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Clinical Significance and Utility of Biochemical Markers in polytrauma patients with pelvic fractures

DOCTORAL THESIS SUMMARY

Doctoral Supervisor:

PROF. UNIV. DR. GREABU MARIA

Doctoral Student:

DUMITRU COSTIN

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   https://view.publitas.com/amph/rmj_2020_1_art-15
2. **Dumitru COSTIN,** Iulia-Ioana STĂNESCU, Alexandra TOTAN, Daniela MIRICESCU, Iulian Constantin CREANGĂ, Maria GREABU - Abordarea pacientului politraumatizat cu fractură de bazin. Romanian Journal of Medical Practice, Vol. XV Issue 1, p37-45, 2020.
   https://rjmp.com.ro/articles/2020.1/RJMP_2020_1_Art-08.pdf
3. **Costin Dumitru,** Alexandra Ripszky Totan, Iulia-Ioana Stănescu-Spînu, Daniela Miricescu, Maria Greabu - Correlation between NT-proBNP, hsCRP and OXSR1: Clinical relevance in evaluating the prognosis of polytrauma patients. Romanian Medical Journal – Volume LXVIII, No. 3, p 406-410, 2021
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LIST OF ABBREVIATIONS

AAST - Asociația Americană pentru Chirurgia Traumatologică

ACTH – Hormon Adrenocorticotrop

AIS - Abbreviated Injury Scale

ANZAST - Asociația pentru Chirurgia Traumatologică din Noua Zeelandă

APACHE II - Acute physiology and chronic health evaluation

APTT - Timp de tromboplastină parțială activată

ATP – Adenozintrifosfat

ATLS – Advanced trauma life support

BTS - Societatea Britanică de Traumă

CCC - Cation-chloride cotransporters

CFTR - Cystic fibrosis transmembrane conductance regulator protein

CK/CPK – Creatinkinaza/Creatinfosfokinaza

CRP - Proteina C-reactivă

DGU - Societatea Germană de Traumă

ESTES - Societatea Europeană pentru Trauma și Chirurgie de Urgență

GCS - Glasgow Coma Score

GGT – Gamaglutamiltransferaza

IL -6 - Interleukina 6

IL-1 – Interleukina 1

IL-3 – Interleukina 3

IL-1RA5 - Intra-Articular Interleukin-1 Receptor Antagonist

INR - International Normalized Ratio

ISS - Injury Severity Score

JIA – Artrită Juvenilă Idiopatică

MSOF - Multiple Systems Organ Failure

MSU – Urat Monosodic

NBCe1- sodium bicarbonate cotransporter

NLPR3 - NLR Family Pyrin Domain-Containing Protein 3

NT proBNP - Peptid natriuretic de tip B NT proBNP

OXSR1 - Oxidative stress–responsive kinase 1

PAMP - Pathogen associated molecular patterns

PRR - Pattern recognition receptors

PT – Timpul de protrombină

RA – Artrită Reumatoidă

SI - Sistemul Sacro Ischiatic

SIC - Sistemul Sacro Ilio Cotiloidian

SLC12 - Sodium/potassium/chloride transporter 12

SOFA - Sequential organ failure assessment score

SP - Sistemul Sacro Pubian

SPAK - Proline-alanine-rich kinase

STK39 - Serine/threonine kinase 39

TCZ – Tocilizumab

TNF – Tumor Necrosis Factor

TS – Tensiunea Sistolică

VS - Vertical Shear

WNK – Lysine deficient protein kinase

Introduction

In today's society, technological progress and the need for population mobility have led to an increase in the number of individuals exposed to high-energy traumatic agents. This can be attributed to several reasons, such as the year-over-year increase in traffic volumes, which, combined with outdated road infrastructure inadequately adapted to modern traffic conditions, leads to a higher number of road accidents; a significant portion of the population working in the construction sector, resulting in more workplace accidents, in addition to traumas from natural disasters or mass accidents.

It is important to note that in the context of current socio-economic development, a significant percentage of patients exposed to high-energy traumatic agents are young individuals, which increases social costs both due to the need for treatment of these victims and due to their temporary or permanent, total or partial work incapacity.

High-energy traumas often result in polytrauma, which has been redefined according to the new Berlin definition as an association of an ISS $\geq$ 16, interpreted as an AIS $\geq$ 3 for two or more body regions, combined with one or more additional variables from the following five physiological parameters: hypotension (systolic blood pressure $\leq$ 90 mmHg), loss of consciousness (GCS $\leq$ 8), acidosis (base excess $\leq$ -6.0), coagulation disorders (partial thromboplastin time $\geq$ 40 s or INR $\geq$ 1.4), and age ($\geq$ 70 years).

It is vital to understand and study the systemic effects of polytrauma, both because if not properly treated, they can lead to acute renal failure, MSOF, and ultimately death, and because, in the event of disasters or calamities, when a significant number of victims occur in a short time, protocols for rapid diagnosis and modern treatment are needed both for individual injuries and to prevent or treat the potentially fatal systemic damage that occurs in polytrauma.

For the rapid, correct, and complete treatment of polytraumatized patients, the existence of a multidisciplinary team, composed of specially trained and experienced doctors in treating patients who are victims of high-energy trauma, and the existence of specific diagnostic and treatment protocols, are extremely important.

In this context, the present doctoral research aims to determine the concentrations of biochemical markers in polytraumatized patients who associate pelvic fractures and to analyze the

potential of these biomarkers as useful tools in diagnosing and especially monitoring these patients, as well as developing a medical management plan for the case.

I. CURRENT STATE OF KNOWLEDGE

The first part of the work consists of two chapters that address general notions about polytrauma and pelvic fractures.

The first chapter is dedicated to pelvic fractures, which include injuries to the pelvic ring: the iliac bone, sacrum, and coccyx. These fractures vary in severity and can lead to severe forms with a high mortality rate due to vascular complications or association with other traumatic injuries. The bony pelvis is structured from the iliac bones, united posteriorly by the sacrum, forming an ogival console that supports the trunk. In a vertical position, the body's weight is distributed in two directions: one horizontal and one vertical, transmitted to the coxofemoral joint. The pelvic ring's resistance zone is represented by the complete pelvic ring, through which the loads are transmitted. The weak areas are important for the localization of fractures and disjunctions of the pelvic belt.

The pelvic ring is partially deformable due to its three joints: the pubic symphysis and the sacroiliac joints. There are two main types of pelvic fractures: pelvic fractures that do not involve the coxofemoral joint, usually resulting from high-energy trauma, and acetabular fractures affecting the acetabular component of the coxofemoral joint. This terminology is debatable, but it has become rooted in orthopedic language due to significant treatment differences between the two types of fractures [2].

