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Endotoxemia in patients with depressed cardiac function who develop septic shock after cardiac surgery. The role of immunomodulation strategies.

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Index:

1. Introduction

Sepsis is a potentially life-threatening condition caused by an infection and an inadequate dysregulation of host immune response. It is one of the leading causes of mortality despite the extensive efforts and many different types of treatments[1].

Sepsis and septic shock are major healthcare problems, impacting millions of people around the world each year and killing between one in three and one in six of those it affects[1,2].

Early identification and appropriate management in the initial hours after the development of sepsis improve outcomes. Identifying and not underestimating the signs and symptoms listed above, along with the detection of some biomarkers (such as C Reactive Protein - CRP, Erythrocyte Sedimentation Rate - ESR, Procalcitonin -PCT, Iterleukin-6 - IL-6), are crucial elements for early diagnosis of sepsis and the timely establishment of its appropriate clinical management[2,3].

The role of the inflammatory response in the pathogenesis of the syndrome has supported the modern concept of sepsis. Nevertheless, a definition of sepsis and the criteria for its recognition is a continuous process, which reflects the growing knowledge of its mechanisms and the success and failure of diagnostic and therapeutic interventions[1,4]. The concepts of sepsis has changed over the time, from the *"systemic inflammatory response syndrome triggered by infection"* to *"a severe, potentially fatal, organic dysfunction caused by an inadequate or dysregulated host response to infection"*[5].

The term sepsis comes from Greek, which means putrefaction or putridity. It was characterized by Hippocrates as a dangerous, odoriferous, biological decay of the body[6].

According to a consensus meeting, published in 1992 and endorsed in 2003, sepsis was defined as the systemic inflammatory response syndrome (SIRS) caused by infection[6,7].

Advances in understanding the pathogenic mechanisms of sepsis, the recognition that inflammatory and anti-inflammatory responses are triggered at the onset of infection, ed to the review of the concept of sepsis in 2016, which defined sepsis as a serious, potentially fatal, organic dysfunction caused by a dysregulated host response to infection and septic shock in a subset of patients in which underlying

circulatory and cellular/metabolic abnormalities are sufficiently profound to substantially increase mortality[4,6].

The new criteria as well as the implications in multi-professional team training processes and intervention strategies are being debated by the scientific community[4,5].

The pathogenesis of sepsis is complex and involves multiple aspects of the interaction between the infecting microorganisms and the host. The recognition of pathogens and the resulting cellular activation are fundamental for infection control. Paradoxically, the host inflammatory response is also the substrate for the pathophysiological changes in sepsis[6,7,8].

2. Sepsis in Cardiac Surgery

In cardiac surgery, the prevalence of sepsis is between 0.39% and 2.5%, with a mortality ranging from 65% up to 79%[6,7]. However, myocardial dysfunction, characterized by biventricular dilatation and reduced ejection fraction, is present in most septic patients, and it seems to be not due to myocardial hypoperfusion but to circulating depressant factors; including the cytokines tumor necrosis factor-alpha (TNF-α) and IL-1β[9,10,11].

Notably, during the perioperative period in cardiac surgery, many factors such as surgical trauma, shear stress, blood contact with cardiopulmonary bypass (CBP), internal drainage system, blood transfusion, and reperfusion after ischemia could influence and impact patients' outcomes[12.13]. In addition, cardiac surgery with cardiopulmonary bypass is associated with gut barrier dysfunction and endotoxin lipopolysaccharide (LPS) release. These factors together can provoke a dynamic systemic immune response[14].

Therefore, several new immunomodulatory approaches have been investigated during the last years, among them the immunostimulation and the extracorporeal blood purification techniques (EBPTs)[15,16].

Several studies and case series supported the use of Polyvalent intravenous Immunoglobulins based on pleiotropic effects on the inflammatory and immune mechanisms and the beneficial effects on hemodynamic and survival[17,18].

Although the evidence for beneficial effects of IgM-enriched Immunoglobulins in patients with severe sepsis and septic shock has not always been supportive, systematic reviews have generally concluded that IgM-enriched immunoglobulin preparations are associated with a reduction in mortality[19,20].

Extracorporeal blood purification techniques have a history of 15 years in treating critically ill patients. Removing or decreasing serum concentration of inflammatory mediators, fragments of gut-derived Gram-negative (lipopolysaccharides or endotoxin) and tissue degradation products from the

systemic circulation can provide beneficial effects (preventing multi-organ dysfunction and immune-paralysis)[21,22].

However, new studies are needed to establish the appropriate technique, patient selection, timing, duration of the treatment, and the effect on solid clinical endpoints (mortality, organ dysfunction).

3. Sepsis Biomarkers

A sepsis marker should be useful in early diagnosis, characterized by a high specificity and sensitivity, be correlated with the severity of the disease and therefore endowed with prognostic value, have a clinically useful half-life, allow differential diagnosis between infectious etiology and not infectious and last but not least be easy to dose. The available markers are numerous: *white blood cell count (WBC), C reactive protein (CRP), Procalcitonin (PCT), Endotoxin, Cytokines, Interleukin-6 (IL-6), Interleukin-1 (IL-1), complement factors, Endothelin-1, ICAM-1 and VCAM-1, phosopholipase A2, Lactoferrin, Neopterin, Elastase, Erythrocyte Sedimentation Rate (ERS)*[22].

However, none of them are specific for sepsis whose diagnosis cannot be concluded on the basis of the presence alone, but must be evaluated in the context of the clinical picture suggesting sepsis. It is therefore essential to evaluate the appropriateness of the request for individual markers in collaboration with the clinician in order to exploit their potential: diagnostic, therapeutic monitoring and prognosis[22].

C-reactive protein is an archetype acute phase protein found in 1930 by Tillett and Francis in patients with pneumococcal pneumonia, whose sera precipitated somatic C-polysaccharide fraction of pneumo- cocci. C-reactive protein is produced by the liver with the maximum production 24-38 hours after inflammation onset[23,24].

CRP binds Gram-positive and Gram-negative bacteria and stimulates their adhesion and complement dependent phagocytosis by leukocytes.

Generally it can be said that the main function of CRP is to bind heterogeneous structures of both endo- and exo-genous origin (altered membranes, cell debris, bacteria, and parasites) and with this bond, trigger defense mechanisms of the macro-organism adherence and modulation of phagocytic cells, activation of complement system, stimulation of opsonization and phagocytosis.

In the past 15 years another protein called procalcitonin has gained ground in clinical practice. Procalcitonin (PCT) is a protein of 116 aminoacid sequence and a prohormone of calcitonin. In

physiological conditions, calcitonin is secreted by C cells of thyroid gland, where it is formed from its precursor i.e. Procalcitonin[25].

The latest guidelines on the management of sepsis (SSC 2021) suggest the measurement of Procalcitonin (PCT) and the evaluation of its trend over time, as it helps in the differentiation of viral infections from bacterial ones and in the correct interpretation of the results of microbiological tests and also provides additional information on the host's response to infection[1,26].

Procalcitonin (PCT) is therefore to be considered as a measure of the patient's response to the infection and indirectly of the extent and severity of the infection.

Furthermore, the measurement of Procalcitonin (PCT) must also be used to support the "de-escalation" of antibiotic therapy following the improvement of the patient's clinical condition, whether he is hospitalized in an ICU or in an ordinary ward[1].

Presepsin (sCD14-ST) is a soluble fragment of CD14, a receptor present on the membrane of phagocytes. It activates the inflammatory cascade in innate immunity and in patients with sepsis[27].

The physiological concentration is <6 mg / L. In case of sepsis, it increases early significantly, but this value has low specificity[28].

Adrenomedullin (ADM) is a peptide hormone encoded by the ADM gene.

It is a powerful vasodilator and controls blood circulation to and from organs and is an early biomarker of organ failure. It increases as a defense response to organ damage and dysfunction, restoring endothelial function; protects against organ damage induced by LPS; stabilizes microcirculation in response to a generalized infection[29,30].

Furthermore adrenomedullin increases in both bacterial and viral infections. It level is correlate with the severity of sepsis and it has a high prognostic power for risk stratification[31].

IL-6 contributes to host defense against infections and tissue injuries. However, exaggerated, excessive synthesis of IL-6 while fighting environmental stress leads to an acute severe systemic inflammatory response known as 'cytokine storm', since high levels of IL-6 can activate the coagulation pathway and vascular endothelial cells but inhibit myocardial function[32,33].

IL-6 is regarded to be a multifunctional hormone: it is a differentiation factor for B lymphocytes, cytotoxic T lymphocytes, and for hematopoiesis in the bone marrow (BM). Furthermore, it can act as a growth factor for plasmacytomas and hybridomas, the property of which is used to determine its concentration in biological fluids. In addition, IL-6 is an endogenous pyrogen[34,35].

However, its main physiologic role is probably to act as a messenger between damaged tissues and the liver, since IL-6 can be released by many cells and is the main inducer of acute-phase responses in the liver[36,37].

3.1 Lps: a biomarker for Sepsis.

Endotoxin, or more accurately termed bacterial lipopolysaccharide (LPS), is recognized as the most potent and common microbial mediator implicated in the pathogenesis of sepsis and septic shock. Yet despite its discovery well over a century ago, the fundamental role of circulating endotoxin in the blood of most patients with septic shock remains enigmatic and a subject of considerable controversy[38,39].

LPS is the most prominent *"alarm molecule"* sensed by the host's early warning system of innate immunity presaging the threat of invasion of the internal milieu by Gram-negative bacterial pathogens. In small doses within a localized tissue space, LPS signaling is advantageous to the host in orchestrating an appropriate antimicrobial defense and bacterial clearance mechanisms[40,41]. Conversely, the sudden release of large quantities of LPS into the bloodstream is clearly deleterious to the host, initiating the release of a dysregulated and potentially lethal array of inflammatory mediators and procoagulant factors in the systemic circulation[38,42]. The massive host response to this single bacterial pattern recognition molecule is sufficient to generate diffuse endothelial injury, tissue hypoperfusion, disseminated intravascular coagulation and refractory shock[39,41]. Numerous attempts to block endotoxin activity in clinical trials with septic patients have met with inconsistent and largely negative results[43,44].

Yet the groundbreaking discoveries within the past decade into the precise molecular basis for LPS-mediated cellular activation and tissue injury has rekindled optimism that a new generation of therapies that specifically disrupt LPS signaling might succeed[38,39,41]. Other microbial mediators found in Gram-positive bacterial and viral and fungal pathogens are now appreciated to activate many of the same host defense networks induced by LPS. This information is providing novel interventions in the continuing effots to improve the care of septic patients.

LPS is able to trigger inflammation through both intracellular and extracellular pathways[42,43,45]. Classical interactions between LPS and host cells first involve LPS binding to LPS binding protein (LBP), a carrier. The LPS-LBP complex then binds to a receptor complex including the CD14, MD2, and toll-like receptor 4 (TLR4) proteins, initiating a signal cascade which triggers the secretion of pro-inflammatory cytokines. However, it has been established that LPS is also internalized by macrophages and endothelial cells through TLR4-independent pathways. Once internalized, LPS is able to bind to the cytosolic receptors caspases-4/5 in humans and the homologous caspase-11 in mice. Bound caspases-4/5 oligomerize and trigger the assembly of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasome followed by the activation of inflammatory caspase-1 resulting in subsequent release of interleukin-1β. Caspases-4/5 also activate the perforin gasdermin D and purinergic receptor P2X7, inducing cell lysis and pyroptosis. Pyroptosis is a notable source of inflammation and damage to the lung endothelial barrier during sepsis[46, 47].

LPS is a major component of the outer membrane of gram-negative bacteria and a pathogen-associated molecular pattern which is recognized by the pattern recognition receptor toll-like receptor 4 (TLR4). Once host cells recognize LPS, they release pro inflammatory cytokines including tumor necrosis factor-α, interleukins (ILs). If an imbalance occurs between pro- and anti-inflammatory mediators, the inflammatory responses are dysregulated leading to the development of sepsis. It should be noted that sepsis can also be caused by gram-positive bacterial infections and in some cases by fungi or parasites. In such cases, LPS would not play a role in pathogenesis[46,48].

While the relationship between extracellular LPS and TLR4 has been well documented, pharmacological interventions to neutralize circulating LPS or to block cell surface TLR4 receptors have failed in clinical trials. One possible explanation for these failures is unabated LPS internalization in diverse cell types. Human lung epithelial cells, endothelial cells (ECs), cardiomyocytes, and macrophages can all internalize LPS in vitro. LPS has been shown to enter the cytoplasm of macrophages and induce inside-out pro-inflammatory signaling independent of TLR4. Once internalized, LPS binds to the inflammatory caspases-4/5 in humans and their ortholog caspase-11 in mice, resulting in the activation of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) and "non-canonical" inflammasomes, leading to pyroptosis, an inflammatory form of cell death[41,44,48].

