestinal mucosal integrity, contributing to improved clinical outcomes (33, 34). There are studies confirming the impact of lower plasma glutamine levels on clinical outcomes after cardiac surgery (35): Buter et al. found that when comparing preoperative with postoperative plasma glutamine concentrations, there was a significant decrease in glutamine levels and this was related to a higher rate of infectious complications. However, according to the studies published in the PubMed and Cochrane

databases, there have been no investigations so far into the effects of postoperative glutamine immunonutrition on these patients and on outcomes after cardiac surgery. This study is the first one to investigate the effects of postoperative immunonutrition on standard elective cardiac surgical patients who, although being at low operative risk as assessed by standardised scales adapted for cardiac surgery, are likely to have a relatively wide range of postoperative complications affecting their quality of life, long-term morbidity and survival after cardiac surgery.

Our patients were randomly divided into immunonutrition (IN) and control (C) groups: the IN group received an immune formula containing glutamine for 5 days in addition to the standard daily meals; the C group received standard daily meals. Common systemic inflammatory markers (such as C-reactive protein (CRP), leukocyte counts and leukogram, procalcitonin, interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor-alpha (TNF-α)) and cellular immunity markers (CD3+, CD4+, CD8+ cells and their ratios) were measured preoperatively in both groups. The ability of these cells to get activated in vitro was also assessed by flow cytometry. After exposure to phytomutagen in vitro, CD4+CD69+ and CD8+CD69+ concentrations in the cells were measured by flow cytometry. CD69 is a very early activation marker of T lymphocytes that has recently emerged in practice. By tracking and measuring it, we can tell how many T lymphocytes, stimulated with antigen in vitro, become activated and can participate in the immune response. SIRS markers and cellular immunity cells and their activation marker were repeatedly measured in both groups on postoperative day 6. Postoperative outcomes and complications were recorded throughout the hospital stay and at 30 days postoperatively, and compared between the groups.

Animal studies (36, 37) and studies in intensive care patients and those with severe trauma or major surgery (38, 39) have demonstrated

beneficial effects of glutamine on cytokine release, T cell count and balance, as well as on improving clinical outcome. However, even though glutamine seems to be beneficial in modulating the immune response after cardiac surgery, there are no studies that have investigated the effects of postoperative immunonutrition in such patients. Also, if the hypothesis that glutamine indeed helps to reduce immunosuppression due to a decrease in T cells is confirmed, it remains unclear whether it is just an increase in T cell counts and what role it plays in their activation. Thus, this study was aimed to better understand the contribution of glutamine immunosuppression to physiological processes and to provide prospects both in terms of supplementing treatment decisions for cardiac surgery patients and the design of new studies.

# 2. RESEARCH METHODS

## 2.1. Patients and enrolment protocol

This is an open-label, randomised controlled study conducted at the Centre for Anaesthesiology, Intensive Care and Pain Management of Vilnius University Hospital, Santaros Klinikos in the period from February 2015 to June 2017. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee (No 158200-12-561-162). The study was also registered on the website of the US National Library of Medicine at the National Institutes of Health (www.clinicaltrials.gov) with the identification number NCT04047095. The study enrolled elective low-risk cardiac surgery patients with lower PhA values by BIA, indicating reduced cell viability.

Before the study was initiated, all patients enrolled in the study were provided with full information about the study and could only be enrolled after signing a study-specific informed consent form.

All patients underwent BIA to measure their cell viability one day before surgery. Patients who met the inclusion criteria and had no exclusion criteria were enrolled in the study after having signed an informed consent form. After enrolment, all patients were entered in an anonymisation log and given identification numbers. Patients were randomised into groups using a simple randomisation sequence: even-numbered patients were allocated to the immunotherapy (IN) group and odd-numbered patients - to the control (C) group. In both groups, blood samples (systemic inflammatory response markers and specific cellular immunity cells) were taken one day before or on the day of surgery. Patients in the IN group received a complex of glutamine, vitamins and antioxidants for immunonutrition and a standard hospital diet for 5 days after surgery. The C group received a standard diet. Blood tests were repeated on day 6 after surgery.

Early postoperative outcomes were followed up throughout the hospital stay or for 30 days after surgery if the patient was discharged home or to a rehabilitation facility earlier than 30 days after surgery.

As for late outcomes, we assessed one-year mortality and the number of hospital readmissions, thus estimating late morbidity.

#### 2.1.1. Inclusion and exclusion criteria

Inclusion criteria:

1. Elective cardiac surgery with cardiopulmonary bypass (CPB): coronary artery bypass grafting (CABG), aortic valve replacement (AVR), mitral valve replacement (MVR), tricuspid valve repair (TVR), combined surgeries (CABG and AVR/MVR);
2. PhA by BIA <5.5;
3. Patient age: 18 - 80;
4. Signed study-specific informed consent form.

Exclusion criteria:

1. Previous cardiac surgery;
2. Left ventricle ejection fraction (LVEF) <40%;
3. Pulmonary artery pressure >55 mmHg;
4. Diagnosis of infectious endocarditis;
5. Pacemaker;
6. Complicated intraoperative course: unplanned surgical intervention or development of low cardiac output syndrome during surgery: failure to wean from CPB, insertion of intra-aortic balloon in the ascending aorta and the use of intra-aortic balloon counterpulsation (IABC) or maintenance of hemodynamic with infusion of two or more sympathomimetics with a cumulative dose exceeding 0.2 mcg/kg/min);
7. Surgery time >6 h.

### 2.1.2. Data and data collection

Demographic data, comorbidities, preoperative laboratory and instrumental tests and surgery details were obtained from medical records.

### 2.1.3. Demographic characteristics

Demographic characteristics:

1. Age;
2. Sex;
3. Body mass index (BMI).

### 2.1.4. Comorbidities and risk scoring scales

1. New York Heart Association (NYHA) classification of heart failure (39);
2. American Society of Anesthesiologists (ASA) classification of physical health (40);
3. Charlson Comorbidity Index (CCI) (41);
4. Estimating the risk of early mortality after cardiac surgery using the EuroScore II calculation (15). In our selected cohort, the mean EuroSCORE II value was below 2%, which was ranked as low risk according to the average presented in the database of the Society of Thoracic Surgeons (25);
5. Percentage risk scores for mortality and early outcome after cardiac surgery according to the Society of Thoracic Surgeons (STS) calculator (42);
6. Smoking;
7. Arterial hypertension;
8. Diabetes;
9. Previous chronic kidney disease (CKD);
10. Previous myocardial infarction.

### 2.1.5. Surgery details

1. Type of heart surgery involving CPB (CABG, AVR, MVR or combined (CABG+AVR, CABG+MVR, CABG+MVR);
2. Intraoperative aortic cross-clamp time (43);
3. Cardiopulmonary bypass (CPB) time (43);
4. Surgery time (the time from the moment of incision until surgical site closure).

### 2.1.6. General preoperative laboratory tests

1. Total leukocyte count (*109/l);
2. Monocytes (*109/l);
3. Neutrophils (*109/l);
4. C-reactive protein (CRP) concentration (mg/l);
5. Haemoglobin concentration (g/l);
6. Creatinine concentration (mmol/l);
7. Creatinine clearance;
8. Glomerular filtration rate;
9. Blood platelet count (*109/l).