Pelvic fractures are often associated with the modern era, motorization, and excessive speed. They represent between 0.3% and 6% of all fractures and occur with a 20% incidence in cases of polytrauma. The main causes include traffic accidents, falls from height, and industrial accidents. These fractures primarily affect young adult men, with a peak frequency between 15 and 30 years old and a second peak between 50 and 70 years old. The overall mortality rate for these fractures varies between 6% and 30%, reaching impressive figures of 50% in the case of open pelvic fractures.

The classification of pelvic fractures can be simple, based on the fracture site, or more complex, depending on the mechanism of production and the degree of stability of the pelvic fractures. There are several classification systems, including the Tile classification and the Young and Burgess classification. Regarding the clinical examination in pelvic fractures, it varies according to the severity of the injuries, from minor injuries that do not endanger the patient's life to severe injuries associated with high mortality [12].

The treatment of pelvic fractures varies according to the type of fracture. In the case of stable fractures, treatment consists of bed rest followed by progressive mobilization. In the case of unstable fractures, the addition of trans-skeletal traction or surgical intervention is necessary to fix the fracture [23]. Methods of stopping bleeding include stabilizing the pelvic fracture, selective embolization angiography, and identifying other sources of bleeding. In conclusion, pelvic fractures are complex injuries with a wide spectrum of severity and require a multidisciplinary approach in their management.

The second chapter addresses the issue of polytrauma, a term introduced by Tscherne and his colleagues in 1966, referring to patients with two or more severe injuries, one of which can be life-threatening. Definitions have diversified over time, with Border and his colleagues defining polytrauma in 1975 as patients with two or more significant injuries [25]. Although the Injury Severity Score (ISS) was not initially included in the definition of polytrauma, it has become a standard global parameter, used for evaluating patients with multiple injuries and for classifying trauma centers [29].

A group of clinicians, under the aegis of important societies such as AAST and ESTES, came together to develop an evidence-based definition of polytrauma. The result was the "Berlin Definition" of polytrauma, which includes five pathological states and auxiliary parameters for describing a patient with multiple injuries, focusing on separating different mortality rates [31]. This definition has been validated and compared with other existing definitions, proving its usefulness in transparently evaluating treatment outcomes and objectively comparing studies.

The improved Berlin definition includes an ISS $\geq$ 16 and an AIS $\geq$ 3 for at least two body regions, plus at least one of five standardized physiological responses. Studies have shown that adding physiological variables to ISS increases its predictive power for mortality. Factors such as age, systolic blood pressure, and the Glasgow Coma Scale have good predictive power for

mortality. In addition, studies emphasize the importance of recognizing the difference between "polytrauma" and "multitrauma," the latter referring to the physiopathological response of the injured person to the injury load.

Polytrauma is associated with more severely altered hemodynamic parameters and the need for more complex urgent procedures. Cranio-cerebral injuries and thoracic traumas are major risk factors, and their combination leads to an exponential increase in mortality. Procedures such as external fixation of the pelvic ring can be used to stabilize fractures and control hemorrhage. In conclusion, polytrauma is a complex condition that requires a multidisciplinary approach and precise definition to improve treatment and patient outcomes.

II. PERSONAL CONTRIBUTION

In Chapter 3, I reviewed the main biochemical markers considered in the studies that will follow in the future chapters to assess the impact of pelvic ring fractures in polytraumatized patients.

Interleukin-6 (IL-6) is a multifunctional cytokine involved in regulating the immune and inflammatory response. Secreted by various cells, including macrophages, T and B lymphocytes, fibroblasts, and endothelial cells, IL-6 plays a crucial role in responding to infections, wounds, stress, and neoplasms. Elevated levels of IL-6 are an early indicator of acute inflammation and can anticipate the onset of complications in cases of surgical stress or undetected injuries.

IL-6 interacts with lymphoid and non-lymphoid cells, having both pro-inflammatory and anti-inflammatory actions. Secreted as a polypeptide of 184 amino acids, with a molecular weight of approximately 21 kDa [46], IL-6 stimulates the hepatic production of acute-phase reactants and intervenes in hepatic regeneration processes [47]. It contributes to hematopoiesis, secretion of ACTH and other pituitary hormones, and influences the formation of osteoclasts, being involved in the pathology of osteoporosis and rheumatoid arthritis [49].

An important aspect of IL-6 is its role in the immune response in polytrauma. Studies from the 80s and 90s showed that tissue injuries in polytrauma trigger a local and systemic inflammatory response, with an increased level of pro-inflammatory cytokines. This explains the increased incidence of infectious complications and adult respiratory distress syndrome in such

cases. IL-6 is an essential marker in diagnosing and monitoring the treatments of immune conditions, in correlation with clinical and paraclinical data.

The development of "Damage control surgery" in orthopedics reflects the importance of carefully addressing the severity of traumas, the biological constitution of the patient, and the necessity of surgical interventions [52]. IL-6 plays a key role in fracture healing, and IL-6-oriented therapy, such as the use of tocilizumab (TCZ), has shown promising results in treating inflammatory diseases, including rheumatoid arthritis and other refractory conditions [58]. Thus, IL-6 and its inhibitors are at the center of modern therapeutic strategies for managing inflammatory diseases and post-traumatic response.

TNF (Tumor Necrosis Factor) is a proinflammatory cytokine known for its ability to destroy tumor cells and induce hemorrhagic necrosis in transplanted tumors. TNF-α (cachectin) and TNF-β (lymphotoxin) were initially described as cytotoxic factors produced by macrophages and lymphocytes, respectively. These mediators act through the same receptors and have similar biological effects [59]. TNF-α is produced not only by macrophages and monocytes but also by other cell types, including smooth muscle cells and certain tumor cell lines. It acts directly through two distinct surface receptors, TNFR I and TNFR II. TNF receptors can be found on most cell types, except erythrocytes. Portions of these receptors are cleaved by proteases, forming soluble receptors that act as endogenous inhibitors of TNF-α and thus regulate the level of extracellular TNF-α [59].

TNF-α influences a wide variety of biological effects, including cytolytic and cytostatic effects on tumor cells, chemotactic effect on neutrophils, increased permeability at the endothelial cells in the inflammation region, activation of various metabolic processes in muscle cells and adipocytes, stimulation of phagocytosis and production of prostaglandin E2 in macrophages, activation of osteoclasts, stimulation of fibroblasts, and synthesis of collagenase.