Despite great efforts and numerous clinical trials, there is still a major need for effective therapies for sepsis. Neutralization or elimination of bacterial toxins remains a promising approach. The understanding of the interaction of the endotoxin (Lipopolysaccharide - LPS) of Gram-negative bacteria with its cellular receptor, namely the CD14/TLR4/MD2 complex, was a major breakthrough. Unfortunately, clinical trials for sepsis on the neutralization of LPS or on the inhibition of TLR4 signaling failed whereas those on LPS removal remain controversial. Recent discoveries of another class of LPS receptors localized within the cytoplasm, namely caspase-11 in mice and caspases-4/5 in humans, have renewed interest in the field. These provide new potential targets for intervention in sepsis pathogenesis. Since cytoplasmic recognition of LPS induces non-canonical inflammasome pathway, a potentially harmful host response, it is conceivable to therapeutically target this mechanism[46,48]. However, a great deal of care should be used in the translation of research on the non-canonical inflammasome inhibition due to multiple inter-species differences. In this review, we summarize the knowledge on endotoxin sensing in sepsis with special focus on the intracellular sensing. We also discuss new potential therapeutic approaches about septic shock [8,41,47,49].

4. The role of Ultrasound in patient with sepsis in Intensive Care Unit

Ultrasonography is an evolving skill in critically ill patients[50]. During cardiac anesthesia, and in the Intensive Care Unit (ICU), echocardiography allows diagnosis of hemodynamic disorders and assessment of the static or dynamic parameters when making decisions regarding surgical procedures and perioperative management[51,52]. Being an important diagnostic tool in cardiac surgery, echocardiography can reveal the type of low CO syndrome and assess ejection fraction, heart volumes, systolic and diastolic function, valve pathology, pulmonary circulation, ventricular filling pressures, pericardial effusion, and fluid responsiveness[53,54].

Although transthoracic echocardiography (TTE) remains the key method for good postoperative patient evaluation, during surgery and in emergent ICU situations, most cardiac anesthesiologists prefer transesophageal echocardiography (TEE) for checking the adequacy of mitral valve repair and other procedures[50,55].

Guidelines published by the *European Society of Intensive Care Medicine (ESICM)* and by the *American Society of Anesthesiologists (ASA)* and *Society of Cardiovascular Anesthesiologists* recommend the use of TEE for patients with persistent hypotension or hypoxia when timely diagnostic information cannot be obtained by TTE or other modalities[50,55].

The use of echocardiography is also encouraged during the initial phase of shock to help identify the main mechanisms involved in "*Low cardiac output syndrome"* (LCOS) and to aid in the selection of appropriate therapy[55,56].

"Ultrasonic cardiac output monitoring" (USCOM) is another technique that applies ultrasound to suprasternal and intercostal sites, facilitating a cross-sectional view of the aortic and pulmonary outflow tracts[52,55]. USCOM is often used after coronary bypass procedures, not involving valvular lesions. However, the technique has questionable accuracy, compared with thermodilution performed using advanced hemodynamic platforms[55,57].

5. Research Project

The purpose of the present study was to assess the effectiveness of a new combined approach, with IgM-enriched Immunoglobulin and extracorporeal blood purification associated with Levosimendan infusion in a cohort of patients developing septic shock after cardiac surgery, measured as effects on modulation of inflammatory cascade, hemodynamic response and optimization of peripheral perfusion.

6. Materials and Methods

6.1 Study Design and Population

We conducted an observational study on patients with depressed cardiac function who developed septic shock after cardiac surgery between November 2020 and August 2022.

The study was conducted at *Heart Center* of *Great Metropolitan Hospital* "*Bianchi-Melacrino-Morelli"* in Reggio Calabria in collaboration with the *"Department of Clinic and Experimental Medicine"* of University of Messina.

6.2 Clinical Management

Patients were treated according to current guidelines for the management of patients with Septic Shock *(Suviving Sepsis Campaign).*

Data of patients enrolled between November 2020 and August 2022 (*Active group*) were compared with data already recorded from a population of patients with depressed cardiac function approach admitted between November 2019 and November 2020 (*Control group*), who developed septic shock after cardiac surgery.

Active Group consolidated treatment at our Institution, according to the current Guidelines, was:

- a cycle of Levosimendan ic ev for 24/h;
- extracorporeal blood purification therapy (*EBPT*) in combination with intravenous administration of IgM enriched immunoglobulin 5 mL/kg/die for at least three consecutive days.

Control Group consolidated treatment according to the previous Guidelines (*SSC 2016*).

Inclusion criteria were:

1. Patients over 18 years of age;

2. Patients undergoing cardiac surgery (from valve dysfunction, bacterial endocarditis, dilated cardiomyopathy, myocardial ischemia due to critical coronary artery disease) with preoperative ejection fraction (EF) lower than 35%;

3. Diagnosis of Septic Shock within one week of surgery, according to Guidelines (ref. SSC guidelines 2021, www.survivingsepsis.org).

Exclusion criteria were:

1. Patients under the age of 18;

2. Pregnant or nursing women;

3. Patients with chronic renal failure and on RRT before surgery;

4. Life expectancy <24 hours;

5. Need for extracorporeal membrane oxygenation (ECMO).

Patients with a diagnosis of septic shock were identified according to *Surviving Sepsis Campaign 2021* criteria[1].

Subjects who satisfied the listed requirements and who developed septic shock within 7 days after heart surgery were included in the study.

The *Sequential Organ Failure Assessment Score* (*SOFA Score*) that is an individual score for each organ to determine progression of organ dysfunction was measured at baseline and at different time points in Intensive Care Unit (ICU): time zero (baseline), 24 hours after start's treatment (T1); 48 hours after start's treatment (T2); 72 hours after start's treatment.

The first group (*Active Group*) received a cycle of Levosimendan ic ev for 24/h and underwent extracorporeal blood purification therapy (*EBPT*) in combination with intravenous administration of IgM enriched immunoglobulin 5 mL/kg/die for at least three consecutive days, in conjunction with *Surviving Sepsis Campaign Guidelines 2021*[1].

In addition, serum Interleukin 6 (IL-6), Endotoxin Activity Assay (EAA), Procalcitonin (PCT), White Blood Cells counts (WBC), Erythrocyte Sedimentation Rate (ESR), C Reactive Protein (CRP), Sequential Organ Failure Assessment Score (SOFA), Temperature (T), Vasoactive Inotropic Score (VIS), Ejection Fraction (EF), Stroke Volume (SV), Cardiac Output (CO) were assessed in both two groups at different time points: time zero (baseline), 24 hours after start's treatment (T1); 48 hours after start's treatment (T2); 72 hours after start's treatment.

SV and CO were measured by ultrasound bedside and were confirmed by hemodynamic platform (*EV1000*® systems*, Edwards LifeSciences*®, Irvine, CA).

6.3 Ethic Committee Approval

The study did not involve medical, pharmacological, or behavioral interventions in addition to hospital standards of care. This research has been carried out in agreement with the principles laid out in the original Declaration of Helsinki and its later amendments.

The study was approved by by the locals Ethics Review Committee: Ethics Committee "*South Area Division*" of the Calabria Region.

6.4 Primary and secondary endpoints

The primary endpoint of the study was to value Blood Endotoxin Activity. EAA™® is a useful test to measure endotoxemia severity in whole blood in critical patients.

The secondary endpoints were:

- assessment of multiorgan dysfunction (SOFA) and the dynamics of biochemical and biohumoral variables (PCT, IL-6, WBC, ESR, CRP, ESR, T) over time observed.

• the study of the performance indicators of the cardiovascular system advancement of hemodynamic dysfunction, such as EF (Ejection Fraction), SV (Stroke Volume), CO (Cardiac Output) and VIS (Vasoactive Inotropic score).

• hospital mortality, length of stay in the ICU and 30-day, 60-day, 90-day survival.

All data were measured at baseline and at 24, 48, 72/h after start's treatment.

6.5 Rationale of multimodal therapeutic strategy

6.5a Levosimendan

The pharmacological effects of Levosimendan, an inodilatator indicated for the treatment of decompensated heart failure, are exerted via three pathways: (a) increased sensitivity of troponin C to calcium in myocardial cells, thereby inducing a cAMP-independent inotropic effect; (b) opening of adenosine triphosphate-sensitive potassium channels (KATP channels) in the smooth muscle cells of the vasculature, so inducing vasodilation; and (c) activation of KATP channels in cardiac mitochondria, hence protecting cells against ischemia/reperfusion injury[58,59].

As it is a calcium sensitizer not a calcium mobilizer, Levosimendan does not increase myocardial oxygen consumption, and prevents myocardial apoptosis and remodeling[59,60].

The drug's impact on mortality in large randomized controlled trials has been inconsistent or inconclusive but, in contrast to conventional inotropes, there have been no indications of worsened survival and some signals of improved heart failure-related quality of life. For this reason, Levosimendan has been proposed as a safer inodilator option than traditional agents in settings, such as advanced heart failure [59,61].

The Surviving Sepsis Guidelines currently support the use of dobutamine to augment vasopressor therapy in the appropriate clinical scenarios, but the evidence for its use is sparse. There certainly are potential downfalls to the use of inotropic agents in septic cardiomyopathy, such as increase myocardial demand and vasodilation. Because of their mechanism of actions, milrinone and

levosimendan have the theoretical advantage of acting outside of the adrenergic pathway which is known to be dysfunction in "*sepsis-induced cardiomyopathy" (SICM)*[1,62,63].

Levosimendan is an attractive therapy for septic cardiomyopathy because it targets cardiomyocyte calcium signaling which is known to be pathologic in septic cardiomyopathy. Moreover, it acts in a catecholamine-independent manner to minimize effects on oxygen demand, arrhythmias, and catecholamine resistance from sepsis. *Morelli et al.* performed a randomized, prospective trial published in 2005 which showed levosimendan improved hemodynamics (LVEF + CI) in patients not responsive to dobutamine at 5 microg/kg/min because of adrenergic hyporesponsiveness[61,64].

They also showed that levosimendan increased gastric mucosal flow, creatinine clearance, urine output, and lactate clearance compared with dobutamine [61,64].

A meta-analysis of 7 trials by Zangrillo published in 2015 showed that levosimendan had a mortality benefit when compared to dobutamine[61,65].

The next major trial to come out was the LeoPARDS trial in 2016. This double-blind RCT compared levosimendan to placebo plus standard of care for sepsis and found no difference in SOFA scores or mortality but a trend toward worse mortality with levosimendan[64,66].

6.5b IgM-enriched immunoglobulin

IgM-enriched immunoglobulin, falls under a group of polyclonal immunoglobulin preparations[67,68].

The preparations (Pentaglobin®) is comprised of immunoglobulin G (IgG) and immunoglobulin A (IgA) while also being enriched by immunoglobulin M (IgM). It must be infused intravenously at 12 mL/h for 3 days continuously[69,70].

The effects of e-IgM have been investigated in different settings and in different populations, especially in Surgery and Intensive Care Units (ICUs). A Cochrane review on 43 studies evaluated the effects of e-IgM as adjunctive therapy in patients with bacterial sepsis or septic shock based on mortality, bacteriological failures and duration of stay in hospital. Unfortunately, clinical heterogeneity prevented a pooled analysis of polyclonal and monoclonal intravenous

immunoglobulins (IVIG) and the reaching of a global statistical significance. Moreover, a significant reduction of mortality in adults with sepsis compared to placebo or no intervention was observed in a subgroup analysis of 10 polyclonal IVIG trials and seven trials on e-IgM preparation (RR 0.81), but not in trials at low risk[71,72]. The authors concluded that polyclonal IVIG reduced mortality among adults with sepsis, but the evidence for e-IgM preparation is still insufficient to support a robust conclusion of benefit, even if the mortality reduction was about 20%[73].

6.5c Extracorporeal blood purification therapy for sepsis - EBPT

The evolution of continuous renal replacement therapies (*CRRTs*) from the initial description to the current technology has permitted worldwide use of extracorporeal therapies in critically ill patients. In intensive care, severe AKI occurs mostly in the context of MOF. The term multiple organ support therapy (MOST), now termed "Extracorporeal Organ Support" (ECOS), encompasses all forms of organ support by an extracorporeal circuit (such as: renal replacement therapies [RRTs], extracorporeal CO2 removal [ECCO2R], venoarterial or veno-venous [VV] extracorporeal membrane oxygenation [ECMO], liver support systems, hemoperfusion, and various blood purification devices)[74,75]. Significant advances in technology and science resulted in the development of new biomaterial, membrane design, and anticoagulation techniques, allowing for optimization of treatment dose and modality[75,76] The possible role of extracorporeal techniques in restoring a balanced immune response by eliminating/deactivating inflammatory mediators was explored[77].