Determination and comparison of these parameters preoperatively allowed us to get an overall picture of the subjects' general condition.

### 2.1.7. Assessment of immunological status:

Cellular immunological response:

1. T lymphocytes: measuring CD3, CD4 and CD8 counts;
2. Determination of a CD4/CD8 ratio;
3. Assessment of T lymphocyte activation using the expression of CD69 marker on the T lymphocyte membrane (CD4CD69 and CD8CD69).

Systemic inflammatory response markers:

1. Tumour necrosis factor α (TNF-α);

2. Interleukin-6 (IL-6);
3. Interleukin-10 (IL-10);
4. Procalcitonin.

Parameters were compared between the groups using mean or median values.

### 2.1.8. Bioimpedance analysis method and phase angle interpretation

All patients underwent bioimpedance analysis prior to being enrolled to the study and the resulting 50 Hz phase angle (PhA) measurement was used as a selection criterion. As there is no validated phase angle indicating normal or abnormal measurements for cardiac surgical patients, we used reference values provided in the population study by A. Bosy-Westphal et al. (26), where a normal mean PhA value for a standard cardiac surgical patient at the Vilnius University Hospital Santaros Klinikos is 5.57° (26). We adjusted this value to 5.5° and set it as a cut-off.

Thus, the study included adult patients with low PhA (<5.5º), which we chose as an indicator of reduced cell viability and frailty.

The BIA analysis was performed with the InBody S10 device (Seoul, South Korea) according to the ESPEN BIA application guidelines (44).

The data obtained were entered in a data collection log and then transferred to an electronic database.

## 2.2. Patient groups and immunonutrition

Patients in the immunonutrition group received a special immunosupplementation in addition to their usual meals for five days after surgery, starting on the morning of the first postoperative day. Each supplementary sachet (22.4 g) contained: glutamine 10 g, vitamin C 250 mg, vitamin E 83 mg, beta-carotene 1.6 mg, selenium 50 µg, zinc 3 mg, and fibre 1 g. The immunonutrients were administered three times a day at a cumulative daily dose of 67.3 g

(glutamine 30 g). The control group was provided only with normal daily meals.

Patients were visited daily before each dose taken. Those who discontinued the immunonutrition diet for one reason or another (indicated in the results section) were excluded from the study.

The main component of our immunosupplementation was glutamine. In smaller amounts, the mixture contained vitamin C (250 mg), vitamin E (83 mg), beta-carotene (1.6 mg), selenium (50 μg), zinc (3 mg), and fibre (1 g).

### 2.3. Assessment of the immune system

#### 2.3.1. Assessment of CD3+, CD4+ and CD8+ subpopulations

Flow cytometry analysis of T cell populations of every patient has been tested twice. The first sample was taken preoperatively in the morning on the day of surgery and the second one – on the sixth postoperative day. The relative percentages of CD3+ (mature T cells) and T cell subpopulations (CD4+, T-helper cells) and (CD8+, T-cytotoxic cells)) were obtained by flow cytometry. Samples were stained using the monoclonal antibodies to surface markers CD3, CD4 and CD8 (BD Biosciences, San Jose, CA, USA). Antibody to CD14/CD45 combination was used for analysis region gating, and to isotype control for marker settings. After erythrocyte lysis with BD FACS lysing solution (BD Biosciences) followed by a wash procedure and a fixation step, the samples were analysed using flow cytometry. Antigen expression was analysed on a FACSCalibur flow cytometer (BD Biosciences) using the CellQuestPro analysis software. Absolute numbers of lymphocyte subsets were calculated by using the absolute lymphocyte counts obtained with the Sysmex 5000i (Kobe, Japan).

### 2.3.2. Assessment of T cell activation

Peripheral blood mononuclear cells (PBMCs) were isolated under sterile conditions using density gradient centrifugation through lymphocyte isolation suspension (LymphoprepTM, Axies-Shield Poc AS, Norway). After the washing step, the PBMCs were suspended in RPMI 1640 medium (RPMI 1640 Medium, HyClone Laboratories, USA) with 20% newborn calf serum (Life Technologies, USA), supplemented with penicillin-streptomycin solution (Biological Industries, Israel) and seeded in 10 $cm^2$ surface activated growth area using TPP tissue culture tubes (TPP Techno Plastic Products AG, Switzerland) for phytohaemagglutinin (PHA-P, Sigma-Aldrich) stimulation testing (PHA concentration 10 μg/ml). Samples were cultured at 37 °C in a humidified 5% $CO_2$ cell culture incubator. After 18 h of incubation, samples were stained using the monoclonal antibodies CD3PerCP / CD14PE / CD45FITC, CD3PerCP / γ1PE / γ2aFITC, CD3PerCP / CD4PE / CD69FITC and CD3PerCP / CD8PE / CD69FITC (BD Biosciences, San Jose, CA, USA). Antigen expression was analysed using the FACSCalibur flow cytometer (BD, USA) and the CellQuestPro analysis software (BD Biosciences, San Jose, CA, USA). Cells were sequentially gated on lymphocytes (based on side scatter (SSC) vs. CD3PerCP dot plot), after which percentages of the activation marker CD69+ on the CD4+ and CD8+ subpopulations were determined.

### 2.3.3. Systemic inflammatory response markers

After thawing, all serum samples were tested simultaneously. The prohormone of calcitonin, procalcitonin (PCT), was measured on the ADVIA Centaur XP random access analyser (Siemens Healthcare Diagnostics) using the ADVIA Centaur® BRAHMS PCT assay (the assay is a two-site sandwich immunoassay using direct chemiluminescent technology that uses three mouse monoclonal antibodies specific for PCT. A direct relationship exists between the

number of PCTs present in the patient sample and the number of relative light units (RLUs) detected by the system). Quantitative measurements of TNF-α, IL-6 and IL-10 were performed on the IMMULITE 1000 random access immunoassay system using TNF-α, IL-6 and IL-10 test kits (Siemens Healthcare Diagnostics) and a solid-phase chemiluminescent immunometric assay.

### 2.4. Assessment of clinical outcomes

#### 2.4.1. Early outcomes

The following was analysed during the study:

1. The most common clinical outcomes after cardiac surgery proposed by the Society of Thoracic Surgeons (STS).
2. Total length of stay in the Intensive Care Unit (ICU);
3. Use of blood products;
4. Incidence of infections during hospital stay. The total number was analysed and compared between the groups. Each case was also analysed separately: source of infection, causative agent;
5. Renal impairment, as based on the RIFLE criteria proposed by KDIGO (45);
6. ICU readmissions during hospital stay;
7. Length of hospital stays after surgery.

#### 2.4.2. Late outcomes

The following was assessed:

1. One-year mortality after surgery;
2. One-year hospital readmissions after surgery.

## 2.5. Statistical analysis

To start our study, we first determined the sample size needed to obtain reliable results. The sample size was determined on the basis of available resources of clinical data and clinical practice. The following formula was used to determine the sample size:

$N = (z_{1-\alpha/2} s_d / \bar{d})^2, \quad \alpha = 0.05, z_{1-\alpha/2} = 1.96,$ where $\bar{d}$ = the difference of measurement means and $s_d$ = the standard deviation of the differences (for quantitative variables). According to the formula, a reliable sample size for the results should consist of at least 12 patients for each group.