The determination of serum TNF-α levels constitutes a marker for systemic inflammatory response (SIRS) associated with sepsis, trauma, and heart failure. There is evidence that inflammation plays an essential role in the early repair of fractures, with TNF-α and other factors expressed at the fracture site. TNF-α promotes bone resorption and inhibits bone formation, and its blockade has the potential to inhibit or reverse bone loss. Anti-TNF therapy has been

proposed as a potential dual treatment for controlling inflammation and preventing osteoporosis and associated fractures in inflammatory diseases [63,64].

C-reactive protein (CRP), discovered in the serum of patients with pneumonia in 1930 and isolated in 1941, is an important marker of inflammation. CRP has opsonizing properties, influencing monocyte recruitment and affecting endothelial function [65]. Its levels increase rapidly following tissue injuries, for example, after orthopedic interventions, highlighting its role in the inflammatory response and immunological defense [67].

The N-terminal prohormone of brain natriuretic peptide (NT-proBNP), a member of the natriuretic peptide family, is essential in regulating hydroelectrolytic balance and circulatory homeostasis, playing significant roles in the response to cardiac stress and trauma. ANP and BNP negatively influence the angiotensin-aldosterone system, promoting diuresis and natriuresis [72]. In particular, BNP, generated in the myocardium following ventricular distension, moderates renal and cardiovascular functions. Its levels fluctuate significantly due to factors such as age, sex, and ethnicity, making it difficult to interpret without considering individual and interindividual variations [72]. These particularities underline the necessity to adjust reference values for NT-pro-BNP, especially in individuals over 50 years, and its relevance in evaluating post-traumatic cardiac response [74].

Oxidative stress responsive kinase 1 (Serine/threonine-protein kinase 1) OXSR1 is a protein kinase encoded by the OXSR1 gene located on chromosome 3 in humans. It plays a role in the regulation of kinases derived in response to environmental stress and may play a role in the regulation of the actin cytoskeleton [75].

OXSR1 is ubiquitously expressed in most tissues, with high levels in the lungs, particularly in the bronchial epithelium [76]. Additionally, OXSR1 is believed to play an important role in regulating the immune response and oxidative stress [77].

The expression of OXSR1 protein was found in soluble, particle, and nuclear fractions of the heart, spleen, liver, kidneys, lungs, testicles, small and large intestines, and stomach [82].

Interleukin-1β (IL-1β) is a proinflammatory cytokine essential for the body's immune responses to infections and traumatic injuries. Predominantly synthesized by cells of the innate immune system, such as monocytes and macrophages [84], IL-1β is initially produced as an

inactive precursor. Its activation is stimulated by a variety of agents, including extracellular ATP, the toxins nigericin and maitotoxin, bacterial infections, monosodium urate crystals, and other pathogens. These stimuli induce the secretion of IL-1β through a series of inflammasomes, including NLRP3, NLRC4, and NLRP1, highlighting its complex role in inflammatory responses [89,90,93,97].

Creatine kinase/Creatine phosphokinase (CK/CPK) is an essential enzyme, predominant in myocardium and skeletal muscles, and present in smaller amounts in the brain.

CK has four forms: mitochondrial and three cytosolic isoenzymes (CK-MM for muscles, CK-MB for myocardium, CK-BB for brain) [99]. CK-MM dominates in healthy muscle tissue, and its variations are useful indicators in diagnosing muscle disorders, including muscle trauma. CK-MB is significant in identifying myocardial injuries and may be elevated in myocardial trauma, myocarditis, and cardiomyopathies. CK-BB, more rarely encountered, is associated with cerebral and neurological impairments. Increases in total CK and its isoenzymes are relevant in the context of traumatic injuries, rhabdomyolysis, malignant hypothermia, and chronic renal failure [102].

Chapter 4 is dedicated to the working hypothesis and general objectives.

The motivation for choosing the theme of this doctoral research is represented by the necessity of identifying new biochemical markers that facilitate the establishment of an early diagnosis in the case of a polytraumatized patient so that the therapeutic conduct to be followed can be established as quickly and accurately as possible. The initiation of correct treatment as early as possible can lead to a decrease in the rate of complications and mortality due to polytrauma.

As a result of extensive bibliographic research, a series of questions arose from which a series of working hypotheses were formulated:

- Could OXSR1 be a useful oxidative stress marker in evaluating the polytraumatized patient?
- Could interleukins-1β, IL-6, and TNF-α be more sensitive and specific biochemical compounds than those usually used in practice?
- Given the hemodynamic instability often encountered in this group of patients, could the NT-proBNP value be prognostic for patient outcomes?

To answer these questions, in addition to the necessary bibliographic studies, quantitative and statistical analyses of results obtained from a study involving 34 patients were conducted, of whom 20 suffered polytrauma that included a pelvic ring fracture and 14 represented the control group.

The general methodology of the research is found in **chapter 5.** The selection of patients from the two cohorts, of ill and non-trauma history patients, was done exclusively with persons admitted to the Clinical Emergency Hospital of Bucharest during the period 2018-2022. The diagnoses of the patients included in the study were established by the attending physicians, based on national and international criteria and protocols in force.

Levels of IL-1beta, IL-6, TNF-α, hsCRP, and OXSR1 were measured in the serum of patients with pelvic ring fractures and subjects from the control group using the sandwich ELISA method and the NT-proBNP biomarker by the competitive ELISA method, using commercial kits and a semi-automatic ELISA analyzer.

The Enzyme-Linked Immunosorbent Assay (ELISA) is a method for capturing the target antigen (or antibody) in samples utilizing a specific antibody (or antigen). This immunological technique enables both the detection and quantification of the target molecule through an enzymatic reaction. The ELISA method is characterized by its sensitivity and specificity, while also being simple, rapid, and cost-effective. The processes of reproducing and interpreting results are streamlined. The ELISA technique is effective even in detecting molecules at low concentrations, thereby minimizing the risk of interferences. This precision stems from the unique ability of an antibody to bind exclusively to its specific antigen, making it a valuable tool in research and clinical diagnostics.

In ELISA, various antigen-antibody combinations are utilized, always including an enzyme-labeled antigen or antibody, and the enzymatic activity is measured colorimetrically. This method's distinctiveness lies in its high degree of sensitivity and specificity, offering simplicity, speed, and cost-efficiency. Reproduction and interpretation of results are simplified processes. ELISA proves efficient even in the detection of low-concentration molecules, minimizing interference risk. This accuracy derives from the unique capacity of an antibody to bind solely to

its specific antigen, rendering it an invaluable instrument in both research and clinical diagnosis contexts.

There are four main variants of the ELISA method:

1. Direct ELISA: Uses an enzymatic antibody directly bound to the fixed antigen on plates.
2. Indirect ELISA: Utilizes a primary antibody and a secondary enzymatic antibody for antigen detection.
3. Sandwich ELISA: Characterized by greater specificity, involving the fixation of an antibody on plates, followed by the addition of the antigen and a second enzymatic antibody.
4. Competitive ELISA: Based on the competition between the antigen in the sample and a fixed competitor on plates for the binding of the primary antibody, followed by detection with a secondary enzymatic antibody.