The strategies included high-volume hemofiltration (HVHF), hemoperfusion, plasma exchange, coupled plasma filtration adsorption (CPFA), and the use of high cut-off (HCO) membranes and membranes with enhanced adsorption properties[74,78].

Different extracorporeal techniques have been studied in recent years in the hope of maximizing the effect of renal replacement therapy in modulating the exaggerated host inflammatory response, including the use of high volume hemofiltration (HVHF), high cut-off (HCO) membranes, adsorption alone, and coupled plasma filtration adsorption (CPFA)[74,79]. These strategies are not widely utilized

in practice, depending on resources and local expertise. The literature examining their use in septic patients is growing, but the evidence to support their use at this stage is considered of low level[79,80].

EBPTs represent a new approach to the adjuvant treatment of severe sepsis, septic shock and MODS. During sepsis, lipopolysaccharide released by gram-negative bacteria induces activation of inflammatory pathways resulting in organ dysfunction and failure. Extracorporeal therapies that remove this molecule and support organ function are useful in critically ill patients in ICUs[74,78].

Extracorporeal blood purification therapies have been proposed as a strategy to improve outcome of septic patients, attenuating the systemic expression of pro- and anti-inflammatory mediators and restoring the immune homeostasis. Among these techniques, haemoadsorption places sorbents in direct contact with blood in an extracorporeal circuit: solutes are attracted by the sorbent through hydrophobic interactions, ionic attractions, hydrogen bonding and Van der Waals interactions. Haemoadsorption acts through a non-specific removal of a broad spectrum of inflammatory mediators, which can also include microbial toxins[75,79].

HCO membranes with high adsorbing properties are the preferred choices of clinicians and intensivists.

Other systems on direct hemoperfusion or plasma filtration/adsorption, shown in the *Table 1*, are for example: CytoSorb® (CytoSorbents Corporation, NJ, USA), EMIC-2® (Fresenius, Bad Homburg, Germany), Toraymyxin® (Toray Industries, Tokyo, Japan), Theranova® (Gambro Dialysatoren, Hechingen, Germany, a subsidiary of Baxter International), Oxyris® (Baxter, Meyzieu, France), HA330® (Zhuhai Lizhu Group of Biological Material Co, Ltd., China), and HFR® [75,77,80] (*Table 1*).

Table 1. Systems for blood purification in sepsis.

Device	***Composition***	***Mechanism***	***Eliminated substances***
Cpfa® *(Bellco)*	Polysulfone Plasma filter for continuous haemofiltration	Adsorption, Filtration Hemofiltration	Cytokines, Circulating myoglobin
Cytosorb® *(Cytosorbents)*	Polistirenedivinilbenzene	Adsorption,	Cytokines, Endotoxins
Toramyxyn® *(Toray)*	Polymyxin B	Adsorption	Endotoxins
Emic-2® *(Fresenius)*	Polysulfone	Adsorption,	Cytokines, Endotoxins, Circulating myoglobin
OXiris® *(Prismaflex)*	Membran based on AN69	Adsorption, Convection	Cytokines, Endotoxins
HA-330/380® *(Jafron)*	Neutro-macroporous resin	Adsorption, Convection	Cytokines, Endotoxins

Cpfa

Coupled plasma filtration adsorption (Cpfa®) is a novel extracorporeal blood purification therapy for sepsis which adsorbs both proinflammatory and anti-inflammatory mediators during sepsis nonselectively. In vitro studies have demonstrated the efficacy of CPFA in adsorbing inflammatory mediators like IL-1β, IL-6, IL-8, IL-10, and TNF-α amongst others[81].

CPFA has been shown to enhance early hemodynamic stability, reduce inotropic support requirement, and improve the immune response in septic patients. However, these trials have so far failed to demonstrate any improvement in hard clinical outcomes[81,82].

CPFA consists of filtration, adsorption and hemofiltration. During the filtration phase, plasma is separated from blood using a plasma filter. This separated plasma then passes through a sorbent cartridge where a specific resin allows nonspecific adsorption of pro and anti-inflammatory mediators and endotoxins. Adsorption is the accumulation of molecules on the surface of a sorbent material

depending on the membrane material, pH, ionic strength and pore size. There is no contact of red blood cells, white blood cells and platelets with the sorbent, thereby preventing treatment induced thrombocytopenia. The plasma filtrate is regenerated and returned to combine with blood thereby avoiding unwanted losses[82]. CPFA can be used with a hemofilter for additional blood purification and removal of excess fluid in the presence of acute kidney injury[81,83].

Cytosorb

CytoSorb® is an International Science Organization 10993 biocompatible device that is approved in the United States under International Science Organization 13485 certification. It is also approved as an extracorporeal cytokine adsorber in the European Union and marketed in 29 countries across the globe for all the indications that are associated with high cytokine levels[84].

It is a CE-approved hemoadsorption device designed to remove excess levels of inflammatory mediators like cytokines and other mid-molecular weight molecules through size selective removal and surface adsorption[84,85].

Unlike metabolic approaches to anti-inflammation, CytoSorb® is able to capture directly and reduce mid-molecular weight inflammatory mediators (approximately 10-60 kDa) in blood, including both pro- and anti-inflammatory cytokines, chemokines and bacterial exotoxins. It is reported to work most effectively when treatment is initiated within 24 h of diagnosed sepsis. As a result of adsorption of inflammatory metabolites like cytokines it is inferred that hemodynamic and metabolic stabilization will follow[84,86].

Polystyrene divinylbenzene copolymer columns have been developed for use with intermittent hemodialysis, continuous kidney replacement therapy (CRRT) machine, cardiopulmonary bypass, or extracorporeal membrane oxygenation (ECMO) blood circuits[85,87]. Although data from small observational studies reported improvements in hemodynamic status/vasopressor dose, organ dysfunction, and cytokine levels (particularly interleukin [IL] 6 and IL 8) in patients with septic shock

and acute respiratory distress syndrome (ARDS), a larger randomized trial of patients with sepsis did not confirm these benefits[84,87].

Toraymyxin

Toraymyxin® or Polymyxin B hemoperfusion therapy (Toray Industries Ltd.®, Japan) is the state-of-the-art therapy for the treatment of patients with endotoxic septic shock who are unresponsive to conventional treatment[88].

It is the reference for the treatment of patients with endotoxic septic shock who are unresponsive to conventional therapies. With its outstanding endotoxin removal capacity, high affinity binding may remove up to 90% of circulating endotoxin after two hemoperfusion treatments[89].

Endotoxin removal therapy with polymyxin B immobilized fiber column (PMX) has been clinically applied for sepsis and septic shock patients since 1994. The effectiveness and usefulness of this therapy have been demonstrated for more than a quarter of a century. However, a documented survival benefit has not yet been demonstrable in a large, multicenter, randomized and controlled trial. Immunomodulatory effects as a result of endotoxin removal and/or other mechanisms of action have been described in literature[88,90].

These effects and other potential immune effects may explain some of the improved effects upon organ dysf PL-B is a polycationic antibiotic and is well known to bind endotoxin and neutralize its toxicity. The binding site of PL-B to endotoxin is reported to be a lipid A portion, with binding via ionic and hydrophobic interactions. Lipid A is the toxic moiety of endotoxin and its structure is much more conserved among gram-negative bacterial species and strains. Therefore, it is rational to use PL-B as a ligand to bind many kinds of endotoxin in gram-negative sepsis. The intravenous use of PL-B is contraindicated, due to its nephrotoxicity and neurotoxicity[88].

Therefore, the PL-B was immobilized on the surface of an insoluble carrier material, so that it maintained its affinity for endotoxin, to obtain a selective adsorbent. Polystyrene and polypropylene conjugated fibers were produced by a melt spinning process, with island-sea type conjugated fibers

and polypropylene (island component) to provide reinforcement to the fibers. The PL-B immobilized knitted fabric was wrapped around a central pipe, which had a number of holes in the sidewall, producing an adsorbent compartment[89,90].

Emic-2

Ultraflux® EMiC-2® is an advanced CRRT therapy combining the advantages of citrate anticoagulated CVVHD with improved removal of middle molecules. "Emic" is an acronym for "*Enhanced Middle Molecule Clearance"*[74,76,77].

The goal is to allow the removal of the following molecules:

- *β2 microglobulin (β2m) 12 kDa*
- *Cystatin C 13 kDa*
- *Myoglobin 17 kDa*
- *Interleukin-1β (IL-1β) 18 kDa*
- *Interleukin-6 (IL-6) 21 kDa*
- *Interleukin-10 (IL-10) 37 kDa*[74,77]

OXiris

The OXiris® set can be used with the PrisMax® and PrismaFlex® systems, and is a filter that performs multiple blood purification therapies simultaneously, including continuous renal replacement therapy (CRRT) and the removal of cytokines and inflammatory mediators from the blood[74].

The Oxiris® filter set has a three-layer membrane structure:

-The enhanced AN69 membrane enables adsorption of cytokines and toxins while providing renal support by diffusion and convection.

-The PEI (polyethyleneimine) surface treatment allows for the adsorption of endotoxins, and provides renal support through removal of fluids and toxins by diffusion and convection.

-The heparin graft on the membrane reduces membrane thrombogenicity.

The Oxiris® Set is authorized by FDA under an Emergency Use Authorization (EUA) for the treatment of patients 18 years of age or older with confirmed COVID-19 admitted to the ICU with confirmed or imminent respiratory failure in need of blood purification, including use in continuous renal replacement therapy, to reduce pro-inflammatory cytokine levels, who have any one of the following conditions[74,77]:

A) Early acute lung injury (ALI)/ early acute respiratory distress syndrome (ARDS);

B) Severe disease, defined as:

- *Dyspnea,*
- *Respiratory frequency ≥ 30/min,*
- *Blood oxygen saturation ≤ 93%,*
- *Partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300 and/or Lung infiltrates >50% within 24 to 48 hours.*

C) Life-threatening disease, defined as:

- *Respiratory failure,*
- *Septic shock,*
- *Multiple organ dysfunction or failure* [74,75,77].

In our study CRRT was performed in continuous veno-venous haemodiafiltration (CVVHDF) mode with citrate-based anticoagulation and the cartridges used was integrated in the CRRT circuit.

7. Variables and Measurements

Data were prospectively collected during the patient's admission and entered into a database for research purposes.

Cardiothoracic surgery techniques included valve surgery, coronary artery bypass graft (CABG), or combined surgery such as surgery for congenital heart diseases, aortic aneurysms, and aortic dissections.

EF (Ejection Fraction), SV (Stroke Volume), CO (Cardiac Output) and VIS (Vasoactive Inotropic score) were used to evaluate the cardiovascular system function.

7.1a EAA - Endotoxin Activity Analysis

Endotoxin is a major component of the cell wall of Gram-Negative bacteria and is an important microbial mediator of sepsis. The Endotoxin Activity Assay (EAA™®) can be used as a diagnostic tool for measuring endotoxin activity and allows for stratification of patients based on endotoxin load[91].

The EAA™® is the gold standard for measuring Endotoxin Activity in human whole blood, and has been utilized in human and veterinary research projects around the world[91,92].

The EAA™TM® is a chemiluminescent bio-assay based on the oxidative burst reaction of activated neutrophils to complement coated LPS-IgM immune complexes.

The IgM antibody, a key reagent, is specific for the Lipid A portion of endotoxin (LPS). In the presence of LPS, the ensuing oxidative burst results in light emission in the presence of luminol.

By using the EAA (Spectral Diagnostics Inc. ®, Toronto, ON, Canada), a quick 30-minutes *in vitro* test that assesses neutrophil response to endotoxin by chemiluminescent reaction, blood endotoxin activity was determined. Sepsis risk and poorer clinical outcomes, both at ICU discharge and hospital discharge, were both related to EAA 0.4.[91,93]

Each EAA™MTM® test relies on 3 reactions: the first reaction is a negative control which allows each patient to be their own control, the second is the test sample and the third is a maximum chemiluminescence calibrator with a high level of exogenous endotoxin[92].

The EAA™MTM® is a semi-quantitative test that will provide an Endotoxin Activity (EA) result. The endotoxin activity result will stratify patients into a low (<0.40), intermediate (0.40-0.59) and high (≥0.60) endotoxin activity level, allowing for stratification of patients based on endotoxin load[92,93].

7.1b PCT - Procalcitonin

PCT is a precursor hormone of calcitonin, that is not detectable in healthy individuals. However, the production of PCT is upregulated in response to bacterial infections and can decrease rapidly during recovery[94,95]. Thus, PCT provides important additional information, which are able to supplement clinical and diagnostic parameters. This in turn, has not only a high impact on decisions regarding treatment of patients with suspected infections or sepsis, but can also influence the duration of antibiotic treatment courses[95,96].

However, the there is no universal consensus on the optimal use of PCT in the setting of sepsis.

