Immunological-Inflammatory Considerations in the Critically Ill Surgical Patient *

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The immunological response in a surgical patient refers to the body's reaction to the stress and trauma caused by surgery. Surgery is considered a significant physiological insult, following the pathology itself as trigger, and the body's immune system plays a crucial role in the healing and recovery process[1-2] The immune response can be divided into two main phases: the immediate or innate response and the delayed or adaptive response.

A – Immediate or Innate Response

Immunological memory is defined as "the ability of the immune system to respond more rapidly and effectively to pathogens that have been encountered previously"[3]. It was long thought that memory was an exclusive property of the adaptive immune system. However, studies in plants, invertebrates, that both lack adaptive immunity and, more recently, in mammals, have shown that innate immune cells can also perform this function[4-6]. The ability of innate immune cells to remember and respond accordingly to secondary challenges has thus been termed "innate immune memory"[7-8]. The innate immune memory phenotype is highly dependent on the nature and intensity of external stimuli and can result either in enhanced activation, also known as "trained immunity"[8], or unresponsiveness, also known as "tolerance". In this context, the term tolerance is distinct from self-tolerance, defined as a lack of immunological response to self-antigens[9-11]. This phase occurs immediately after surgery[12-13] and involves a non-specific response by the immune system to the tissue injury and the presence of foreign material, such as sutures and implants.

Key components of the innate immune response include:

A1 Inflammation: The release of pro-inflammatory molecules, such as cytokines and chemokines, leads to localized swelling, redness, and heat at the surgical site. This inflammatory response helps recruit immune cells to the area and plays a role in tissue repair.

A2 Neutrophils: These are the first immune cells to arrive at the site of injury. They play a crucial role in phagocytosing (engulfing and digesting) bacteria and cellular debris to prevent infection and initiate the healing process.

A3 Macrophages: Following neutrophils, macrophages take over the task of phagocytosis and also release growth factors that promote tissue repair.

A4 Natural Killer (NK) cells: NK cells are important in the early defense against viruses and tumor cells. They also contribute to tissue repair and wound healing.

A5 Monocytes[14-15] are a type of white blood cell, which is an essential component of the immune system. They are a part of the innate immune response and play a significant role in defending

the body against infections and other harmful substances. Monocytes are produced in the bone marrow and circulate in the bloodstream. When needed, they can migrate into various tissues throughout the body and differentiate into macrophages or dendritic cells, depending on the specific signals they receive.

Key functions of monocytes include:

1) Phagocytosis - Monocytes are capable of engulfing and digesting foreign particles, such as bacteria, viruses, and other pathogens. This process helps to eliminate potential threats to the body.
2) Antigen Presentation - Monocytes can also present antigens (pieces of pathogens) to other immune cells, such as T cells. This presentation is crucial for initiating a specific immune response and activating T cells to target and destroy infected cells.
3) Inflammation - Monocytes play a role in the inflammatory response by releasing various cytokines and chemokines. These signaling molecules help recruit other immune cells to the site of infection or injury, contributing to the body's defense mechanisms.
4) Tissue Repair and Healing - Once they differentiate into macrophages, monocytes also play a role in tissue repair and wound healing by removing debris and assisting in tissue remodeling.

Monocyte levels can be assessed through a complete blood count (CBC) test, and elevated levels can indicate various conditions, including infections, autoimmune diseases, and certain cancers. Understanding the role of the monocytes and their functions is important for comprehending the immune response and the body's defense mechanism against diseases.

B – Delayed or Adaptive Response

Immunological memory is a fundamental aspect of the adaptive immune system, which helps the body recognize and respond more effectively to previously encountered pathogens. This memory allows the immune system to mount a faster, stronger, and more targeted response upon re-exposure to the same pathogen. There are two main types of adaptive immune responses that exhibit immunological memory[16-18].