Each variant has specific applications, with Sandwich ELISA being particularly sensitive and suitable for precise analyses, while Competitive ELISA is optimal for determining low molecular weight compounds.

Statistical analysis was performed using IBM SPSS Statistics 25 and Microsoft Office Excel/Word 2013. Quantitative variables were tested for distribution using the Shapiro-Wilk test and were expressed in the form of means with standard deviations or medians with interpercentile ranges.

Chapter 7 includes the first study of the **research "Evaluation of the inflammatory status in polytraumatized patients with pelvic ring fractures".**

7.1 Introduction (Working Hypothesis and Specific Objectives)

Polytrauma is a major traumatic event that invariably associates with a systemic inflammatory response, which can readily progress to multiple organ failure. Although the exact mechanism through which local tissue destruction can induce distant organ dysfunction is not fully elucidated, polytrauma, by definition, involves multi-organ impairment. Nevertheless, a multitude of biomarkers have been identified, proven to be more or less accurate indicators of the progression, prognosis, and survival chances of polytraumatized patients with pelvic fractures. The present study focused on the following biomarkers associated with the inflammatory syndrome: IL-1 beta, IL-6, TNF-alpha, hsCRP.

The specific objectives of this study were:

1. To determine the level of IL-1 beta in blood samples of polytraumatized patients with pelvic ring fractures and to compare it with reference values.
2. To determine the level of IL-6 in blood samples of polytraumatized patients with pelvic ring fractures and to compare it with reference values.
3. To determine the level of TNF-α in the blood of polytraumatized patients with pelvic ring fractures and to compare it with reference values.
4. To determine the level of hsCRP in the blood of polytraumatized patients with pelvic ring fractures and in patients constituting the control group, and to compare these two values.

7.3. Results

Table VII.1 Values of IL-1 beta, IL-6, and TNF-alpha – Comparison Between Study Values and Reference Averages

Biomarker	Reference mean	Mean ± SD	Mean difference (95% C.I.)	p*
IL-1 beta (p=0.642**)	3.31	14.19 ± 2.49	10.88 (9.71-12.04)	**<0.001**
IL-6 (p=0.081**)	2.5	8.96 ± 1.37	6.46 (5.81-7.10)	**<0.001**
TNF alfa (p=0.106**)	1.323	16.71 ± 2.30	15.39 (14.31-16.47)	**<0.001**

***One-Sample T-Test, **Shapiro-Wilk Test**

Table VII.2 Comparison of hsCRP Values Between the Control Group and the Patient Group

Group / hsCRP	Mean ± SD	Median (IQR)	Mean rank	p**** (p=0.003***)
Control (p=0.978**)	0.746 ± 0.29	0.7 (0.5-0.95)	-	**<0.001**
Lot – Patients (p=0.089**)	5.73 ± 1.87	5.5 (4.12-6.45)	-	

****Shapiro-Wilk Test, ***Levene's Test for Equality of Variances, ****Welch T-Test**

The data from Tables VII.1 and VII.2 and Figures VII.1-4 represent the comparison of the investigated parameters in relation to the reference population averages. The distribution of hsCRP values was normal in both groups according to the Shapiro-Wilk test (p>0.05). The following results were observed for the tested group:

- Significantly higher IL-1 beta values compared to the reference population (p<0.001), with a mean difference of 10.88 (95% C.I.: 9.71-12.04);

- Significantly higher IL-6 values compared to the reference population (p<0.001), with a mean difference of 6.46 (95% C.I.: 5.81-7.10);

- Significantly higher TNF-alpha values compared to the reference population (p<0.001), with a mean difference of 15.39 (95% C.I.: 14.31-16.47);

- hsCRP values were significantly higher in the patient group from the study (5.73 ± 1.87) than in the control group (0.746 ± 0.29) according to the Welch T-Test ($p<0.001$);

7.4 Discussions

C-Reactive Protein (CRP), the first acute phase protein identified, is a sensitive marker of inflammation and tissue lysis[105]. Discovered for its ability to precipitate the C polysaccharide of Streptococcus pneumoniae, CRP is exclusively produced by hepatocytes, predominantly regulated by IL-6. Hepatic production of CRP is triggered rapidly after a stimulus, with a significant serum increase within 6 hours and peaking at about 48 hours[106]. Its plasma concentration, with a constant half-life of approximately 19 hours, directly reflects the intensity of pathological processes. CRP levels are affected by hepatic integrity, and in patients with fulminant hepatic failure, even in the presence of significant septic pathology, CRP may be undetectable. Other influencing factors include sex, age, weight, blood pressure, lipid profile, smoking, and ethanol consumption.

CRP has been associated with the development of type II diabetes mellitus (DMTII), suggesting an inflammatory role in the pathogenesis of this disease[109]. IL-6 and CRP, markers of subclinical systemic inflammation, are correlated with hyperglycemia, insulin resistance, and DMTII. This association is particularly relevant in the context of the hypothesis that DMTII could be a disease of the innate immune system[112].

CRP, used as a marker of inflammation, is a significant predictor for cardiovascular diseases in asymptomatic populations. Research has highlighted a correlation between CRP levels and the prognosis of patients with various cardiovascular conditions, emphasizing the importance of CRP in cardiovascular pathophysiology[115].

Circulating CRP is produced as a soluble pentamer, which, under certain conditions, dissociates into monomers (mCRP) with distinct structural and functional properties. Recent studies have explored the interactions between mCRP, lipids, and other natural substances[120].

In the inflammatory response, CRP plays a crucial role by activating the complement pathway and opsonizing pathogens. The complement, involved in the elimination of particles and

foreign organisms, has three activation pathways: classical, lectin, and alternative, with most components synthesized in the liver[122]. The role of CRP in activating the complement pathway has been intensively studied, being an essential component in evaluating systemic inflammatory processes. Inflammation-related factors such as cytokines and the IL-1 family receptors are particularly important in activating the inflammatory response, with IL-1β having a strong inflammatory effect. IL-1β is usually present in circulation only at very low levels [124], most likely due to its dangerous vasomotor effects [125]. As can be seen in Table VII.1 and Figure 7.1, the IL-1β values in the patients included in the study are approximately four times higher than the reference average, which could be one of the explanations for why patients present marked hemodynamic instability.