For many physicians, determining the duration of an antibiotic therapy is a challenging decision, due to the fact that clinical signs and symptoms lack sensitivity and specificity to ensure differentiation between self-limited and mild viral infections from more severe bacterial infections[94,96].

The recent Guidelines of Surviving Sepsis Campaign (SSC 2021) underline the importance of PCT determination in the de-escalation process of antibiotic therapy[1,95,97].

Several observational studies assessed the prognostic ability of PCT. A 2015 systematic review and meta-analysis of PCT in predicting mortality in sepsis included 23 observational studies with 3,944 patients. Studies had different PCT cut-offs but all measured PCT serially. The authors found that elevated PCT and non-clearance of PCT were associated with increased mortality in septic patients with pooled relative risks of 2.60 (95% CI, 2.05–3.30) and 3.05 (95% CI, 2.35–3.95) respectively[98].

The two largest studies were the TRIAGE[99] and MOSES[100] studies. TRIAGE was a multicenter prospective observational study of 6,970 undifferentiated adult medical patients presenting to the EDs of three tertiary-care hospitals in Switzerland, France and the USA. Irrespective of presenting diagnosis and independent of underlying infection, PCT was a strong and independent predictor of 30-day mortality, with increased mortality as well as ICU admission and hospital readmission seen with higher PCT values. PCT also improved the prognostic accuracy of the quick sequential organ failure assessment score[1, 101].

Similarly, the ED and ICU based MOSES study of 858 patients, showed when PCT did not decrease by > 80% from baseline to day 4, 28-day mortality doubled[100].

7.1c T - Temperature

Systemic infections may alter the host body temperature, and a pre-existing altered body temperature may modulate the host response to infection[1,3].

Body temperature monitoring is an important parameter in the identification and treatment of patients diagnosed with Sepsis and Septic Shock[2,4].

The continuous measurement in intensive care can concern both the external and internal temperature.

The first can be obtained through the use, for example, of an infrared skin probe.

The second through the positioning of probes at the bladder, rectal, axillary and external auditory canal levels.

Central intravascular catheters equipped with a thermistor can also be used (e.g. PICCO® or EV1000® femoral arterial catheter).

Through continuous measurement it is useful for obtaining feedback on the clinical trend and efficacy of the therapies and for promptly treating hyperpyrexia and hypothermia conditions [1,2,3,4].

Hyperpyrexia is responsible for vasodilation resulting in hypotension and the need for volemic or pharmacological resuscitation drug treatment[1].

Hypothermia is responsible for peripheral vasoconstriction and consequent hypoperfusion[1].

7.1d VIS - Vasoactive Inotropic Score

The vasoactive-inotropic score (VIS) is a scale showing the amount of vasoactive and inotropic support calculated by a simple formula. Recently, it was suggested that the VIS after cardiac surgery predicts morbidity and mortality in patients.

The necessity and degree of hemodynamic support with vasoactive drugs is frequently considered as a marker of disease severity, and it is well known that mortality rates increase when high doses of vasoactive drugs are required.

Notably, need for vasoactive drugs is included in mortality prediction scores such as the Sequential Organ Failure Assessment (SOFA) score, European System for Cardiac Operative Risk Evaluation (EuroSCORE) II or the pRedicting mortality in patients undergoing veno-arterial Extracorporeal MEMBrane oxygenation after coronary artEry bypass gRafting (REMEMBER) score.

A total of three studies, two of which performed in the pediatric population, validated VIS in patients with septic shock[102].

The largest study was performed by McIntosh and colleagues and investigated 138 children (age 60 days – 18 years) admitted to ICU for sepsis and requiring vasoactive support. 41 Co-primary outcomes were ICU length of stay and ventilator-free days. They assessed VIS upon admission, and after 6, 12, 24 and 48 hours, as well as highest VIS in the first 48 hours, and they determined that VIS at 48 hours was the strongest predictor of the primary outcomes, while VIS at 12 hours was independently associated with a composite outcome of cardiac arrest, need for ECMO, or inhospital mortality[102,103].

Vasoactive-inotropic medications were typically started and used at the discretion of the attending anesthesiologist based on the patient's pathophysiological state, hemodynamic variables derived from the pulmonary artery catheter and blood pressure usually directly measured in the radial artery, and transesophageal echocardiographic findings. There was no dictated protocol for vasoactive and inotropic medications, but they were managed under general institutional principles[103,104].

The VIS is a numerical scale showing the amount of vasoactive and inotropic support calculated using a simple formula. The inotrope score (IS) was initially proposed by Wernovsky and colleagues in 1995 as a scale to quantify the amount of cardiovascular support received by patients after arterial switch operations[104].

The Vasoactive Inotropic Score (VIS) was calculated using the following formula:

dopamine dose (μg/kg/min) + dobutamine dose (μg/kg/min) + 100 × epinephrine dose (μg/kg/min) + 100 × norepinephrine dose (μg/kg/min) + 10,000 × vasopressin dose (U/kg/min/) + 10 × milrinone dose (μg/kg/min) + enoximone dose (μg/kg/min) + 50 × levosimendan dose (μg/kg/min) + 25 × olprinone dose (μg/kg/min) + 20 × methylene blue dose (mg/kg/h) + 10 × phenylephrine dose (μg/kg/min) + 10 × terlipressin dose (μg/min) + 0.25 × angiotensin II dose (ng/kg/min)[104].

7.1e EF - Ejection Fraction

The prognostic implications of myocardial dysfunction in patients with sepsis and its association with mortality are controversial. Several tools have been proposed to evaluate cardiac function in these patients, but their usefulness beyond guiding therapy is unclear[105].

Ejection fraction (EF) is a measurement, expressed as a percentage, of how much blood the left ventricle pumps out with each contraction. An ejection fraction of 60 percent means that 60 percent of the total amount of blood in the left ventricle is pushed out with each heartbeat.

A normal heart's ejection fraction may be between 50 and 70 percent, according to the American Heart Association[106].

Is possible to have a normal ejection fraction measurement and still have heart failure.

An ejection fraction measurement under 40 percent may be evidence of heart failure or cardiomyopathy.

An EF from 41 to 49 percent may be considered "borderline". It does not always indicate that a person is developing heart failure. Instead, it may indicate damage, perhaps from a previous heart attack.

In severe cases, ejection fraction can be very low.

Sepsis-induced cardiomyopathy is encountered in the intensive care unit (ICU), and its prevalence in septic patients ranges from 10 to 70%. This variety between studies is likely due to a lack of formal diagnostic criteria and under recognition. Moreover, the epidemiologic variance emphasizes the complex factors in sepsis: source and severity, promptness of resuscitation, and different antimicrobial and hemodynamic treatment approaches[105,107].

Septic patients with lower EF had higher mortality rates than other septic patients.

Decreased systolic contractility during sepsis limits ventricular ejection and stroke volume. Initially, this effect is compensated for by increased diastolic filling during volume resuscitation. Reduced afterload due to arterial vasodilation also compensates so that cardiac output can be maintained or increased. Recent results recognize the importance of diastolic dysfunction, reduced ventricular diastolic compliance that impedes ventricular filling. Diastolic dysfunction becomes increasingly important as severity of septic shock increases. When impaired ventricular ejection is coupled with limited diastolic filling, stroke volume must decrease[107,108].

7.1f SV and CO - Stroke Volume and Cardiac Output

In physiology, the systolic volume or stroke volume is the amount of blood pumped from a ventricle at each ventricular systole. Normally it increases as the force of contraction of the ventricle itself increases[106].

It is calculated from measurements of the volume of the ventricles obtained with echocardiography, subtracting the volume of blood that remains in the ventricle after systole (called the end-systole volume) from the volume of blood present in the ventricle just before the systole (end-diastolic volume). The term "systolic volume" can be applied to each of the two ventricles, although in most cases it refers to the left ventricle. The systolic volume is usually the same in the two ventricles, about 70 ml in a healthy 70 kg man[106].

Systolic volume is an important determinant of cardiac output, which is the product of systolic volume and heart rate, and is also used to calculate the ejection fraction, which is equal to the systolic volume

divided by the end-diastolic volume. The systolic volume is reduced in particular pathological conditions and is closely related to cardiac function[109].

The value is obtained by subtracting the end-systolic volume (ESV) from the end-diastolic volume (EDV) of the ventricle examined. The formula is: VS = EDV-ESV.

In a 70 kg healthy man, the end-diastolic volume is approximately 120 mL and the end-systolic volume is approximately 50 mL, with a difference of 70 mL which corresponds to the systolic volume.

Cardiac output is the amount of blood the heart pumps in 1 minute, and it is dependent on the heart rate, contractility, preload, and afterload[106,109].

7.2 The role of Hemodynamic platform

The transpulmonary thermodilution technique is used in VolumeView/EV1000® (*Edwards LifeSciences®, Irvine, CA*) systems, and involves the injection of a cold saline bolus into the jugular or subclavian vein followed by measurement of the resulting temperature changes in a femoral artery using a thermistor-tipped arterial catheter[110,111]. The mathematical analysis of the thermodilution curve allows calculation of CO, global end-diastolic volume (GEDV), global ejection fraction, cardiac function index, extravascular lung water (EVLW), and pulmonary vascular permeability index[112]. These parameters provide additional information about the patient's hemodynamic status and can serve as therapeutic targets[111].

7.2 *Statistical Analysis*

Descriptive and comparative analysis of all considered outcomes were performed between *Active and Control groups*. The Kolmogorov–Smirnov test was performed to estimate the normal distribution of all outcomes. Since the variables were not normally distributed, a non-parametric approach was applied. Absolute and relative frequencies of categorical variables were calculated, while median values with interquartile ranges (IQ1-IQ3) were evaluated for the continuous variables. Mann

Whitney's test was applied to compare continuous variables between two groups. For each group, the Friedman test were applied to compare all the continuous outcomes at the different time points.

Fully adjusted Cox proportional hazards regression models were generated for mortality outcomes. The Cox models were adjusted for therapeutic strategy (*Active groups and Control group*).

A p-value of <0.05 was considered statistically significant. The statistical analysis was performed with SPSS version 23.0 (IBM Corp., SPSS Statistics, Armonk, NY, USA).

8. Results

8.1 Baseline and Clinical Characteristics

Among 79 patients, 41 (%) received the *new standard of care treatment* for septic shock plus blood purification and IgM enriched Immunoglobulins (*active group*), and the remaining 38 (%) patients with the *precedent standard of care treatment* (*control group*).

The two groups did not significantly differ in age and gender as well as in the prevalence of acute renal insufficiency (AKI), Hypertension, Chronic obstructive pulmonary disease (COPD), Left Ventricular Ejection Fraction (EF), Cardiopulmonary bypass (CBP) and Aortic Cross Clamp (ACC) Times *(Table 2).*

No statistically significant changes were observed in the two groups, except for VIS (35 vs 39; p=0,020).

Furthermore at baseline, circulating levels of PCT and IL-6, SOFA score, WBC and EAA were similar between the two groups.

Also the hemodynamics parameters EF, CO, VIS were also overlapping and showed no differences in the two groups.

However, the Vasoactive Inotropic Score was significantly higher in patients of the active group than in the control group (*Table 2*).

The two groups of patients are also similar in type of surgery. In the active group 18 patients (43,9%) underwent valvular surgery; 13 (31,7%) coronary artery bypass graft; 8 (19,5%) ascending aorta and 2 (4,8%) frozen elephant trunk (FET).

In the control group 16 patients (42,1%) underwent vavular surgery; 14 patients (36,8%) coronary artery bypass graft; 7 patients (18,4%) ascending aorta and 1 patient (2,6%) frozen elephant trunk (FET).

Colonization was present in 14 patients (34,15%) in the active group compared to 13 patients (34,2%) in the control group.

Multiple drug resistance (MDR) Bacteria in the active group were 18 (44 %) compared to 16 (42,1%) in the control group.

Considering the whole population, *Methicillin-Resistant Staphylococcus Aureus (MRSA)* was present in 3 patients (7,3%) in the active group and in 2 (5,2%) patients in control group.

Microbiological identification of bacterial micro-organisms in both groups is reported in *Table 3*.

In the active group, 3 patients (7,31%) was treated with Coupled Plasma Filtration and Adsorption (CPFA ®) together with Cytosorb® Cartridge and Toraymyxin® Cartridge, 18 patients (43,9%) were treated with continuous venovenous hemodiafiltration (CVVHDF) together with Cytosorb®, 15 patients (36,6%) were treated with Hemoperfusion together with Toraymyxin® and after with CVVHDF, 4 patients (9,7%) were treated with Hemoperfusion + Toraymyxin®, 2 patients (4,9%) were treated with Ultraflux® EMIC2 Filter (*Table 4).*

8.2 Characteristics of patients by group overtime

8.2.1 Primary outcome

EAA were evaluate at 24h, 48h and 72h.

Median EAA significantly decreased from 1.4 at baseline to 0.6 at 72h in active group (*Table 5*) and from 1.4 at baseline to 1.2 at 72h in control group (Table 6).