Cell-mediated immunity (T cell memory)

This involves the activation and expansion of specific T cells, which are responsible for recognizing and destroying infected cells. T cells that encounter a specific antigen during an initial infection undergo clonal expansion, producing a population of memory T cells. These memory T cells remain in the body for an extended period, sometimes for a lifetime, ready to respond rapidly upon re-exposure to the same antigen. They can quickly recognize and eliminate infected cells, preventing the spread of the pathogen.

Humoral Immunity (B cell memory)

B cells are responsible for producing antibodies, which are proteins that bind to specific antigens on pathogens, marking them for destruction by other immune cells or neutralizing their activity. During an initial infection or vaccination, B cells that recognize the pathogen undergo clonal expansion and

differentiation into plasma cells, which produce large amounts of antibodies. Some of these B cells also become memory B cells. If the same pathogen enters the body again, memory B cells can quickly differentiate into plasma cells, producing a rapid and robust antibody response. This helps to eliminate the pathogen before it can cause widespread infection. Immunological memory is the basis for the effectiveness of vaccines. Vaccines contain weakened or inactivated forms of pathogens, or specific components of pathogens, that stimulate the immune system to produce memory cells without causing disease. When a vaccinated individual is later exposed to the actual pathogen, their immune system can mount a rapid and effective response, often preventing illness or reducing its severity. Overall, immunological memory is a crucial aspect of the immune system's ability to protect the body from recurring infections and provides the basis for long-lasting immunity against many diseases. The adaptive immune response takes longer to develop and is specific to the particular pathogens or foreign substances encountered during surgery. It involves the activation of T and B lymphocytes and leads to the formation of memory cells for long-term protection.

Key components of the adaptive immune response include:

B1 T lymphocytes: T cells recognize specific antigens presented by antigen-presenting cells (APCs) and can activate other immune cells or directly attack infected or damaged cells.

B2 B lymphocytes: B cells produce antibodies that can neutralize pathogens and foreign substances encountered during surgery.

B3 Memory cells: Some of the T and B cells formed during the adaptive response become memory cells, which "remember" the encountered pathogens or antigens. This results in a quicker and more robust immune response if the same pathogen is encountered in the future.

Factors Affecting Immunological Response in Surgical Patients.

Several factors can influence the immunological response in surgical patients:

– *Age*: Older patients may have a weaker immune response compared to younger individuals.
– *Nutritional Status*: Malnutrition can impair the immune system, leading to increased susceptibility to infections and delayed wound healing.
– *Pre-existing Health Conditions*: Chronic illnesses or immunosuppressive conditions may compromise the immune response.
– *Type and Duration of Surgery*: The extent and complexity of the surgery can impact the immune response.
– *Anesthesia*: Certain anesthetic agents can affect the immune system.
– *Post-operative Care*: Proper wound care and infection prevention measures are essential for optimal immune function and recovery. It's important to note that while the immune response is vital for healing and defense against infection, an excessive or prolonged immune response can lead to complications, such as excessive inflammation or immunosuppression. Thus, providing appropriate surgical care and managing the patient's immune response are critical aspects of ensuring a successful surgical outcome[19-22].

We analyzed 37 evaluations of 23 patients admitted to our Emergency Surgery Department from August 2022 to June 2023. Hematologic biomarkers were evaluated, including white blood cells

(WBC), lymphocyte, monocyte count and serum concentrations of interleukin IL-6 (IL-6), procalcitonin (PCT) and C-reactive protein (CRP). All these variables were measured at various time points: admission, prior to any therapeutic intervention, immediately after surgery and during postoperative follow-up.

All samples were tested for routine complete blood count conducted by using a Sysmex, DASIT XN (Sysmex, Kobe Japan) automatic blood cell analyzer.

Serum concentrations of IL-6, were measured with the Roche Elecsys IL6 assay, chemiluminescent immunoassay evaluation run on the Cobas e801 immunoassay analyzer. The stated reference interval is <7 pg/mL.