The serum response of interleukin-6 (IL-6) to surgical injuries has been extensively characterized, with Shenkin and colleagues [126] observing increases in IL-6 within 90 minutes of skin incision, peaking at 4 hours after cholecystectomy. Studies by Cruickshank et al. [127] indicated an increase in IL-6 between 2 and 4 hours, peaking between 6 and 12 hours following various surgical procedures. They correlated the maximum serum concentrations of IL-6 with the extent of tissue trauma, rather than with the duration of the surgical intervention or anesthesia. Kossmann et al. [129] demonstrated high concentrations of IL-6 in blood and cerebrospinal fluid after traumatic brain injuries, while other research indicated bleeding as a sufficient stimulus for increased IL-6, but not to the level of tissue injuries [130]. In polytraumatized patients with pelvic fractures, bleeding and tissue trauma are important factors in IL-6 secretion, as observed in Figure 7.2.

Martic C. and colleagues [131] found that patients with high levels of IL-6 have a higher risk of developing nosocomial infections, especially pneumonia. In our study, patients with high IL-6 values had a slower recovery. Liu C and co-authors [132] reported that traumatic injuries cause high plasma levels of TNF-alpha and soluble receptors (TNFR1 and TNFR2) in the early phases of trauma, remaining high until the third or fifth day after injury. TNF-α, as part of the inflammatory response, initiates the activation of other cytokines and growth factors, with a quicker release than other proinflammatory cytokines [132]. High levels of TNF-α post-trauma are harmful, inducing central sensitization and hyperalgesia [130], confirmed in our study by the increased consumption of analgesic and anti-inflammatory drugs in the study group [134].

8. : Biochemical Markers of Muscle Lysis in Polytraumatized Patients with Pelvic Ring Fractures

8.1 Introduction (Working Hypothesis and Specific Objectives)

Natriuretic peptides (NP), including atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), are essential in maintaining cardiorenal homeostasis and hydroelectrolytic balance. Their discovery has changed the perspective on the heart, now seen as an endocrine organ [139]. ANP and BNP, primarily produced in the atria, reduce vascular resistance and increase diuresis and sodium excretion, thus influencing blood pressure [141]. The BNP gene encodes a preproBNP precursor, which is converted into proBNP. A fragment of the preproBNP signal peptide has been suggested as a circulating biomarker for cardiac ischemia and myocardial infarction [143]. BNP, responding to cardiac wall stretching and volume-related strains, exists in circulation in multiple proteolytic and glycosylated forms. Commercial tests for BNP and NT-proBNP detect all fragments containing the epitope recognized by the used antibodies, without absolute specificity [144].

BNP and NT-proBNP, useful indicators in the diagnosis of chronic heart failure, are elevated in patients with left ventricular dysfunction. BNP, with a half-life of about 20 minutes, and NT-proBNP, with a half-life of 1-2 hours, reflect the current cardiac status.

Creatine kinase (CK), with 4 isoenzymes (mitochondrial, CK-MM, CK-MB, CK-BB), plays a significant diagnostic role. CK-MM is present in skeletal muscles, CK-MB in the myocardium, and CK-BB in brain tissue, urinary, and digestive tract. The serum level of CK is a diagnostic marker in myocardial infarction (CK-MB) and muscular damage (CK-MM). Serum elevation of CK-BB can indicate pulmonary infarction, pulmonary or breast adenocarcinoma, or cerebral conditions. CK-MB is increased in acute myocardial infarction, myocarditis, cardiac surgery, defibrillation, ventricular arrhythmias, cardiomyopathies, and myocardial ischemia. The CK-MM isoenzyme increases in rhabdomyolysis, compartment syndrome, muscle trauma, seizures, intense physical effort, and hypokalemia.

The specific objectives of this study consisted of:

1. Determining the level of NT-proBNP in the blood of polytraumatized patients with pelvic ring fractures and in patients constituting the control group, and comparing the two values.

2. Determining the level of CK in the blood of polytraumatized patients with pelvic ring fractures and in patients constituting the control group, and comparing the two values.

The determination of CK was carried out using the spectrophotometric method. According to the manufacturer's insert, the method has very high sensitivity and specificity in detecting CK concentrations, and the detection limit of the kit is 9.2 U/L, with the detection method being colorimetric.

The principle of the determination method is as follows: CK catalyzes the phosphorylation of ADP in the presence of creatine phosphate, resulting in the formation of ATP and creatine. The concentration is determined by the rate of formation of NADPH, measured at 340 nm, through the following coupled reactions, catalyzed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH):

1. ATP, produced by the CK reaction, is used by HK to phosphorylate glucose, forming glucose-6-phosphate.

2. Glucose-6-phosphate is then oxidized by G6P-DH in the presence of NADP+, resulting in the production of gluconolactone-6-phosphate and the reduction of NADP+ to NADPH.

The increase in NADPH concentration, directly proportional to the CK activity in the sample, is quantified by measuring the absorbance at 340 nm. This absorbance measurement correlates with the concentration of CK in the blood sample. The use of HK and G6P-DH in these coupled reactions ensures high specificity for the detection of CK, as these enzymes specifically interact with the products of the CK-catalyzed reaction. The spectrophotometric method, therefore, provides a reliable and accurate assessment of CK levels in the blood, which is essential for diagnosing conditions like myocardial infarction, muscle damage, and other related disorders.

Creatine phosphate + ADP (catalyzed by CK) → Creatine + ATP

Glucose-6-Phosphate + NADP+ (catalyzed by Glucose-6-Phosphate Dehydrogenase, G6P-DH) → 6-Phosphogluconate + NADPH + H+