A significant greater reduction was observed in the active group compared to the control group both at 48h (-0,3 vs 0; p= 0,006) and at 72h (-0,7 vs 0,3; p= 0,003) (*Table 8, Table 9, Figure 1*)

8.2.2 Inflammation Outcome

PCT, IL-6, CPR, ESR, WBC were evaluated at 24h, 48h and 72h.

Median PCT significantly decreased from 12 at baseline to 5.6 at 72h in active group (*Table 5*) and from 14 at baseline to 6.6 at 72h in control group (*Table 6, Figure 2*).

Median (IQR) IL-6 significantly decreased from 1,6-4,0 at baseline to 1,4-3,4 at 72h in active group (Table 5) and from 2,3 to 2,2 in control group (*Table 6, Figure 3*).

No significant statistical variation was observed in the active group compared to the control group of PCT and of IL-6 a 24h, 48h e 72h (*Table 7, Table 8, Table 9*).

Median CRP and ESR significantly decreased from 61,3 at baseline to 36 at 72h in active group (Table 5) and from 63,6 to 51,0 at 72h in control group (*Table 6*).

A significant greater reduction of ESR was observed in the active group compared to the control group at 24h (-4 vs - 1,7; p= <0,001), at 48h (-14 vs -5,5; p= <0,001) and at 72h (-26 vs -8,6; p= <0,001) (*Table 7, Table 8, Table 9, Figure 4*).

A significantly greater reduction in CPR was observed in the active group compared to the control group at 24h (-4 vs -2; p= <0,001), at 48h (-14 vs -6; p= <0,001) and at 72h (-25,3 vs -10,1; p= <0,001) (*Table 7, Table 8, Table 9, Figure 5*).

Median WBC significantly decreased from 26.666 at baseline to 19.780 at 72h in active group (p= <0,001) (Table 5) and significantly increased from 27.950 at 24h to 30.620 at 72h in control group (p= 0,001) (*Table 6*).

A significant greater reduction of WBC was observed in the active group compared to the control group at 24h (-4000 vs 2400; p= <0,001), at 48h (-5500 vs 4450; p= <0,001) and at 72h (-11000 vs 1385; p= <0,001) (*Table 7, Table 8, Table 8, Figure 6*).

Median SOFA score significantly decreased from 11,7 at baseline to 8,2 at 72h in active group (p= <0,001) (Table 5) and from 12,7 at baseline to 10 at 72h in control group (p= <0,001) (Table 6).

A significant greater reduction of SOFA score was observed in tha active group compared to control group at 72h (- 4,2 vs - 2,5; p= 0,001) (*Table 9, Figure 7*).

Median Temperature significantly decreased from 38,4°C at baseline to 37°C at 72h in active group (p= <0,001) (*Table 5*) and from 38,4°C at baseline to 38,3°C at 72h in control group (p= <0,001) (*Table 6*).

A significant greater reduction of Temperature was observed in the active group compared to control group a 24h (- 1,1 vs - 0,1; p= <0,001); a 48h (-1,2 vs -0,5; p= <0,001); a 72h (-1,5 vs -0,3; p= <0,001) (*Table 7, Table 8, Table 9, Figure 8*).

8.2.3 Hemodynamic Outcome

We also evaluated hemodynamic and cardiovascular performance parameters.

Median VIS significantly decreased from 35 at baseline to 16 at 72h in active group (p= <0,001) (Table 5) and significantly increased from 39 at baseline to 43 at 72h in control group (p= <0,001) (*Table 6*).

A significant major reduction of VIS was observed in the active group compared to control group at 48h (-8 vs 6; p= <0,001) and at 72h (-18 vs 7; p= <0,001) (*Table 8, Table 9, Figure 9*).

Median EF significantly increased from 25 % at baseline to 35% at 72h in active group (p= <0,001) (Table 5) and from 25% at baseline to 30% at 72h in control group (p= <0,001) (*Table 6*).

No significant statistical variation was observed in the active group compared to control group at 24h, at 48h and at 72h (*Table 7, Table 8, Table 9, Figure 10*).

Median SV significantly increased from 33 at baseline to 43 at 72h in active group (p= <0,001) (Table 5) and from 33 at baseline to 40 at 72h in control group (p= <0,001) (*Table 6*).

A significant greater increase of SV was observed in the active group compared to control group at 48h (5 vs - 1; p= <0,001) and at 72h (9 vs 5,5; p= <0,001) (*Table 8, Table 9, Figure 11*).

Median CO significantly increased from 3 at baseline to 3,6 at 72h in active group (p= <0,001) (Table 5) and from 3 at baseline to 3,6 at 72h in control group (p= <0,001) *(Table 6*).

A significant greater increase of CO value was observed in the active group compared to control group at 24h (0,1 vs -0,3; p=<0,001), at 48h (0,4 vs - 0,2; p= <0,001) and at 72h (0,7 vs 0,2; p= <0,001) (*Table 7, Table 8, Table 9, Figure 12*).

8.3 Survival Analysis

A secondary endpoints were hospital mortality, length of stay in the ICU and 30-day, 60-day, 90-day survival. During the follow-up period (median 90 days), 26 patients died.

Among these, 12 deaths were observed in the active group (29,3 %) and 14 in the control group (36,8 %). In the active arm and in the control group, all death cases were due to multiple organ failure (*Table 10*).

In the active group, 5 patients died within the first 30 days in ICU.

In the control group, 8 patients died within the first 30 days in ICU.

We observed a lower percentage of patients who died in the active group at 30, 60 and 90 days and a greater survival of the active group compared to the control group (*Figure 13*).

However, no statistically significant difference was observed between the two groups (p-value= 0,474)

The data show that patients in the active group have a lower risk of dying from the start of the therapeutic strategy than in the control group (*Figure 14*).

9. Discussion

Sepsis-induced myocardial dysfunction is one of the significant predictors of morbidity and mortality of sepsis, and it is present in more than 40% of cases of sepsis[1,3,4,5].

Endotoxin is considered a key signaling molecule in the pathogenesis of sepsis and septic shock. Anti-endotoxin therapies may result in the improvement of a patient's clinical condition and lower mortality. The pressing clinical challenge is to identify patients for whom endotoxin elimination would be the most beneficial. An endotoxin activity assay (EAA) is available for detection of endotoxins, allowing identification of patients at high risk in intensive care units (ICU)[39,41,42,44].

Despite great efforts and numerous clinical trials, there is still a major need for effective therapies for sepsis. Neutralization or elimination of bacterial toxins remains a promising approach[47,49].

The understanding of the interaction of the endotoxin Lipopolysaccharide - LPS) of Gram-negative bacteria with its cellular receptor, namely the CD14 / TLR4 / MD2 complex, was a major breakthrough[42,44,49]. Unfortunately, clinical trials for sepsis on the neutralization of LPS or on the inhibition of TLR4 signaling failed whereas those on LPS removal remain controversial. Recent discoveries of another class of LPS receptors localized within the cytoplasm, namely *caspase-11* in mice and *caspases-4/5* in humans, have renewed interest in the field. These provide new potential targets for intervention in sepsis pathogenesis[44,49].

The next few years will be crucial for the development of new rapid approaches to the diagnosis and treatment of sepsis.

In recent years, the attention of many researchers has shifted to mitigating the effect of mediators and cytokines on the cardiovascular system to treat and control septic myocardial dysfunction through newly developed techniques such as EBPT and intravenous immunoglobulins[92].

Blood purification therapy is a non-selective and broad-spectrum strategy able to remove both pro-inflammatory and anti-inflammatory mediators from the bloodstream, thus restoring the immune homeostasis. In addition, by performing a concentration-dependent removal of molecules, blood purification acts as a self-tailored therapy[113].

The administration of intravenous immunoglobulins (IVIG) represents a possible additional supportive treatment for the management of acute severe bacterial infections, even though a general consensus based on scientific evidence is still lacking[17,19,20].

Therefore, our results should be considered exploratory and need confirmation by future studies.

Our study did not contemplate a sample size calculation, a limitation that suggests caution when interpreting the study results, particularly those that did not achieve statistical significance.

In this research project, postoperative cardiac surgery patients who received Levosimendan, extracorporeal therapy and IgM enriched immunoglobulins showed improvement in cardiac function and hemodynamics stability.

Additionally, patients undergoing extracorporeal therapy or RRT experienced a more rapid drop in temperature due to the treatment.

This may have resulted a reduction in inflammatory processes, vasoplegia and VIS.

10. Conclusions

The study has several limitations. First, the observational design prevent the possibility to demonstrate any cause-effect relationship between therapeutic strategy and cardiovascular changes: we cannot exclude that the improvement in hemodynamic stability in the Active Group was a result of other therapies.

Second, the small sample size may be underpowered to detect significant changes in some variables. However, this was conceived as an exploratory observational study aimed to detect any improvement in the patients during therapy in sepstic shock and these findings warrant further investigations.

Third, a longer follow-up period (take samples after 72h) could have been required to observe changes in some variables like: IL-6, EAA, GB, PCT, CRP, ESR.

Measurement of combinational biomarkers may require reliable and cost-effective technology development. Selection of biomarkers plays a crucial role in technology development; consequently, assessment of combination of biomarkers like procalcitonin, sCD14-ST, Endotoxemia and other new biomarkers can be made use in evaluation of septic shock in all age groups.

Combination of emerging new biomarkers with PCT could be used in terms of good clinical judgement based on which antimicrobial therapy may suggested, thus reducing the prescription and duration of antibiotic treatment. Combinational biomarker guided antibiotic stewardship is necessary to develop a safer and affordable strategy for diagnosis of sepsis and its prognosis[1,3,4].

The guidelines of the SSC 2021 do not provide certain indications regarding the use of adsorbing HCO filters and the use of IgM-enriched immunoglobulin preparations[1].

The difficulty faced in the most important clinical trials up to now has been to guarantee a correct timing of the start of treatment, which unfortunately cannot be the same for all patients, as it depends on a correct and fast diagnosis of septic shock. The search for new and rapid biomarkers, such as LPS / Endotoxin, could be useful in improving the problem of the time, to confirm the diagnosis and speed up the initiation of multimodal treatments.

Although difficult, further studies could support the role of these "tailored" therapeutic strategies in the patient with septic shock.

11. Tables and Figures

Table 2. Main characteristics of the study population. The table shows comorbidities, the demographic and baseline clinical data of the study.

Baseline Variable		Active Group N=41	Control Group N=38	P-value
Age		71 (65-75)	68 (61-71)	0.080
Sex		28 (68,3)	19 (50,0)	0.098
Hyperthension		38 (92,7)	37 (97,4)	0.343
COPD		30 (73,2)	25 (65,8)	0.476
LVEF		30 (28-35)	30 (27-33)	0.709
CPB		91 (78-104)	89 (80-111)	0.844
ACC Time		72 (60-83)	71 (56-82)	0.648
EAA		1,4 (0,9-2,2)	1,4 (1,2-1,9)	0.753
PCT		12 (8-19)	14 (8-24)	0.589
IL-6		2,3 (1,6-4,0)	2,3 (1,3-3,6)	0.833
WBC		26.666 (20.333- 33.000)	27.950 (23.475-31.200)	0.429
SOFA		11,7 (9,8-13,8)	12,7 (10,3- 13,5)	0.468
VIS		35 (32- 44)	39 (34- 46)	0.020
EF %		25 (25-30)	25 (25-30)	0.816
SV		33 (29-39)	33 (29-40)	0.825
CO		3 (2,6- 3,6)	3 (2,6-3,8)	0.783
T		38,4 (38,0-39,0)	38,4 (38,0- 39,0)	0.887
Type of surgery:				
	Valve	18 (43,9 %)	16 (42,1 %)	
	Cabg	13 (31,7 %)	14 (36,8 %)	
	Ascending Aorta	8 (19,51 %)	7 (18,4 %)	
	FET	2 (4,8 %)	1 (2,6 %)	

List of abbreviations: COPD: Chronic Obstructive Pulmonary Disease; LVEF: Left Ventricular Ejection Fraction; CPB: Cardiopulmonary Bypass; ACC Time: Aortic Cross-Clamp Time; EAA: Endotoxin Activity Assay; PCT: Procalctonin; IL-6: Interleukin-6; WBC: White Blood Cell; SOFA: Sequential Organ Failure Assessment; VIS: Vasoactive Inotropic Score; EF: Ejection Fraction; SV: Stroke Volume; CO: Cardiac Output; T: Temperature; CABG: Coronary artery bypass graft; FET: Frozen Elephant Trunk.

Table 3A. Microbiological identification of bacterial micro-organisms.