The statistical data analysis was performed using the SPSS 20.0 (IBM Corp., Armonk, NY, USA) statistical package for capturing and analysing data. Patients were placed into groups using a random sampling technique. Descriptive statistics were used to structure and present baseline characteristics: variables were described as means ± standard deviations, medians and interquartile ranges, minimum and maximum values, and data distribution and dispersion.

The normality of quantitative variable distributions was tested using the Kolmogorov–Smirnov test. Student's t-test was used to compare the means of independent samples according to their normality of distribution. Variables that were not normally distributed were systematised by calculating medians and differences between the third and first quartiles. The Mann–Whitney–Wilcoxon test was used to compare values between the groups. Categorical variables were expressed as rates and percents (n (%)). The Pearson chi-square test ($\chi 2$) or Fisher exact test was used for comparison of data distributions.

Pearson's correlation coefficient was used to measure the strength of linear relationships between variables. A linear regression model was constructed to identify the relationship between immunonutrition and elevation in T cell counts. Logistic regression was also used to define

the correlation between individual variables and to predict the analysed variables. The logistic regression model was considered appropriate if the Wald Chi-Square p-value was less than 0.05. The logistic regression model was used to calculate the odds ratio (OR), showing how the probability of Y = 1 changes.

A Cox proportional hazards model was built to examine one-year survival rates. The statistical significance level throughout the study was set at $\alpha = 0.05$.

# 3. RESULTS

## 3.1. Patient allocation to the groups

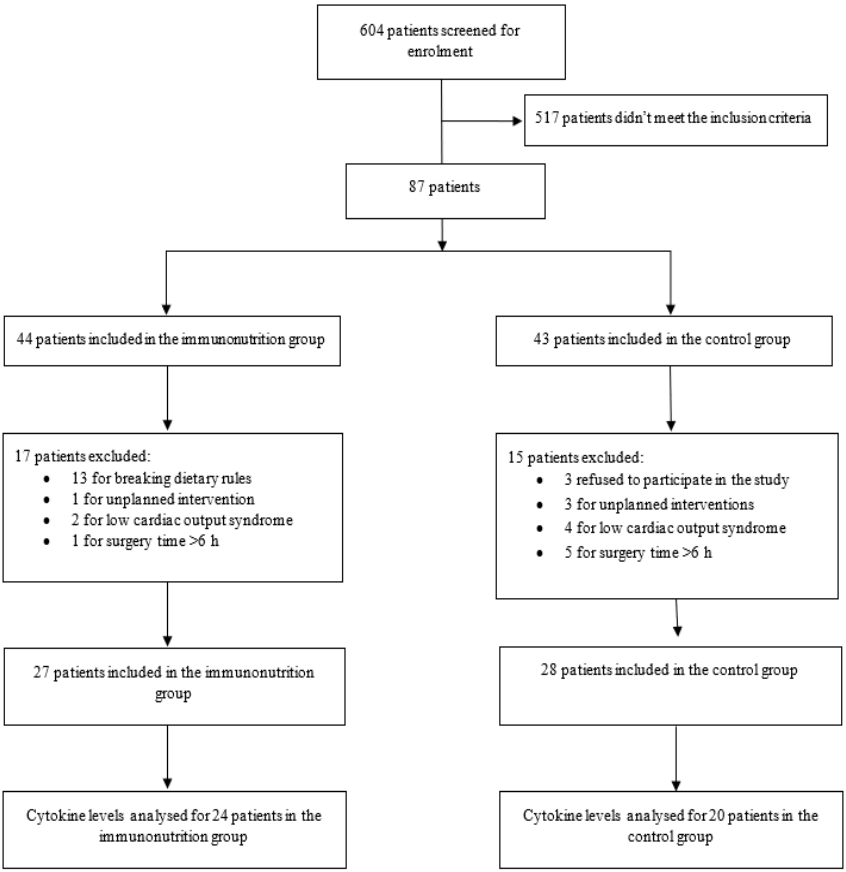


**Figure 1.** Flowchart of patient selection

### 3.1.1. Patients by risk scales and comorbidities

Patients' operative risk, physical condition, comorbidities, severity of heart failure, preoperative laboratory tests, and the likelihood of early postoperative outcomes according to the STS were assessed before

surgery. Before intervention, the groups were compared with each other on all general characteristics. There were no statistical differences found between the groups before the intervention. This indicates that the randomised groups were homogeneous (Table 1).

**Table 1**. General characteristics of patient population

| | **Immunonutrition group, n=27** | **Control group, n=28** | **P** |
|---|---|---|---|
| Characteristics | | | |
| Demographic profile | | | |
| Age (years) | 68.3 (6.9) | 71.0 (5.4) | 0.112 |
| Sex:<br>Male<br>Female | <br>14 (51.9)<br>13 (48.1) | <br>14 (50.0)<br>14 (50.0) | 0.891 |
| Body mass index (kg/m$^2$) | 28.6 (5.1) | 27.8 (4.5) | 0.550 |
| Phase angle (º) | 5.2 [5.04-5.32] | 5.11 [4.72-5.30] | 0.117 |
| Co-morbidities | | | |
| NYHA classification:<br>Class II<br>Class III<br>Class I and IV | <br>3 (11.1)<br>24 (88.9)<br>0 (0) | <br>0 (0)<br>28 (100.0)<br>0 (0) | 0.07 |
| Arterial hypertension | 23 (85.2) | 26 (92.9) | 0.362 |
| Renal failure | 2 (7.4) | 1 (3.6) | 0.531 |
| Myocardial infarction | 9 (33.3) | 5 (17.9) | 0.188 |
| Diabetes | 7 (25.9) | 7 (25.0) | 0.937 |
| Operative characteristics | | | |
| EuroScore II | 1.97 (0.8) | 1.99 (0.8) | 0.918 |
| Operation type:<br>CABG<br>Aortic valve<br>Mitral valve<br>Tricuspid valve<br>Combined | <br>17 (63.0)<br>4 (14.8)<br>3 (11.1)<br>1 (3.7)<br>3 (11.1) | <br>18 (64.3)<br>4 (14.3)<br>1 (3.6)<br>0 (0)<br>5 (17.8) | >0.05 |
| Aortic cross-clamp time (min) | 75.5 (29.3) | 75.2 (25.4) | 0.966 |
| Cardiopulmonary bypass time (min) | 112.6 (39.3) | 109.2 (34.2) | 0.735 |
| Surgery time (min) | 257.4 (73.5) | 258.75 (62.4) | 0.944 |
| Preoperative laboratory parameters | | | |
| Leukocytes (*10$^9$/l) | 6.41 (1.41) | 5.82 (1.7) | 0.181 |
| Monocytes (*10$^9$/l) | 0.55 (0.27) | 0.45 (0.19) | 0.099 |