8.3 Results

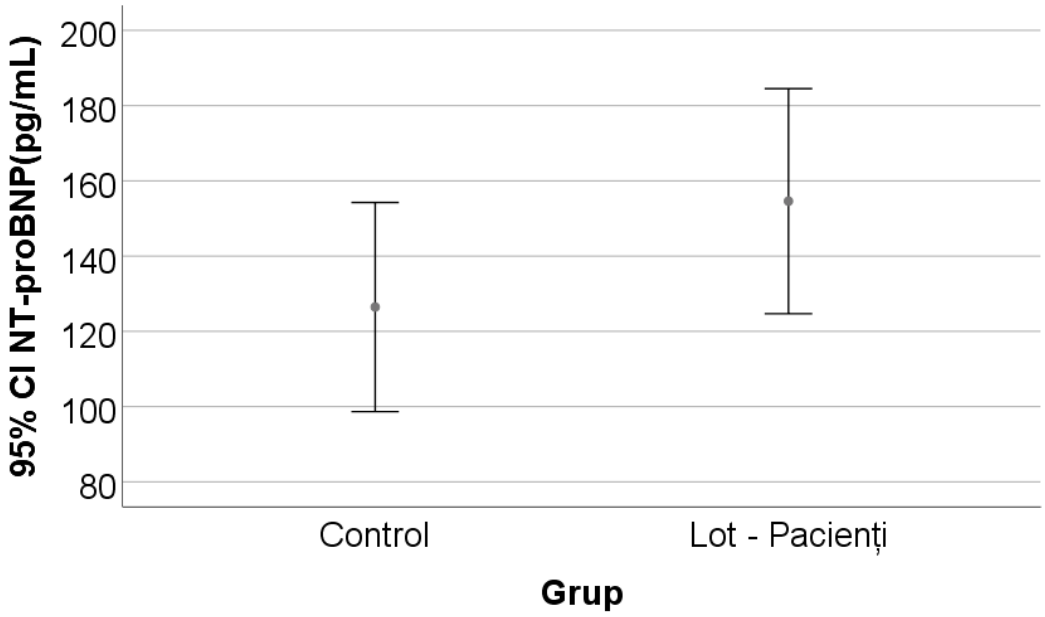

Fig. 8.1. The NT-proBNP Values for the Analyzed Groups

Tabel VIII.1 NT-proBNP Values in the Two Studied Groups

Group / NT-proBNP	**Mean ± SD**	**Median (IQR)**	**Mean rank**	**p*** (p=0.264***)
Control (p=0.462**)	126.46 ± 46	121 (85.5-173.5)	-	0.180
Patients (p=0.235**)	154.6 ± 63.91	148.5 (93.25-203)	-	

The data presented in Table VIII.1 and Figure 8.1 compare the NT-proBNP values between the analyzed groups. To identify the appropriate statistical test for comparing the two groups, the Shapiro-Wilk test was applied for each parameter to determine if the data set follows a Gaussian distribution, and the Student's t-test was used to compare the means of the two groups.

The differences between the groups were not significant according to the Student's t-test, indicating that the NT-proBNP values in the analyzed patients did not differ significantly between the control group (126.46 ± 46 pg/mL) and the study group (154.60 ± 64 pg/mL).

The distribution of NT-proBNP values was normal in both groups according to the Shapiro-Wilk test ($p>0.05$). Based on the results, the differences in NT-proBNP values were not statistically significant between the two groups as per the Student's T-Test ($p=0.180$);

Table VIII.1 Analysis of CK Values

Biomarker	Median reference	Median (IQR)	p*
CK (p<0.001)**	**131**	**815 (469.5-3437.75)**	**<0.001**

In the case of CK, the data set contains atypical values (e.g., 28420 U/L, 11984 U/L) which, however, cannot be deemed as outliers and thus excluded from the data set because these values are consistent with the clinical picture of the respective patients. In this case, the appropriate mathematical model for assessing the central tendency is the median. The above table demonstrates that the central tendency value expressed by the median (815 U/L) is far outside the biological reference range for this parameter (30 -135 U/L, median 131). Due to objective reasons, mainly represented by the COVID-19 pandemic, a control group could not be established for this parameter, and the obtained values were reported against the average population values extracted from the specialized literature, which were used as a reference interval.

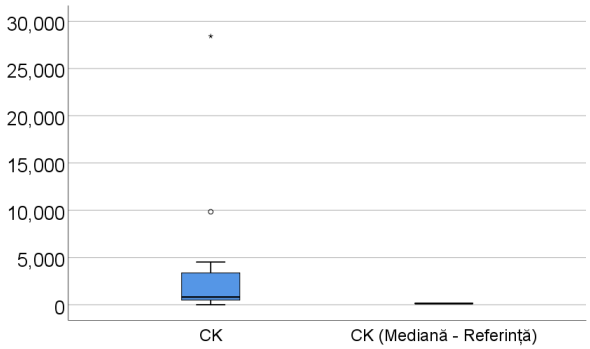

Fig. 8.2. Graphical Representation of NT-proBNP Values Between the Control Group and the Patient Group

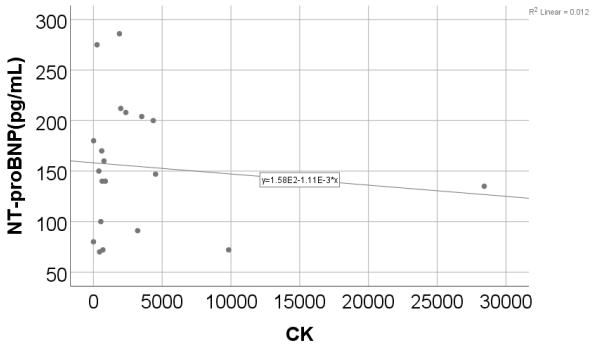

Fig. 8.3 Correlation Between NT pro-BNP and Creatine Phosphokinase

In Figure 8.2, the graphical representation of CK values in the two analyzed groups is depicted, and it is notable that in the study group, CK values were significantly higher than in the control group. The median CK (815) in the testing group was significantly higher compared to the reference population (131) (p<0.001).

In the study group, all patients had altered CK values, which is consistent with the literature and confirms the value of this marker in the diagnosis and monitoring of conditions involving muscle destruction, such as pelvic ring fractures in the context of polytrauma.

8.4 Discussions

The family of natriuretic peptides, especially BNP and NT-proBNP, are commonly used for:

- Monitoring the progression of heart failure (HF)
- Diagnosis, risk stratification, and monitoring the progression and treatment of myocardial infarction

The data obtained indicate that in the case of NT-proBNP, values have a normal distribution in both the study and control groups, but the differences between the groups are not statistically significant.

As Figure 8.3 suggests, there is a slight positive correlation between NT-proBNP and CK values in the studied groups, which could lead to the consideration of NT-proBNP as a marker of muscle lysis in traumatic contexts. However, the lack of statistical significance in the differences between the analyzed groups leads us to assert that the use of NT-proBNP as a biomarker for muscle lysis requires further research; currently, we cannot recommend this marker for monitoring the progression of polytraumatized patients with pelvic fractures.

In the case of CK, the values obtained in the study group did not have a Gaussian distribution. This is most likely due to the fact that the CK-MM value is strongly dependent on the volume of the affected muscle, which is a parameter with very high variability in polytraumatized patients, who by the definition of the pathology associate injuries in different areas of the body, all of which can increase the CK value.

Furthermore, in polytraumatized patients, the association of brain injuries, myocardial contusion, abdominal trauma, etc., which can lead to the increase of CK-MB and CK-BB isoenzymes and, consequently, to the increase of the total CK value, is common.

The evolution of CK values is generally parallel to the clinical evolution of the patient, and this is well highlighted in current practice where it is considered that a decrease in CK value by approximately 30% daily is a positive prognostic factor, while an increasing trend in these values is a negative prognostic factor and requires reevaluation of the therapeutic and surgical strategy as there are pathological elements that sustain muscular suffering.