Pathogen	**Active group** (n = 41)	**Control group** (n = 38)
Acinetobacter Baumani	6	6
Acinetobacter Baumani plus Klebsiella pneumoniae	2	1
Citrobacter Youngae	2	1
Enterobacter cloacae	1	2
Enterococcus faecium	2	1
Enterococcus faecium + Staph. Epidermidis	1	2
Escherichia Coli	2	2
Klebsiella Pneumoniae	5	6
Pseudomonas Aeurginosa	4	3
Pseudomonas Aeuruginosa plus Proteus Mirabilis	0	2
Staphylococcus Aureus plus Acinetobacter Baumanii	4	3
Staphylococcus Aureus	4	3
Staphylococcus Meticillin resistant	1	1
Staphylococcus Meticililin Resistant plus Acinetobacter Baumanii	2	1
Streptococcus Pneumoniae	3	2
Serratia Marciscens	2	2

Table 3B. Cause of septic shock.

Pathogen	**Active group** (n = 41)	**Control group** (n = 38)
Ventilator Associate Pneumonia	17	15
Blood Stream Infection	16	17
Abdominal Infection	7	5
Urinary infection	1	1

Table 4. Extracorporeal blood purification therapy for sepsis - EBPT.

Extracorporeal blood purification therapy for sepsis - EBPT	**Treatment's Time**	**Active Group** (n = 41)
CPFA® + Toraymyxin® and CVVHDF + Cytosorb®	24h+48h+48h	3
CVVHDF + Cytosorb®	48h	18
Hemoperfusion with Toraymyxin® and CVVHDF	24+48h	18
Hemoperfusion with Toraymyxin®	48h	4
CVVHF with Ultraflux® EMIC-2®	72h	2

List of abbreviations: CPFA: Coupled plasma filtration adsorption; CVVHF: Continuous Veno-Venous Hemofiltration; CVVHDF: Continuous Veno-Venous Hemo-Dia-Filtration.

Table 5. Characteristics of "*active group"* patients by time-points.

Variable	Baseline	24h	48h	72h	P-value
EAA	1,4 (0,9-2,2)	1,0 (0,9-1,8)	1,0 (0,6-1,4)	0,6 (0,4-1)	<0.001
PCT	12 (8-19)	11 (7,1-17,5)	7,4 (5,3 -10)	5,6 (4-7,5)	<0.001
IL-6	2,3 (1,6-4,0)	3 (2,4-4,5)	2,7 (2,1 - 4)	2,3 (1,4-3,4)	<0.001
CRP	61,3 (58-66)	57 (52-60,5)	47,1 (44,5-51,4)	36 (33-38,7)	<0.001
ESR	61,3 (58-66)	57 (52-60,5)	47,1 (44-51,5)	36 (33-39)	<0.001
WBC	26.666 (20.333-33.000)	24.000 (15.500-30.250)	23.000 (17.650-30.750)	19.780 (16.000-26.150)	<0.001
SOFA	11,7 (9,8-13,8)	10 (7,6- 13)	10 (9-11,8)	8,2 (5-8,4)	<0.001
VIS	35 (32- 44)	36 (31-43)	25,8 (20,2-36)	16 (12,2-25,0)	<0.001
EF %	25 (25-30)	25 (20-30)	30 (25-35)	35,0 (28,0-37,5)	<0.001
SV	33 (29-39)	35 (31-42)	39 (33,5-45,5)	43 (39-49,5)	<0.001
CO	3 (2,6- 3,6)	3 (2,6-3,6)	3,3 (2,9-3,9)	3,6 (3,3-4,3)	<0.001
T	38,4 (38,0-39,0)	37,3 (36,8-37,6)	37,1 (36,7-37,4)	37 (36,5-37,5)	<0.001

List of abbreviations: EAA: Endotoxin Activity Assay; PCT: Procalctonin; IL-6: Interleukin-6; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White Blood Cell; SOFA: Sequential Organ Failure Assessment; VIS: Vasoactive Inotropic Score; EF: Ejection Fraction; SV: Stroke Volume; CO: Cardiac Output; T: Temperature.

Table 6. Characteristics of *"control group"* patients by time-points.

Variable	Baseline	24h	48h	72h	P-value
EAA	1,4 (1,2-1,9)	1,3 (1,2-1,7)	1,4 (1,1-1,7)	1,2 (0,8-1,4)	<0.001
PCT	14 (8-24)	11,6 (7,1-22,3)	8,9 (5,9- 21,6)	6,6 (4,2-16,8)	<0.001
IL-6	2,3 (1,3-3,6)	2,9 (2,4-3,8)	2,4 (2,1-3,4)	2,2 (1,3-2,7)	<0.001
CRP	63,6 (58-66,9)	61,2 (56,5-65,0)	56,4 (53,0-61,0)	51,0 (47,8-57,7)	<0.001
ESR	61,7 (58-66,2)	60,6 (56,1-64,4)	55,8 (51,2-60,5)	51,2 (48-57,5)	<0.001
WBC	27.950 (23.475-31.200)	29.000 (24.975-35.775)	32.950 (27.075-38.700)	30.620 (23.695-38.000)	0.001
SOFA	12,7 (10,3- 13,5)	11,5 (10,6-12,8)	11,0 (9,8-11,9)	10 (9,1-10,8)	<0.001
VIS	39 (34- 46)	40,5 (37-49)	44 (39-50,8)	43 (30,3-49,8)	<0.001
EF %	25 (25-30)	26,5 (20-30)	30 (25-33,5)	30 (28-35)	<0.001
SV	33 (29-40)	36 (31-42)	34,5 (31-41,5)	40 (35,8-42,3)	<0.001
CO	3 (2,6-3,8)	2,8 (2,3-3,2)	2,8 (2,5-3,1)	3,2 (2,9-3,4)	<0.001
T	38,4 (38,0- 39,0)	38,4 (38,10-38,6)	38 (37,4-38,3)	38,3 (37,6-38,5)	<0.001

List of abbreviations: EAA: Endotoxin Activity Assay; PCT: Procalctonin; IL-6: Interleukin-6; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White Blood Cell; SOFA: Sequential Organ Failure Assessment; VIS: Vasoactive Inotropic Score; EF: Ejection Fraction; SV: Stroke Volume; CO: Cardiac Output; T: Temperature.

Table 7. Comparison in the change between the two groups of all variables studied at 24h.

Variables	Active Group Change Value at 24h	Control Group Change Value at 24h	P-value
EAA	-0,1 (-0,8/0,3)	0,1 (-0,3/0,5)	0.091
PCT	-0,6 (-3/1,1)	-0,6 (-3,2/2,5)	0.543
IL-6	0,5 (0,3/0,7)	0,4 (0,3/0,7)	0.701
CRP	-4 (-6/-3,1)	-2 (-2,7/-1,4)	<0.001
ESR	-4 (-6/-3,3)	-1,7 (-2/-1)	<0.001
WBC	-4.000 (-7.400/ 2665)	2.400 (630/4.625)	<0.001
SOFA	-2,1 (-3,8/0,2)	-0,7 (-1,5/-0,3)	0.053
VIS	2 (-0,8/4)	3 (2/4)	0.395
EF %	0 (-3,5 /2,5)	0 (-3/3)	0.764
SV	2 (1,5/3)	2 (1,8/3)	0.895
CO	0,1 (-0,2/0,2)	-0,3 (-0,5/0)	<0.001
T	-1,1 (-1,9/-0,6)	-0,1 (-0,8/0,3)	<0.001

List of abbreviations: EAA: Endotoxin Activity Assay; PCT: Procalctonin; IL-6: Interleukin-6; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White Blood Cell; SOFA: Sequential Organ Failure Assessment; VIS: Vasoactive Inotropic Score; EF: Ejection Fraction; SV: Stroke Volume; CO: Cardiac Output; T: Temperature.

Table 8. Comparison in the change between the two groups of all variables studied at 48h

Variables	Active Group Change Value at 48h	Control Group Change Value at 48h	P-value
EAA	-0,3 (-1,1/0)	0 (-0,3/0,5)	0.006
PCT	-3 (-11/,0,7)	-2 (-9,6/2,5)	0.141
IL-6	0,1 (-0,1/-0,4)	0,1 (-0,1/0,4)	0.863
CRP	-14 (-16,7/-11,4)	-6 (-7,5/-4,4)	<0.001
ESR	-14 (-16,6/-11,5)	-5,5 (-7/-4,7)	<0.001
WBC	-5.500 (-8.000/-383)	4.450 (0/8.482)	<0.001
SOFA	-2,4(-4,4/0,8)	-1,6 (-2,2/-0,4)	0.429
VIS	-8 (-10/-3,7)	6 (4/7,3)	<0.001
EF %	3 (0/5)	4 (0/5)	0.960
SV	5 (4/7)	1 (0/3,3)	<0.001
CO	0,4 (0,1/0,5)	-0,2 (-0,5/0)	<0.001
T	-1,2 (-2,1/-0,8)	-0,5 (-0,9/0)	<0.001

List of abbreviations: EAA: Endotoxin Activity Assay; PCT: Procalctonin; IL-6: Interleukin-6; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White Blood Cell; SOFA: Sequential Organ Failure Assessment; VIS: Vasoactive Inotropic Score; EF: Ejection Fraction; SV: Stroke Volume; CO: Cardiac Output; T: Temperature.

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Table 9. Comparison in the change between the two groups of all variables studied at 72h.

Variables	Active Group Change Value at 72h	Control Group Change Value at 72h	P-value
EAA	-0,7 (-1,3/-0,3)	-0,3 (-0,7/0,1)	0.003
PCT	-5,8 (-15/-1,4)	-3,9 (-12,7/0,8)	0.263
IL-6	-0,4 (-0,8/0)	-0,4 (-0,8/0)	0.844
CRP	-25,3 (-29,8/-22,7)	-10,1 (-11,8/-8,0)	<0.001
ESR	-26 (-29,3/-22,2)	-8,6 (-10,7/-8,0)	<0.001
WBC	-11.000 (-14.500/-2.720)	1.385 (-2.790/7.850)	<0.001
SOFA	-4,2 (-7,3/-2)	-2,5(-3,5/-0,4)	0.001
VIS	-18 (-27/-10)	7 (-9,3/12)	<0.001
EF %	5 (3/10)	5 (3/10)	0,636
SV	9 (8/11,5)	5,5 (3/9)	<0.001
CO	0,7 (0,5/0,9)	0,2 (-0,2/0,5)	<0.001
T	-1,5 (-2,0/-0,9)	-0,3 (-1/0,2)	<0.001

List of abbreviations: EAA: Endotoxin Activity Assay; PCT: Procalctonin; IL-6: Interleukin-6; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White Blood Cell; SOFA: Sequential Organ Failure Assessment; VIS: Vasoactive Inotropic Score; EF: Ejection Fraction; SV: Stroke Volume; CO: Cardiac Output; T: Temperature.

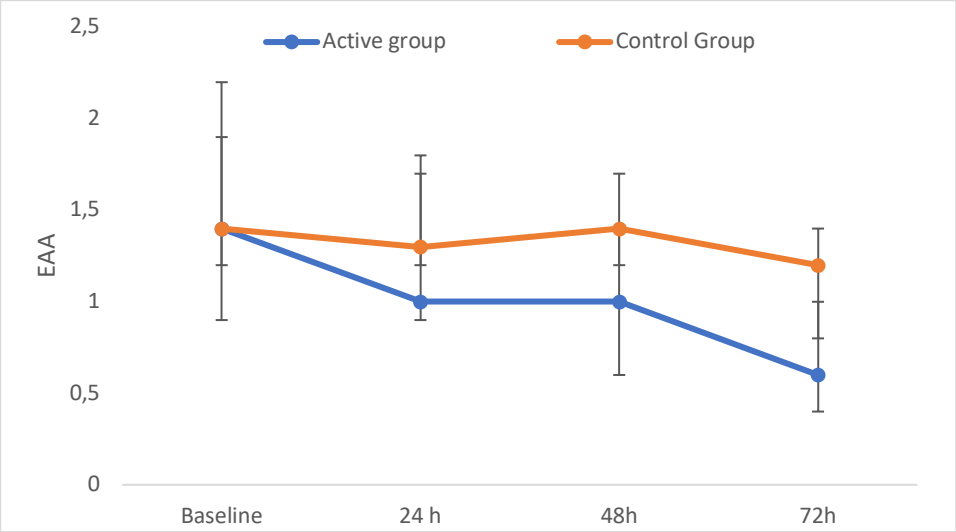

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Figure 1. Endotoxemia measured by EAA™