| | **Immunonutrition group, n=27** | **Control group, n=28** | **P** |
|---|---|---|---|
| Neutrophils (*$10^9$/l) | 3.29 (1.06) | 2.75 (1.03) | 0.064 |
| CRP (mg/l) | 1.87 [0.8-4.7] | 1.33 [0.70-3.25] | 0.429 |
| Haemoglobin (g/l) | 127.92 (11.18) | 129.14 (14.77) | 0.735 |
| Creatinine (mmol/l) | 81.41 (24.85) | 80.92 (19.17) | 0.940 |
| Platelets (*$10^9$/l) | 215.31 (54.86) | 219.11 (53.38) | 0.798 |
| Preoperative immunological parameters | | | |
| Lymphocytes (*$10^9$/l) | 1.76 (0.77) | 1.39 (0.69) | 0.064 |
| $CD3^+$ T cells (%) | 82.15 (7.84) | 82.68 (10.35) | 0.832 |
| $CD4^+$ T cells (%) | 54.96 (9.83) | 56.01 (10.42) | 0.702 |
| $CD8^+$ T cells (%) | 26.99 (9.45) | 26.16 (12.87) | 0.783 |
| CD4+/CD8+ ratio | 2.41 (1.25) | 2.76 (1.46) | 0.340 |
| $CD3^+$ T cells (*$10^9$/l) | 1.44 (0.61) | 1.15 (0.62) | 0.093 |
| $CD4^+$ T cells (*$10^9$/l) | 0.95 (0.39) | 0.76 (0.41) | 0.091 |
| $CD8^+$ T cells (*$10^9$/l) | 0.48 (0.28) | 0.38 (0.29) | 0.191 |

Patients were preoperatively assessed for the percentage probability of mortality and early clinical outcomes after cardiac surgery using the STS calculator. The highest probability was for a short hospital stay (<7 days), with a mean of 30.37 ± 14.15% (Table 2).

**Table 2.** Preoperative assessment of early clinical outcomes (%) in the study groups

| STS clinical outcomes | Immunonutrition group, n = 27 | Control group, n = 28 | P-value |
|---|---|---|---|
| Mortality (median [first and third quartiles]) | 2.46 [1.69; 4.16] | 1.95 [1.43; 3.54] | 0.281 |
| Renal failure (median [first and third quartiles]) | 3.39 [2.29; 17.29] | 2.64 [1.44; 5.25] | 0.195 |
| Ischemic cerebral infarction (median [first and third quartiles]) | 1.29 [0.97; 3.22] | 1.3 [0.79; 1.87] | 0.381 |
| Prolonged ventilation (>24 h) (median [first and third quartiles]) | 12.21 [9.57; 17.95] | 11.86 [9.06; 16.24] | 0.400 |
| Infectious complications (median [first and third quartiles]) | 0.26 [0.14; 0.35] | 0.23 [0.15; 0.39] | 0.993 |
| Resternotomy (median [first and third quartiles) | 6.53 [4.34; 7.98] | 6.25 [4.15; 8.42] | 0.625 |
| Short hospital stay (<7 days) (mean ± SD) | 29.5 ± 13.29 | 31.22 ± 15.13 | 0.657 |
| Long hospital stay (>14 days) (mean ± SD) | 7.93 ± 3.41 | 8.43 ± 5.2 | 0.676 |

Summarising the preoperative assessment of patients according to the risk scales, we can see that the rate of perioperative complications was not prognostically high. Scales adapted for cardiac surgery using the EuroScore II model also showed a low risk of perioperative mortality. Assessment based on the STS criteria showed a high probability of a short hospital stay; risks under other criteria were also below the mean values. However, according to standard methods of assessing severity of condition (NYHA, ASA scores, Charlson Comorbidity Index), patients' condition was not mild. This confirms the fact that cardiac

surgical patients tend to be older, with more comorbidities, i.e. “frail” and with higher risks of morbidity and late mortality (46).

### 3.2. Non-immunological laboratory findings on postoperative day 6

Analysis of laboratory findings (blood cell count and biochemical blood test) on postoperative day 6 revealed no significant differences between the groups (Table 3).

**Table 3.** Laboratory findings on day 6

| | **Immunonutrition group, n = 27** | **Control group, n = 28** | **P-value** |
|---|---|---|---|
| Postoperative haemoglobin, g/l (mean ± SD) | 99.70 ± 12.13 | 102.79 ± 12.24 | 0.353 |
| Postoperative haematocrit, % (mean ± SD) | 29.54 ± 4.18 | 30.90 ± 3.53 | 0.198 |
| Creatinine (mmol/l) (median [first and third quartiles]) | 78.00 [66.00; 118.00] | 96.50 [79.00; 118.75] | 0.140 |

### 3.3. Assessment of immunological parameters on day 6

#### 3.3.1. Leukogram and T cell count on postoperative day 6

The same tests as before surgery were repeated on postoperative day 6. The results were analysed and compared between the groups. There was a significant increase found in CD3+ T cells and CD4+ T cells in the IN group. The data are presented in Table 4 and Figures 2, 3, 4.

**Table 4.** Postoperative laboratory findings

| | **Immunonutrition group, n = 27** | **Control group, n = 28** | **P-value** |
|---|---|---|---|
| Leucocytes (*$10^9$/l) (mean ± SD) | 8.49 ± 3.03 | 8.43± 2.31 | 0.923 |
| Monocytes (*$10^9$/l) (median [first and third quartiles]) | 0.84 [0.72; 1.03] | 0.88 [0.79; 1.13] | 0.368 |
| Neutrophils (*$10^9$/l) (median [first and third quartiles]) | 4.46 [3.61; 6.67] | 4.94 [3.65; 6.34] | 0.736 |
| Basophils, *$10^9$/l (median [first and third quartiles]) | 0.02 [0.01; 0.04] | 0.03 [0.02; 0.05] | 0.146 |
| Eosinophils, *$10^9$/l (median [first and third quartiles]) | 0.29 [0.13; 0.41] | 0.29 [0.18; 0.55] | 0.495 |
| Lymphocytes (*$10^9$/l) (mean ± SD) | 1.87 ± 0.67 | 1.55± 0.74 | 0.103 |
| CD4+/CD8+ ratio (median [first and third quartiles]) | 2.28 [2.04; 4.01] | 2.47 [2.06; 4.73] | 0.533 |
| CD3+ T cells (*$10^9$/l) (mean ± SD) | **1.42 ± 0.49** | **1.12 ± 0.56** | **0.035** |
| CD4+ T cells (*$10^9$/l) (mean ± SD) | **1.02 ± 0.36** | **0.80 ± 0.43** | **0.048** |
| CD8+ T cells (*$10^9$/l) (mean ± SD) | 0.40 ± 0.21 | 0.30 ± 0.18 | 0.066 |