Although the specificity of CK in monitoring muscle lysis in polytraumatized patients with pelvic fractures is low, it is still considered an important and essential biomarker for evaluating the progression of patients, and the research team considers it a standard for researching new markers with superior specificity in the diagnosis and monitoring of the evolution of this pathology.

9. Biochemical Markers for Evaluating the Redox Status in Polytraumatized Patients with Pelvic Ring Fractures

9.1 Introduction (Working Hypothesis and Specific Objectives)

The management and monitoring of polytraumatized patients, including those with pelvic ring fractures, are complex and present a significant challenge in emergency departments. This complexity is due to a cascade of clinical, pathophysiological, and biochemical events that negatively influence the survival rate of these patients [145]. Oxidative stress (OS) and impaired redox status are significant biochemical mechanisms contributing to cell and tissue damage. Recognized for its role in various pathological processes such as cancer and diabetes, OS occurs when the balance between prooxidants and antioxidants is disturbed, tipping the balance in favor of prooxidants [146,149].

OS results from the overproduction of reactive oxygen species (ROS), exceeding the antioxidant defensive system, and/or depletion of antioxidants. Studies have shown that polytraumatized patients exhibit an overproduction of ROS, affecting various biochemical processes and contributing to the deterioration of cells and tissues. This overproduction can affect cell membranes and intracellular functions or can trigger inflammatory signaling, leading to multiple organ dysfunction syndrome (MODS) [150-153].

In this context, an important objective was to evaluate the redox status by determining a novel SO biomarker, OXSR1, and a well-established biomarker, GGT. The aim was to highlight a possible correlation between these biomarkers and the progression of polytraumatized patients with pelvic ring fractures [149].

The specific objectives of this study consisted of:

1. Measuring GGT activity values in the blood of polytraumatized patients with pelvic ring fractures, which were taken from the patients' records with reference values.

2. Determining the level of OXSR1 in the blood of polytraumatized patients with pelvic ring fractures and in patients constituting the control group, and comparing the two values.

9.2 Materials and Methods

The analysis through ELISA and the statistical processing of data are described in the "Research Methodology" chapter.

Two groups of patients were established:

- The study group, Group 1, included patients admitted on an emergency basis with the diagnosis of POLYTRAUMA WITH PELVIC RING FRACTURES at the Clinical Emergency Hospital of Bucharest, during the period 2018-2022.

- The control group, Group 2, included patients admitted for other pathologies, EXCLUDING POLYTRAUMA WITH PELVIC RING FRACTURES.

9.3 Results

Table IX.1. GGT Values in the Analyzed Groups

Biomarker	Mean reference	Mean ± SD	Mean diference (95% C.I.)	p*
GGT (p=0.866**)**	24	49.7 ± 15.38	25.7 (18.5-32.9)	**<0.001**

***One-Sample T-Test, **Shapiro-Wilk Test**

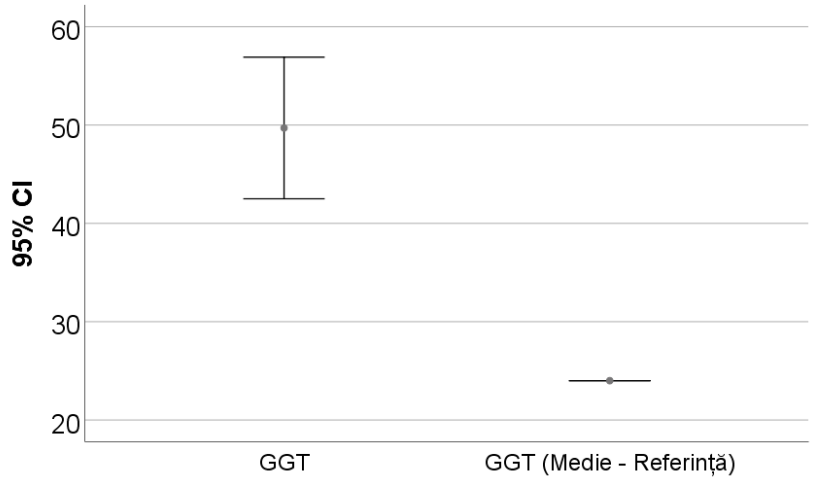

Fig. 9.1. GGT Value in the Study Group Illustrated Alongside the Reference Average

Table IX.1 and Fig. 9.1 represent the GGT values in the study group compared to normal values. The values have a non-parametric distribution in the study group according to the Shapiro-Wilk test (p=0.866). The differences between the groups are significant according to the Mann-Whitney U test (p<0.001), showing significantly higher values of GGT in the study group (49.7 ± 15.38 U/L) compared to normal values. From the patient group, 95% had pathological values.

Table IX.2. OXSR1 Values in the Control Group and the Patient Group

Group / OXSR1	Mean ± SD	Median (IQR)	Mean rank	p*****
Control (p=0.211**)	83.72 ± 32.07	72.1 (56.2-110.9)	24.54	**<0.001**
Patients (p=0.016)**	39.9 ± 27.31	31.2 (21-55.22)	12.10	

***Student T-Test, **Shapiro-Wilk Test, ***Levene's Test for Equality of Variances, ****Welch T-Test, *****Mann-Whitney U Test**

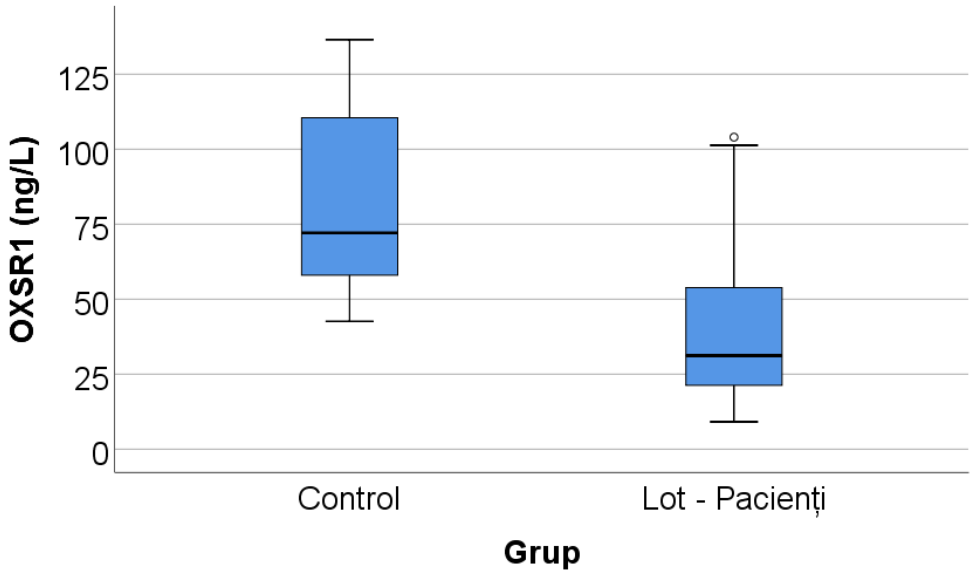

Fig. 9.2. Comparison of OXSR1 Values Between the Control Group and the Patient Group

The data from Table IX.2 and Fig. 9.2 represent the comparison of OXSR1 values between the analyzed groups. The values have a non-parametric distribution in the study group according to the Shapiro-Wilk test (p=0.211). The differences between the groups are significant according to the Mann-Whitney U test (p<0.001), with significantly lower OXSR1 values observed in the study group (39.9 ± 27.31 ng/L) compared to the control group (83.72 ± 32.07 ng/L).