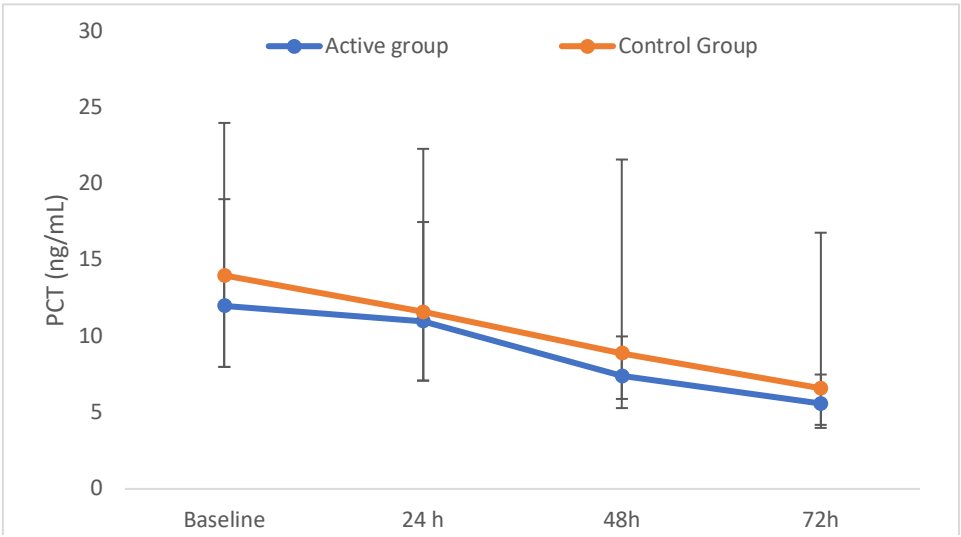

Figure 2. Procalcitonin (PCT)

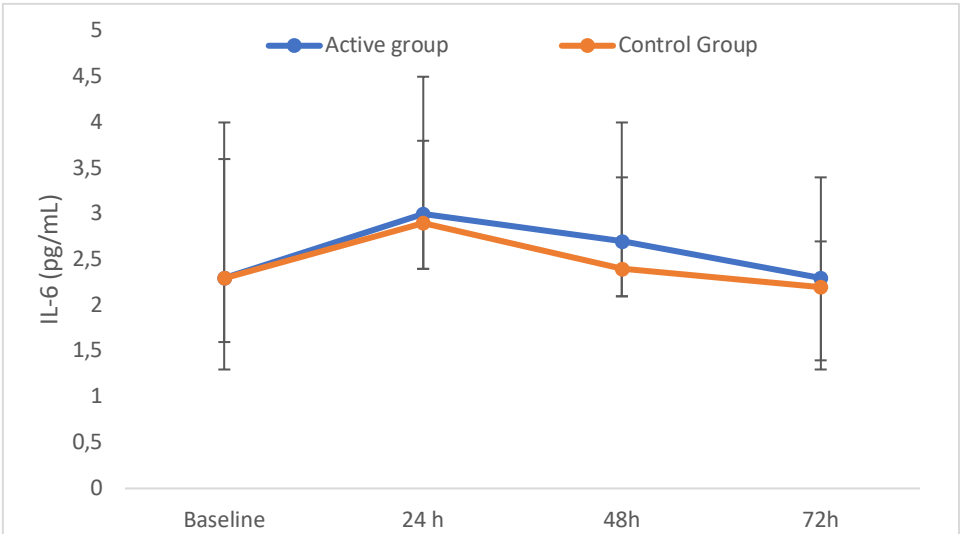

Figure 3. Interleukin-6 (IL-6)

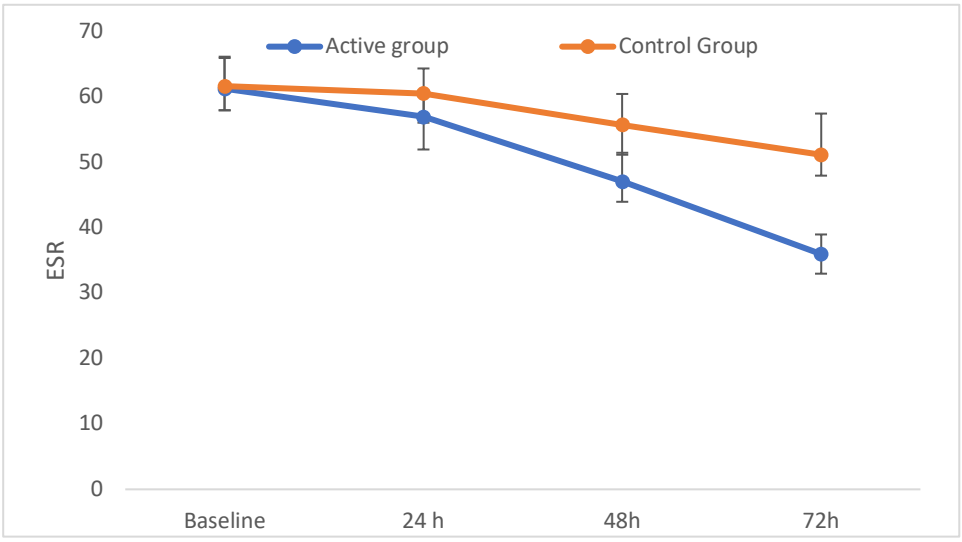

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Figure 4. (ESR)

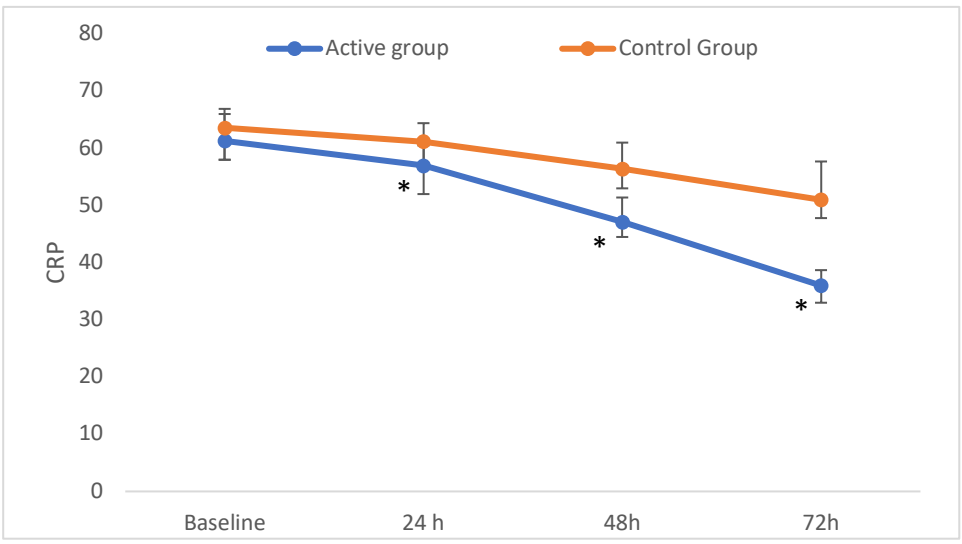

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Figure 5. C-Reactive Protein (CRP)

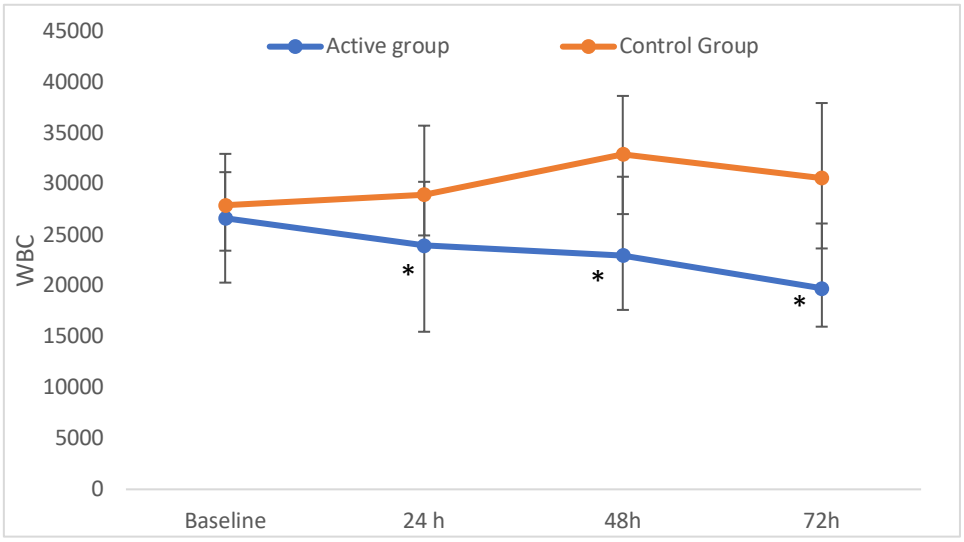

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Figure 6. White Blood Count (WBC)

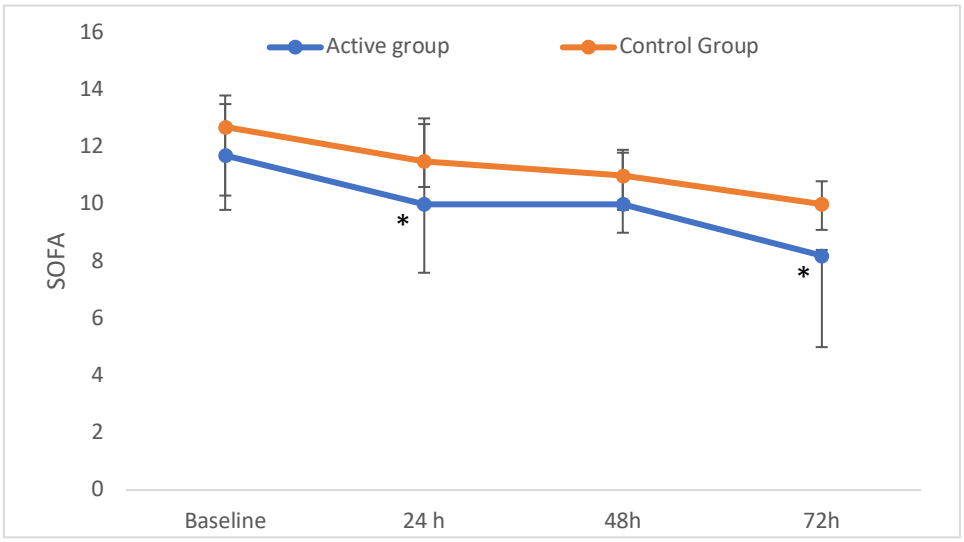

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Figure 7. SOFA score

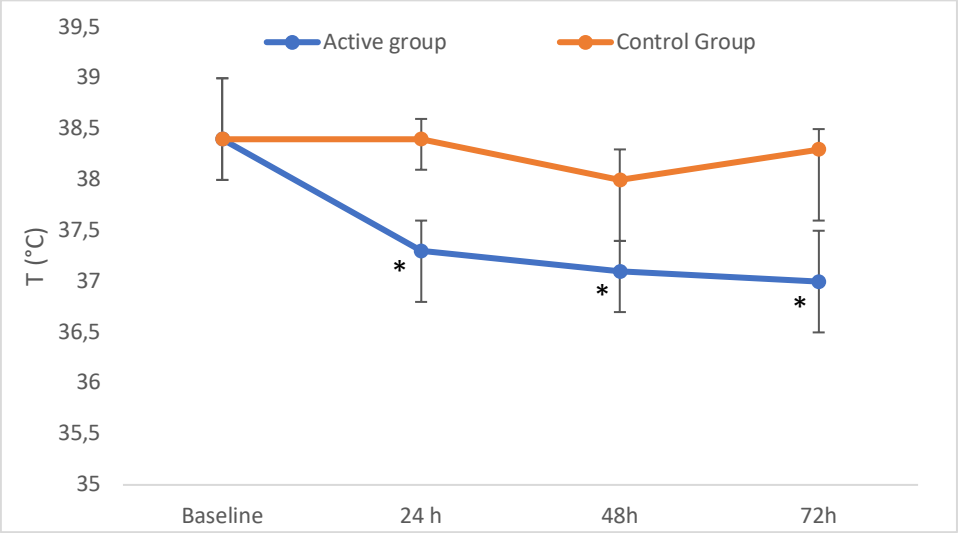

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Figure 8. Temperature (T)