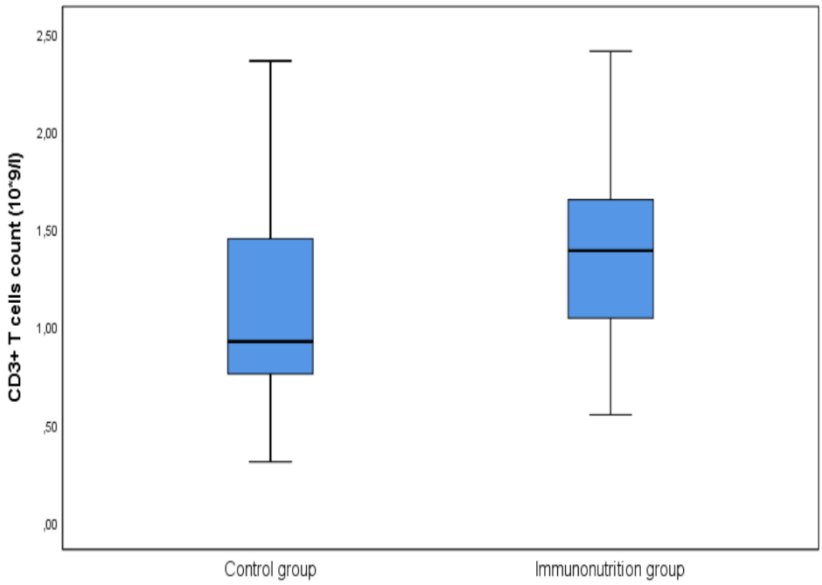


**Figure 2.** CD3+ T cell count on postoperative day 6

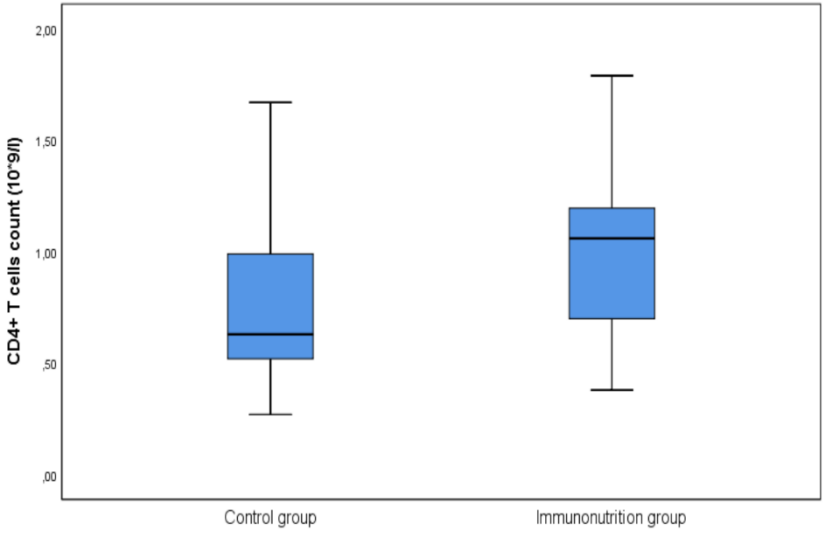


**Figure 3.** CD4+ T cell count on postoperative day 6

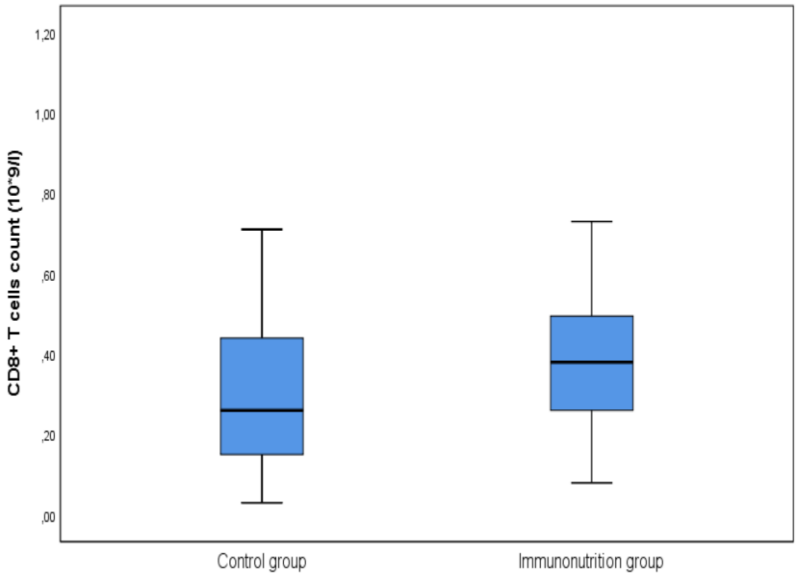


**Figure 4.** CD8+ T cell count on postoperative day 6

3.3.1.1. T cell activation – CD69+ marker

The expression of the CD69+ marker was examined to determine activation in T lymphocyte subsets. It was examined whether the increase in CD4+ cell levels on postoperative day 6 was associated with their activation. The CD69+ marker was calculated in absolute counts (number of cells *109/l) and as a percentage. No significant differences between the groups were obtained (Table 5).

**Table 5.** Activation of T cell subsets by the preoperative and postoperative expression of the CD69+ marker between the groups

| | Before surgery | | | After surgery | | |
|---|---|---|---|---|---|---|
| | **Immunonutrition group** | **Control group** | **P-value** | **Immunonutrition group** | **Control group** | **P-value** |
| CD4+CD69+ T cells (*$10^9$/l) | 0.40 ± 0.31 | 0.30 ± 0.25 | 0.304 | 0.25 [0.16; 0.50] | 0.22 [0.13; 0.41] | 0.578 |
| CD4+CD69+ T cells (%) | 21.1 ± 11.85 | 20.7 ± 9.4 | 0.876 | 17.4 ± 11.1 | 18.0 ± 9.7 | 0.813 |
| CD8+CD69+ T cells (*$10^9$/l) | 0.31 ± 0.46 | 0.15 ± 0.15 | 0.121 | 0.13 [0.06; 0.3] | 0.09 [0.05; 0.14] | 0.178 |
| CD8+CD69+ T cells (%) | 12.3 [5.4; 17.1] | 7.4 [5.53; 10.75] | 0.210 | 7.1 [3.2; 14.7] | 6.45 [3.75; 9.07] | 0.578 |

Data presented as means ± SD or medians [first and third quartiles].

As there was no increase in the CD69+ expression and there was no significant difference between the groups on postoperative day 6, it can be assumed that no further differentiation of T cells into their subtypes occurred.

#### 3.3.1.2. Non-specific immune response – cytokines

Procalcitonin was assessed to exclude the systemic inflammatory response induced by infection and the associated increase in cytokine levels. Cytokine levels were not measured in 11 patients due to the lack of frozen plasma. No significant differences in preoperative and

postoperative cytokine levels were found between the groups (Table 6).