For PCT analysis, we used a non-competitive chemiluminescent immunoassay, the Elecsys BRAHMS PCT assay, on the Cobas e801 (Roche Diagnostics, Mannheim, Germany), an electro-chemiluminescence immunoassay (ECLIA) in association with a ruthenium complex-conjugated antibody. The reference interval for PCT is <0.5 ng/mL.

For CRP evaluation we used an immunoturbidimetric assay: the Elecsys CRP4 assay, on the Cobas c702; the reference interval is <5 mg/L.

Lymphocyte subsets were assayed using peripheral blood of patients collected in EDTA, percentages and absolute count of CD3 T cells, CD4 T lymphocytes (T-helper cells), CD8 T cells (Cytotoxic T cells), CD16/ CD56 Natural Killer cells and CD19 B-lymphocytes were evaluated by AQUIOS CL “Load & Go” flow cytometer (Beckman Coulter, Miami, FL, USA).

We documented, as expected, a higher-than-normal values of IL-6, PCT and CRP in serum samples of patients studied. Absolute number of WBC and monocytes are increased, whereas lymphocyte levels were found pronounced reduced (Fig. 1).

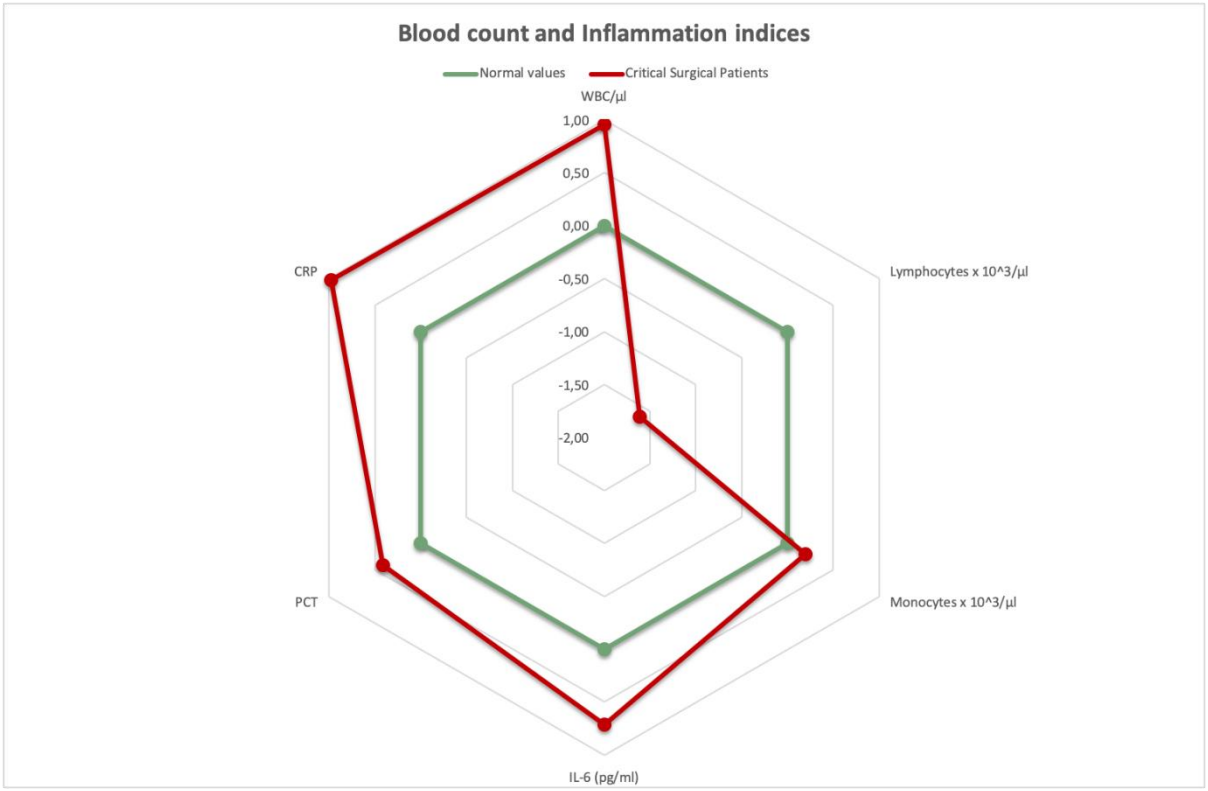

Fig. 1

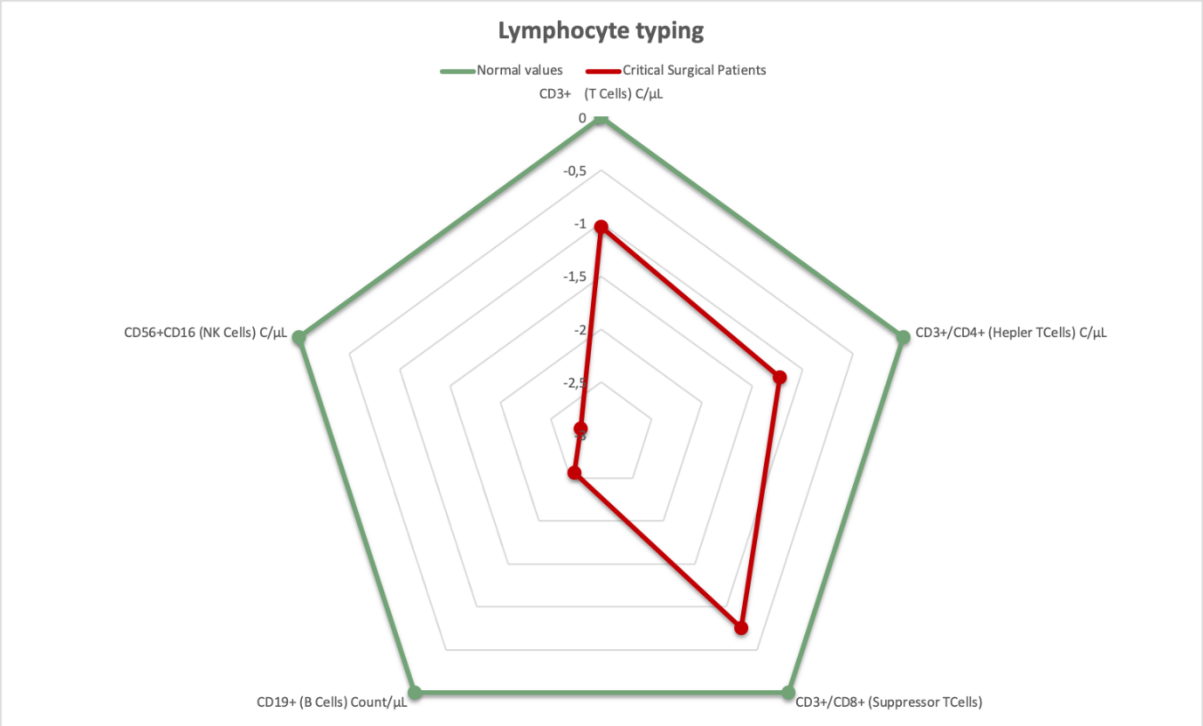

Fig. 2

We investigated the status of the patient's immune system through the lymphocyte typing. Lymphocytes are an essential part of the adaptive immune response to infection and their subsets play a complex and well-documented role in sepsis.[15-16] We observed a drop in the absolute number of total lymphocytes in patients studied, which is associated with a decrease in all lymphocyte populations investigated (Total T-cells, T-helper cells, Cytotoxic T cells, CD16/ CD56 Natural Killer and CD19 B-lymphocytes). In particular, we observed pronounced reduction for Natural Killer and B-lymphocytes. We documented a decrease for CD3 T cells, CD4 T lymphocytes, CD8 T cells, CD16/ CD56 Natural Killer cells and CD19 B-lymphocytes (Fig. 2).