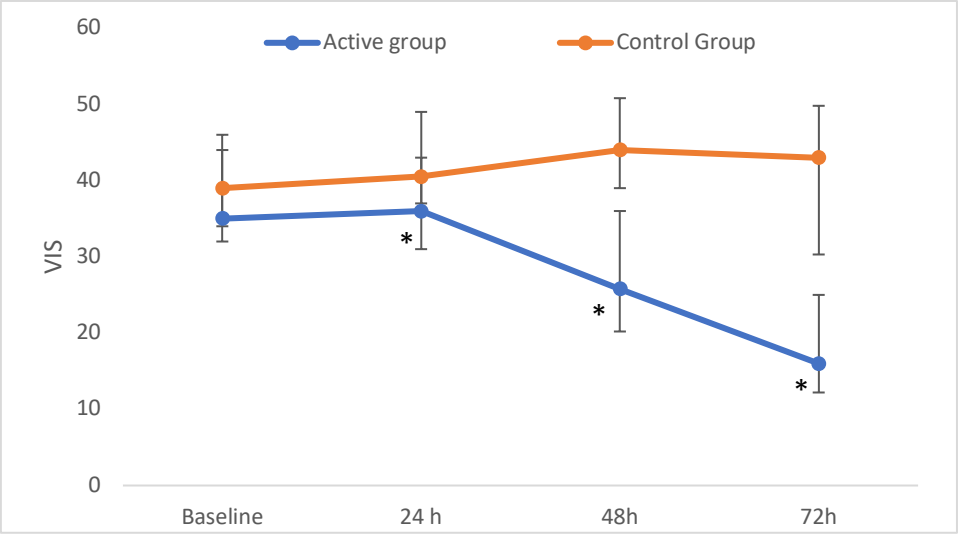

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Figure 9. Vasoactive Inotropic Score (VIS)

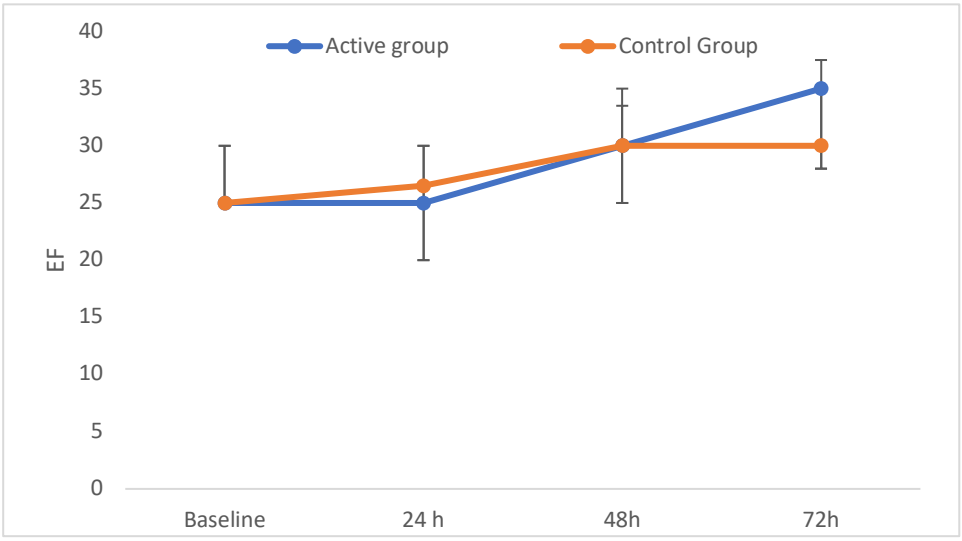

Figure 10. Ejection Fraction (EF)

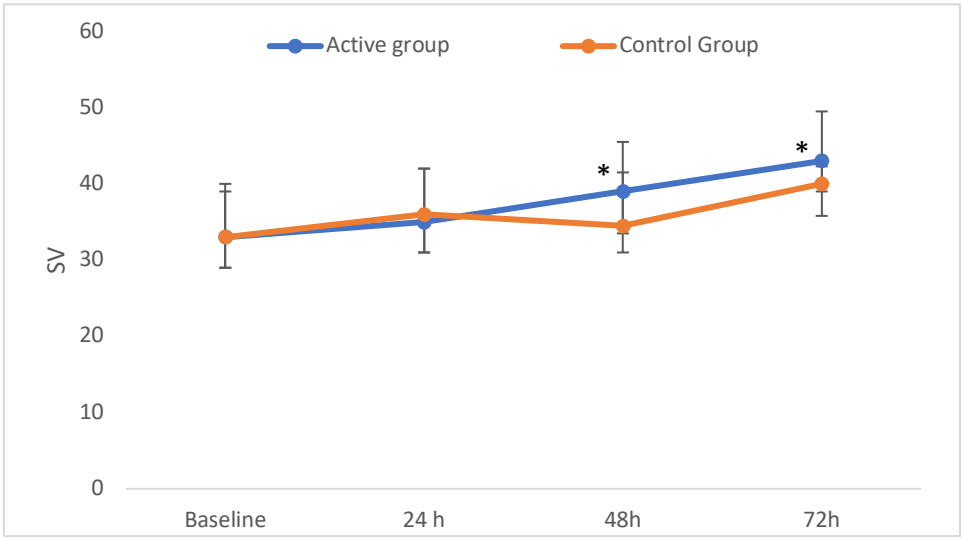

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Figure 11. Stroke Volume (SV)

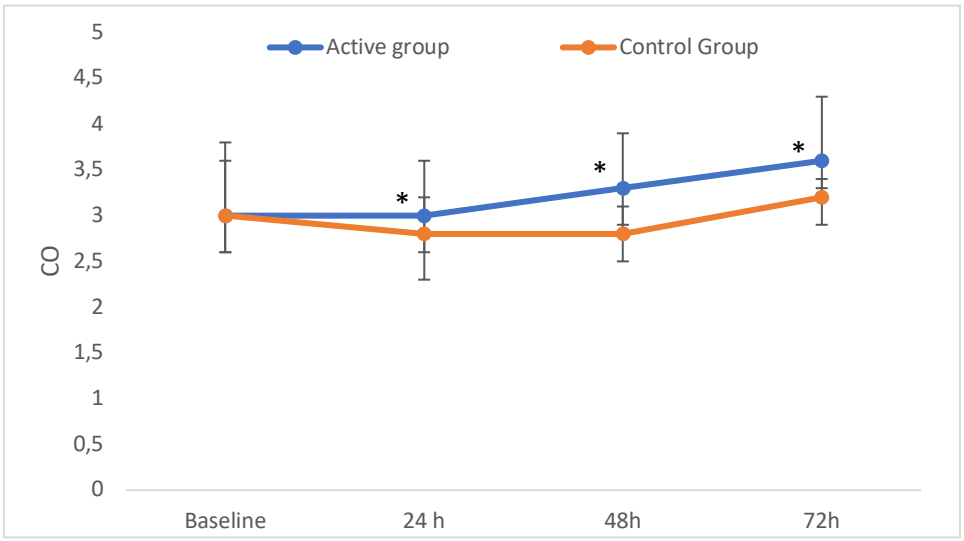

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Figure 12. Cardiac Output (CO)

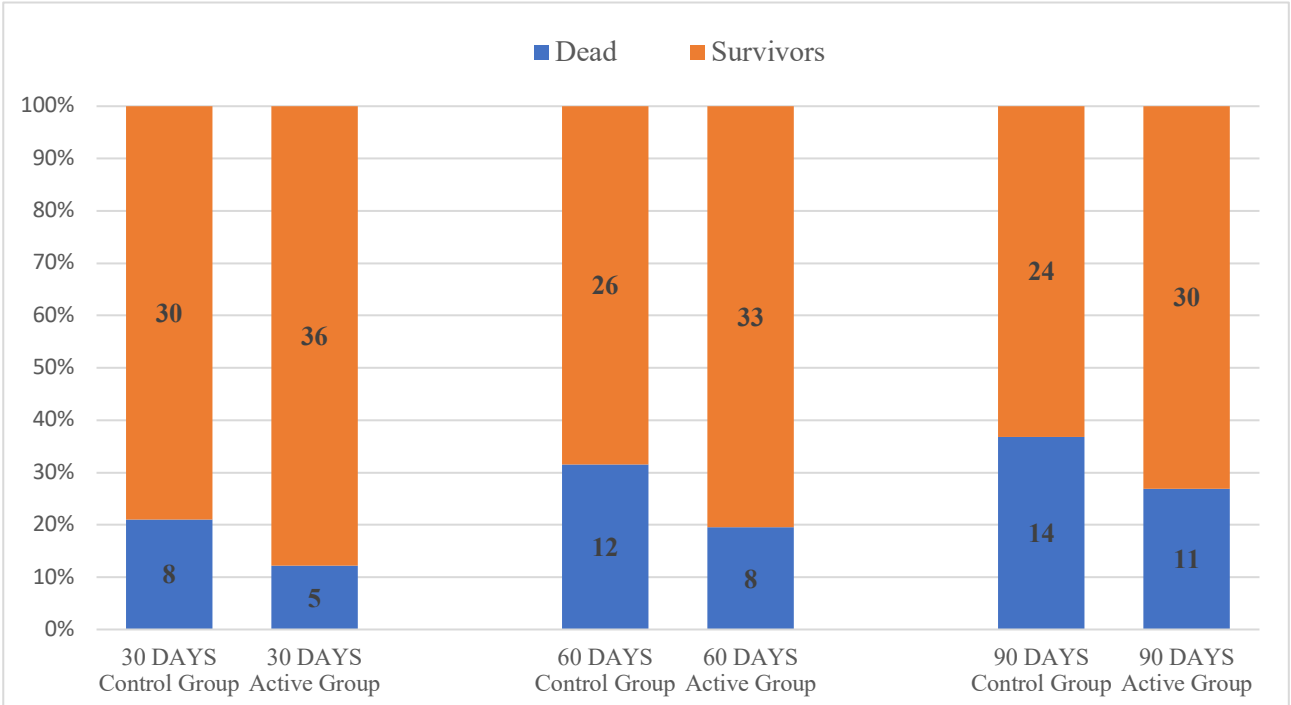

Figure 13: Survival analysis.

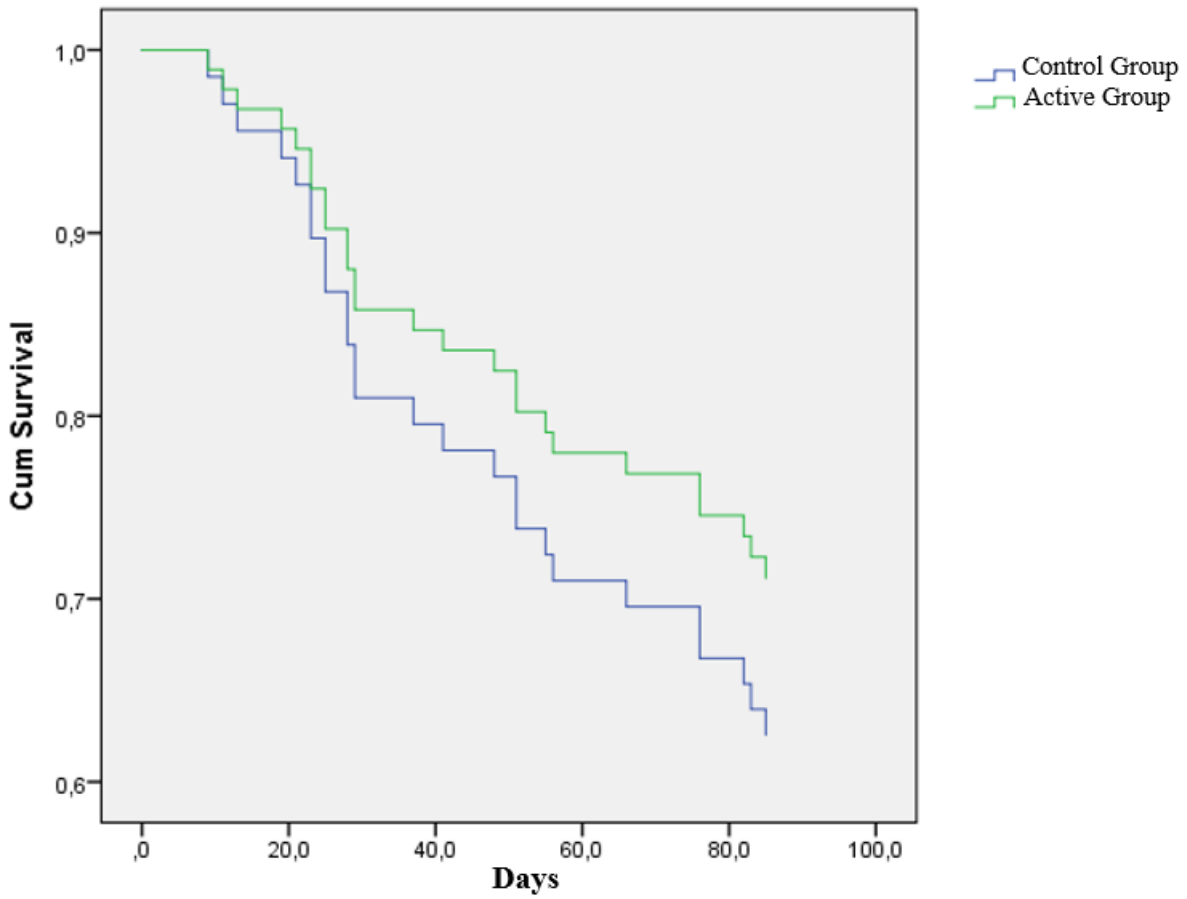

Figura 14: Survival curve.

12. Acknowledgement

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