**Table 6.** Levels of procalcitonin, C-reactive protein and cytokines

| | Before surgery | | | After surgery | | |
|---|---|---|---|---|---|---|
| | **Immunonutrition group** | **Control group** | **P** | **Immunonutrition group** | **Control group** | **P** |
| PCT (mcg/l) | 0.01 [0.01; 0.01] | 0.01 [0.01; 0.01] | 0.898 | 0.03 [0.01; 0.09] | 0.05 [0.03; 0.08] | 0.352 |
| CRP (mg/l) | 1.87 [0.80; 4.70] | 1.33 [0.70; 3.25] | 0.429 | 62.7 [34.2; 106.0] | 63.7 [32.9; 91.0] | 0.840 |
| TNFα (ng/l) | 7.23 ± 3.23 | 8.03 ± 3.05 | 0.406 | 8.13 [7.32; 10.31] | 8.78 [7.65; 11.2] | 0.300 |
| IL-6 (ng/l) | 3.21 [2.61; 4.71] | 3.15 [2.43; 7.67] | 0.588 | 14.65 [9.28; 18.95] | 12.25 [8.55; 22.50] | 0.786 |
| IL-10 (ng/l) | 5.0 [5.0; 5.0] | 5.0 [5.0; 5.0] | 0.192 | 5.0 [5.0; 5.0] | 5.0 [5.0; 5.0] | 0.343 |

Data presented as means ± SD or medians [first and third quartiles].

It is worth noting that CRP levels remained elevated on postoperative day 6 (mean values in the immunonutrition and control groups were 62.7 mg/l and 63.7 mg/l respectively). As procalcitonin levels were not elevated, we assume that the increase in CRP was due to the residual expression of the systemic inflammatory response caused by surgery.

3.3.1.3. Correlation and regression analysis of T cell counts

A correlation analysis was performed to include in the models and analyse only those variables that showed a correlation with postoperative T cell counts. Variables with a correlation coefficient >0.2 were included in linear regression models. The models were constructed so that to find the best combination of variables for obtaining significant changes in cell counts. Significant patterns were found between immunonutrition and changes in CD3+ and CD4+ T lymphocyte levels and other variables. Immunonutrition significantly increased CD3+ T cell counts by 0.264 units (calculated as *109/l) with other variables included in the model being set at fixed values (Table 7).

**Table 7.** Regression analysis for CD3+ T cell count dynamics

| | | **CD3+T cell count** | | | |
|---|---|---|---|---|---|
| **F = 9.388, p< 0.001, adjusted $R^2$ = 0.318** | | | | | |
| | **B** | **Beta** | **t** | **CI 95%** | **P** |
| Constant | 1.710 | | 2.177 | 0.133–3.287 | 0.034 |
| Immunonutrition | 0.264 | 0.245 | 2.123 | 0.014–0.514 | 0.039 |
| Age | -0.022 | -0.256 | -2.207 | -0.042–(-)0.002 | 0.032 |
| Preoperative platelets | 0.004 | 0.435 | 3.842 | 0.002–0.007 | <0.001 |

Another model showed an increase in CD4+ T cell count of 0.232 units in the immunonutrition group (calculated as *109/l), while other variables in the model were set at fixed values (Table 8). No statistically significant patterns could be found for changes in CD8+ T cells.

**Table 8.** Regression analysis for CD4+ T cell count dynamics

| | | CD4+T cell count | | | |
|---|---|---|---|---|---|
| **F = 9.159, p< 0.001, adjusted $R^2$ = 0.232** | | | | | |
| | **B** | **Beta** | **t** | **CI 95%** | **P** |
| Constant | 0.062 | | 0.288 | -0.368–0.492 | 0.774 |
| Immunonutrition | 0.232 | 0.283 | 2.372 | 0.036–0.427 | 0.021 |
| Preoperative platelets | 0.003 | 0.435 | 3.644 | 0.002–0.005 | 0.001 |

## 3.4. Clinical outcomes

### 3.4.1. Early outcomes

#### 3.4.1.1. STS clinical outcomes

Analysis of clinical outcomes, as provided by the STS, during postoperative hospital stay did not show significant differences between the groups (Table 9).

**Table 9.** Patients' clinical outcomes during postoperative hospital stay by the STS criteria

| **STS clinical outcomes** | **Immunonutrition group, n = 27** | **Control group, n = 28** | **P** |
|---|---|---|---|
| Mortality, n (%) | 0 (0) | 0 (0) | |
| Renal failure, n (%) | 0 (0) | 0 (0) | |
| Ischemic cerebral infarction, n (%) | 0 (0) | 1 (3.6) | |
| Prolonged ventilation (>24 h), n (%) | 2 (7.4) | 3 (10.7) | 0.670 |
| Infectious complications, n (%) | 4 (14.8) | 4 (14.3) | 0.956 |
| Resternotomy, n (%) | 0 (0) | 0 (0) | |

| STS clinical outcomes | Immunonutrition group, n = 27 | Control group, n = 28 | P |
|---|---|---|---|
| Short hospital stay (<6 days), n (%) | 0 (0) | 0 (0) | |
| Long hospital stay (>14 days), n (%) | 7 (25.9) | 10 (35.7) | 0.432 |
| Comorbidity, n (%) | 5 (18.5) | 5 (17.9) | 0.949 |

3.4.1.2. Postoperative infectious complications

There was no statistically significant difference detected in the rate of infectious complications between the groups. It is of note that in the C group, as many as 3 localised infections were complicated by sepsis, while in the IN group infections were localised and treated with a single course of antibiotics (Figure 5).

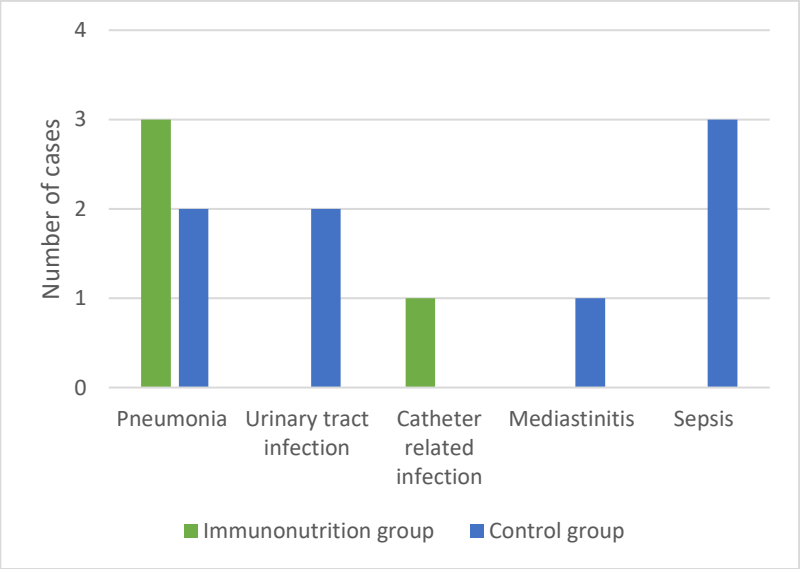


**Figure 5.** Rate of infectious complications between the groups

### 3.4.1.3. Renal failure

Creatinine levels, as recommended by the STS outcome registries, were monitored in patients throughout the hospital stay - the worst renal function parameters were recorded and analysed throughout the hospital stay and compared between the groups. A statistically significant decrease in postoperative creatinine clearance (worsening of renal function) was detected in the control group.

Renal impairment was assessed according to the RIFLE criteria, which allow classification of acute kidney injury according to the severity of injury. RIFLE defines three classes describing the grade of kidney injury (Risk (R), Injury (I), Failure (F)) and two classes describing the outcome of kidney injury (Loss (L) - loss of function persisting >4 weeks, End-Stage Renal Disease (E) - loss of function persisting >3 months). Based on this classification, the results for our patient cohorts are shown in Table 10.