A strong inflammatory response may affect immune homeostasis[23-24]. Immunosuppression is a risk factor for poor prognosis of critically ill patients, but current monitoring of the immune status in clinical practice is still inadequate[25-28]. Profound and persistent lymphopenia, can occur for many reasons: migration of cells into infection sites, associated with expansion of immunosuppressive cell populations such as regulatory T-lymphocytes and myeloid-derived suppressor cells or excess catabolism, caused by several factors including chemotherapy, immunosuppressive therapy, certain viral infections, septic shock, extended burns and systemic granulomatosis[25-30]. Previous studies indicated that lymphopenia may occur early in the course of sepsis and that decreases of specific lymphocyte subsets may help identify fragile patients at higher risk of disease progression[31]. Our analysis reveals an additional line of evidence that it's helpful to investigate lymphocyte, in comparison with other biochemical markers commonly used to stratify outcome of patients in the evolution of sepsis. Consequently, lymphocyte subsets can be regarded as a valuable tool in the establishment of an immune prognostic score for sepsis. Upcoming studies will aim to explore

different trajectories of above-mentioned hematologic biomarkers, and evaluate their relationship with prognosis in critically ill surgical patients.

Every year, worldwide, 50 million patients develop sepsis. Thanks to the huge advances in resuscitation and to the growing understanding of the mechanisms of sepsis, the in-hospital and 28-day mortality of sepsis is decreasing, although the late mortality is still high. Thank to these advances in the ICU assistance, patients passed by a single organ failure from the failure of two or more organ, a clinical entity recognized in 1970s and defined as Multiple Organ Failure (MOF). MOF has always been considered as the result of activation of proinflammatory mediators during infections and sepsis. At the dawn of its identification, it was defined as the epilogue of an uncontrollable infection[24]. But as time passes, it starts to be investigated whether MOF was the cause or the consequence of an infectious phenomenon.

It was only in 1990s that MOF was described as a bimodal event[27], in which an initial insult causes the early MOF that is followed by a period of improvement until a second late MOF in two-thirds of cases[28-31]. To explain this phenomenon, the SIRS/CARS model was proposed. According to this model, when the patient suffers an insult, the immunity system is activated with the production of pro-inflammatory mediators, configuring the Systemic Inflammatory Response Syndrome (SIRS). If this insult is severe enough, patients develop MOF. All this process is a reaction to the exposure to infectious products, such as pathogen-associated molecular patterns (PAMPS), or endogenous danger signals (damage-associated molecular patterns [DAMP] and alarmins) acting through TLR or nucleotide-binding oligomerization domain (NOD) signaling pathways. These processes result very dangerous and complex in peritoneum[32-34]. The early MOF[27-31] that follows is characterized by the overproduction of multiple proinflammatory mediators, the PMN activation, an endothelial injury with an inadequate perfusion and consequently a tissue damage. The result is the activation and overexpression of early response genes, driven in large part through nuclear factor JB (NF-kB) activation[19-22]. When compensatory anti-inflammatory response is activated, causing the Compensatory Anti-inflammatory Response Syndrome (CARS), the doors to late MOF and multiple infections are opened[22,25-29]. While the early MOF and SIRS seem to be associated to an excessive innate immune response, the development of late MOF and CARS seem to be associated to a suppressive adaptative immune response. The adaptative immune changes in severe sepsis are: an increase in T-Reg cells with a parallel T-cells anergy, a macrophage paralysis with a decrease in cytokines production and bacterial clearance and a lymphocyte depletion[26-31]. Despite therapeutic efforts, several patients didn't recover from the late MOF, rather they developed a Chronic Critical Illness (CCI), configuring a new late MOF phenotype, defined as Persistent-Inflammation-Immunosuppression-Catabolism Syndrome (PICS)[26,28,30,34-36.]

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