**Table 10.** Grade of kidney injury by the RIFLE criteria between the groups

| **RIFLE criteria** | **Immunonutrition group, n = 27** | **Control group, n = 28** | **P-value** |
|---|---|---|---|
| Risk, n (%) | 2 (7.4) | 9 (32.1) | 0.023 |
| Injury, n (%) | 2 (7.4) | 3 (10.7) | 0.673 |
| Failure, n (%) | 0 (0) | 0 (0) | |
| Risk + Injury, n (%) | 4 (14.8) | 12 (42.9) | 0.023 |

#### 3.4.1.3.1. Logistic regression

A logistic regression model was developed to assess the relationship between immunonutrition and the risk of kidney injury. The model

was analysed and found to correctly classify 70.91% of the values. Nagerkelke $R^2$ was 0.134, and the p-value of the likelihood ratio was 0.02, indicating that the model has good explanatory power (Table 11).

**Table 11.** Results of the logistic regression model in terms of relationships between immunonutrition and risk of kidney injury

| | **Coefficient** | **Standard deviation** | **Wald criterion** | **P-value** | **Odds ratio** |
|---|---|---|---|---|---|
| Glutamine | 1.462 | 0.663 | 4.862 | 0.027 | 4.312 |
| Constant | -1.749 | 0.542 | 10.426 | 0.001 | |

The data in the table show that patients without glutamine supplementation had a 4.312-fold increased risk of kidney injury. The adequacy of the model was also demonstrated by the area under the ROC curve, which was 0.67, see Figure 6.

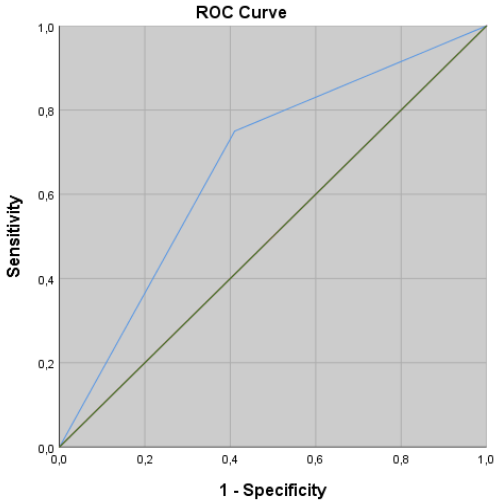


**Figure 6.** ROC curve of the logistic regression model

3.4.1.4. Duration of postoperative treatment and ICU readmissions

Another clinical outcome was the duration of treatment and ICU readmission rate (Table 12).

**Table 12.** Length of stay in ICU and ICU readmission between the groups

| | **Immunonutrition group, n = 27** | **Control group, n = 28** | **P-value** |
|---|---|---|---|
| ICU length of stay (in days), (mean ± SD) | 2.59 ± 1.60 | 2.46 ± 1.64 | 0.770 |
| Total length of hospital stay (in days) (median [first and third quartiles]) | 13 [9; 15] | 13 [9.5; 16] | 0.576 |
| ICU readmissions, n (%) | 1 (3.7) | 5 (17.9) | 0.095 |

ICU – Resuscitation and intensive care unit

### 3.4.2. Late outcomes

#### 3.4.2.1 One-year readmission

Analysis of hospital readmissions within one year after surgery showed a significantly higher rate of readmission of patients from the control group (1 vs 7 patients), p = 0.026 (Figure 7). We considered this criterion as late morbidity and, based on the results, concluded that glutamine-based immunonutrition had an impact on late morbidity, i.e. reduced it.

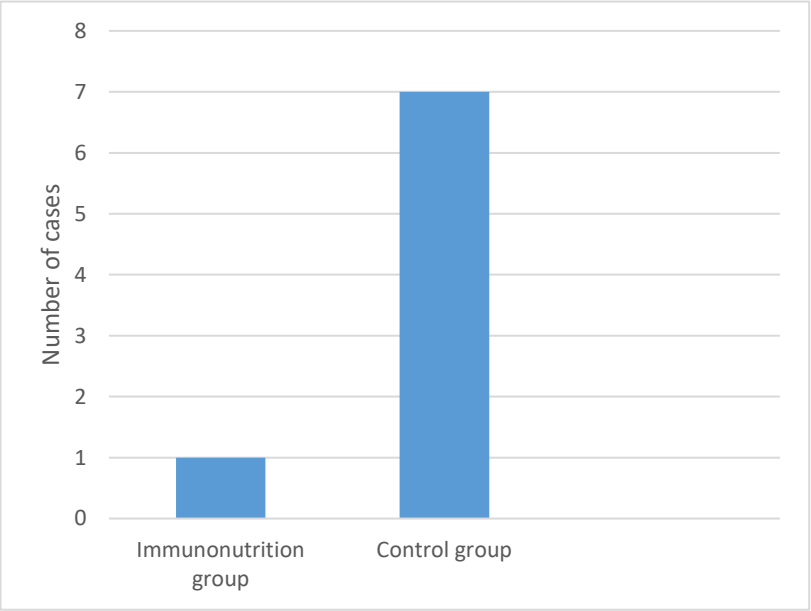


**Figure 7.** ICU readmissions and one-year rates between the groups

### 3.4.3. One-year survival

A total of 4 patients died within a year after surgery. One patient was from the immunonutrition group and 3 patients were from the control group.

A Cox proportional hazards model was developed to assess survival rates. Three variables were included in the model: age, immunonutrition and postoperative kidney injury according to the RIFLE criteria (Risk + Injury). The resulting model is shown in Table 13 and the survival curve is illustrated in Figure 8.

**Table 13.** Cox proportional hazards model for one-year survival

| | Coefficient B | Standard deviation | Wald criterion | p-value | Exp(B) | 95%. Exp(B) confidence interval |
|---|---|---|---|---|---|---|
| Patients with glutamine supplementation | 0.716 | 1.212 | 0.349 | 0.555 | 2.046 | (0.190; 21.993) |
| Age | 0.102 | 0.113 | 0.823 | 0.364 | 1.108 | (0.888; 1.382) |
| Risk + Injury* | -0.722 | 1.054 | 0.469 | 0.493 | 0.486 | (0.062; 3.834) |

*Postoperative renal function according to the RIFLE criteria

Unfortunately, the results obtained gave no grounds to conclude that the aforementioned variable influenced patient survival rates. Although the descriptive statistics and figure showed that one-year mortality was higher in the group without nutritional supplementation, the chance of survival decreases over time.

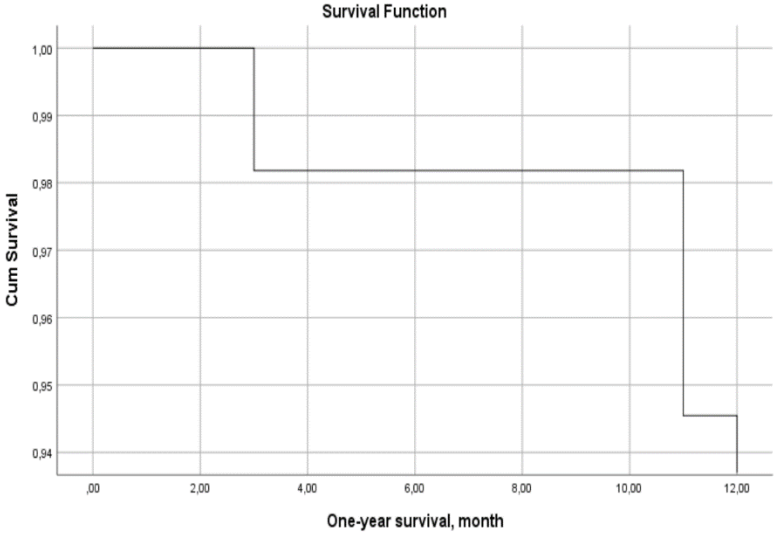


**Figure 8.** Cox proportional hazards model survival curve

Thus, to sum up the results, it is important to highlight that cardiac surgery patients who received immunonutrition in the early postoperative period demonstrated a significant elevation in T helper lymphocytes in the blood, despite steadily decreasing systemic inflammatory response markers and the definite absence of clinical or subclinical infection. We believe this has enhanced their migratory potential and increased their ability to respond more quickly to potential threats. The literature suggests that glutamine is involved in the restoration of body homeostasis and stabilisation of metabolic processes, so we believe that, together with the enhancement of cellular immunity potential, glutamine leads to a lower risk of developing kidney injury and statistically lower one-year morbidity in low-risk but “frail” cardiac surgery patients. No complications or adverse events were observed.

### 3.5. Study limitations

The major limitation to the study was the small sample size, which means that the immunological data are very scattered. When assessing the results, we have seen many trends that could be tested with a larger sample. Certainly, given the experience we have gained from the study, it is likely that with a larger cohort, the number of excluded patients would also increase, mainly due to non-compliance with dietary rules. I would like to emphasise that the results of the study should be seen as explanatory and exploratory, shedding light on some of the possible immunological mechanisms and ways of adjustment in patients after cardiac surgery, and on their relationships with clinical outcomes.

# CONCLUSIONS

6.1. Postoperative immunonutrition had a positive impact on the development of the cellular immune response. Increased numbers of T lymphocytes are likely to have enhanced the migratory potential of T lymphocytes and their ability to respond more quickly to potential threats.

6.2. Glutamine- and antioxidant-based immunonutrition did not influence the course of the systemic inflammatory response after cardiac surgery.

6.3. Postoperative glutamine- and antioxidant-based immunonutrition did not have any effect on the most common outcomes after cardiac surgery reported by the STS. There were no statistically significant differences identified between the groups in terms of length of ICU stay, postoperative hospital stay, ICU readmissions, or rates of infectious complications during hospital stay. There was a significantly lower creatinine clearance in the control group. According to the RIFLE criteria for acute kidney injury, we estimated that immunonutrition based on glutamine and antioxidants reduces the likelihood of kidney injury or risk of kidney injury by a factor of 4.3 after cardiac surgery.

In terms of late outcomes at one year, glutamine- and antioxidant-based immunonutrition has been found to have no effect on one-year mortality after cardiac surgery, but statistically reduced the incidence of hospital readmissions, i.e. late morbidity after cardiac surgery.

# PRACTICAL RECOMMENDATIONS

The results of the study suggest that early postoperative oral immunonutrition with glutamine and antioxidant supplementation is beneficial in low-risk cardiac surgical patients with reduced cell viability, i.e. “frail” patients, when used at the recommended doses and taking into account contraindications to its administration (acute renal and hepatic failure).

I would like to point out that this postoperative immunonutrition regimen did not at all disrupt the routine in elective cardiac surgery, as patients are usually prepared for surgery on an outpatient basis; they are hospitalised the day before or on the day of surgery and stay in hospital for more than 5 days after surgery. Therefore, I can safely say that this immunonutrition would be easily implemented by the attending physician without additional human resources.

Thus, we recommend that early postoperative oral glutamine- and antioxidant-based immunonutrition, at recommended doses and subject to contraindications to glutamine administration, should be administered to low-risk cardiac surgery patients with reduced cell viability.

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## SCIENTIFIC PUBLICATIONS

1. Svetikiene M, Ringaitiene D, Vezeliene J, Isajevas V, Trybe D, Vicka V, Malickaite R, Jurgauskiene L, Norkuniene J, Serpytis M, Sipylaite J. The efficacy of early postoperative enteral immunonutrition on T-lymphocyte count: A randomised control study in low-risk cardiac surgery patients. Clin Nutr. 2021 Feb;40(2):372-379. doi: 10.1016/j.clnu.2020.05.009. Epub 2020 May 15. PMID: 32513480.

2. Svetikienė M., Trybė D., Strioga M., Veželienė J., Isajevas V., Malickaitė R., Jurgauskienė L., Ringaitienė D., Šerpytis M. and Šipylaitė J. (2021) "Impact of Immunonutrition on T Cell Activation:

A Randomized Control Study in Cardiac Surgery Patients", Acta medica Lituanica, 28(2), p. 16. doi: 10.15388/Amed.2021.28.2.16.0

## ORAL PRESENTATIONS

1. Svetikiene M, Isajevas V, Vicka V, Rackauskaite S, Ringaitiene D, Sipylaite J. Nutritional immunomodulation and short-term postoperative outcomes in malnourished patients undergoing cardiac surgery. 8th International Baltic Congress of Anaesthesiology and Intensive care. 1-3 december 2016 Talinn.

2. Svetikiene M, Isajevas V, Vicka V, Rackauskaite S, Ringaitiene D, Sipylaite J. Nutritional immunomodulation in malnourished patients undergoing cardiac surgery. 8th International Baltic Congress of Anaesthesiology and Intensive care. 1-3 december 2016 Talinn.

3. Svetikienė Marija; Ringaitienė Donata; Vėželienė Jevgenija; Trybė Dainius; Vicka Vaidas; Malickaitė, Radvilė; Jurgauskiene,Laimutė Genovaitė; Norkūnienė Jolita; Šerpytis Mindaugas; Šipylaitė Jūratė. Role of nutritional immunomodulation in immune responses in malnourished patients undergoing cardiac surgery. Acta medica Lituanica. Vilnius : Lietuvos mokslų akademijos leidykla. ISSN 1392-0138. eISSN 2029-4174. 2018, vol. 25, suppl. 1, p. 79.

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5. Svetikienė Marija; Ringaitienė Donata; Vezeliene Jevgenija; Isajevas Viktoras; Trybė Dainius; Vicka Vaidas; Malickaitė Radvilė; Jurgauskienė Laimutė Genovaitė; Norkūnienė Jolita; Šerpytis Mindaugas; Šipylaitė Jūratė. The efficacy of early postoperative enteral immunonutrition on T-lymphocyte count in cardiac surgery patient. 2020 ESPEN Virtual Congress September 19-21.Clinical nutrition research symposium. Virtual presentation. https://espencongress.com/wp-content/uploads/2020/09/Clinical-nutrition-research-symposium.pdf.

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