9.4. Discussions

Recent studies on OXSR1, comparing cells from oral squamous cell carcinoma with healthy ones, have highlighted a significant increase in OXSR1 in tumor cells [154]. In patients with severe

polytrauma, the overproduction of reactive oxygen species (ROS) affects essential cellular processes, such as lipid function and protein synthesis, and a decrease in ROS levels has been correlated with a better prognosis [149]. However, the signaling pathways and antioxidant responses related to ROS and how they contribute to cellular homeostasis are not fully understood.

OXSR1 may have both antioxidant and pro-oxidant roles, influencing gene expression and redox signaling. Biological redox reactions are seen as a duality, with roles in both physiological signaling and cellular pathology. OXSR1, involved in both scenarios, is crucial in controlling cellular redox status [149,154]. ROS modulate key metabolic enzymes and control the transition from autophagy to apoptosis, influencing the expression of associated genes [149,154]. Additionally, OXSR1 plays a role in angiogenesis and is involved in cellular protection mechanisms, with damaged cells becoming less tolerant to oxidative attacks [149,154]. The OSR1 protein, activated in oxidative stress, is involved in angiogenesis and protection mechanisms, playing a significant role in apoptosis and inhibiting tumor development [155-159].

The study revealed a decrease in OXSR1 levels in the study group, suggesting protective effects through molecular pathways [149,154].

GGT, a glycoprotein located on the surface of cell membranes, is involved in amino acid transport and the gamma-glutamyl cycle. It plays a crucial role in the degradation of glutathione (GSH), a major intracellular antioxidant, and maintains an optimal concentration of GSH in the cytoplasm, protecting against oxidative stress [160-163]. An increase in GGT activity is associated with various neoplasms and the adaptive response to oxidative and toxic stress [164, 165]. GGT, as a marker of oxidative stress, plays an important role in attenuating its effects by maintaining cellular glutathione metabolism and homeostasis [166].

The research results showed a significant increase in GGT levels in the study group, indicating the onset of oxidative stress, in line with the specialized literature on patients with polytrauma.

Chapter 10: Relationships and Correlations Among Biochemical Parameters Tested in Polytraumatized Patients with Pelvic Ring Fractures

10.1 Introduction:

In the present study, I will exemplify some of the relationships and correlations observed between the biochemical markers followed in polytraumatized patients with pelvic fractures diagnosed, admitted, and treated at the Clinical Emergency Hospital of Bucharest during the period 2018-2022.

Statistical analysis was conducted using IBM SPSS Statistics 25 and Microsoft Office Excel/Word 2013. Quantitative variables were tested for distribution using the Shapiro-Wilk test and were expressed as means with standard deviations or medians with interpercentile intervals.

The comparison of parameters in cases without a control group was conducted in relation to the reference population using the One-Sample T-Test (illustrating compared means and the difference of means with confidence intervals) for variables with normal distribution and the One-Sample Wilcoxon Signed Rank Test (illustrating compared medians) for variables with non-parametric distribution.

In cases with a control group, variables from the test group with a normal distribution were compared between the two groups using the Student T-Test or Welch T-Test (depending on the equality of variances observed according to the Levene's test) while variables with non-parametric distribution were compared between the two groups using the Mann-Whitney U test.

Correlations were also performed between variables; for those with normal distribution, these were quantified using the Pearson correlation coefficient, and for variables with non-parametric distribution, correlations were quantified using Spearman’s rho correlation coefficient.

1. The main objective of this study is to identify correlations between the followed biochemical markers with the aim of developing a diagnostic and treatment protocol for patients with pelvic ring fractures. This protocol is intended to support the evaluation and rapid and comprehensive diagnosis of this pathology and at the same time to assist the team managing such a case in the correct, complete, and rapid assessment, as well as in choosing the optimal therapeutic plan.

10.2 Results

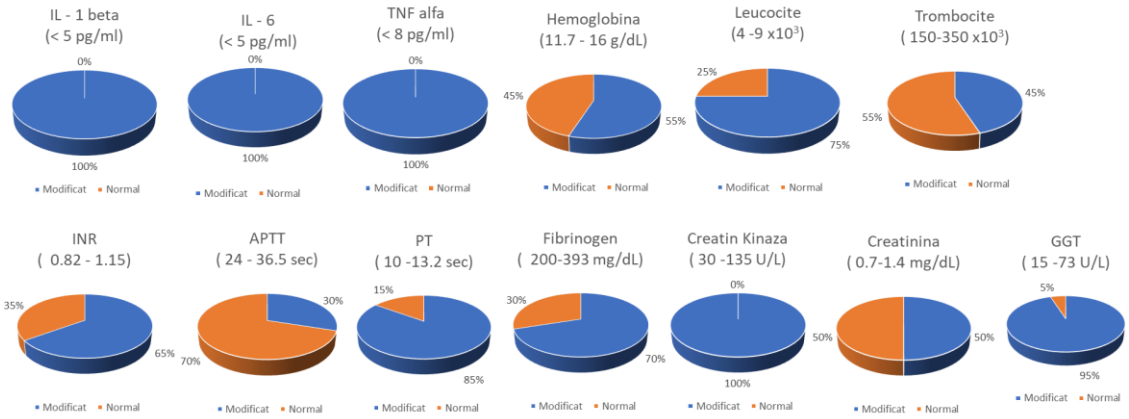

Fig. 10.1 - Percentile distribution for the analyzed parameters within the Biological Reference Range and/or below/above the normal threshold

In Fig. 10.1, it can be observed that the majority of the analyzed parameters (with the exception of APTT and thrombocytes) and **all the biochemical markers that were the objective of this doctoral research are outside the reference range, which demonstrates the important impact of polytraumatism on the body on a multitude of levels.**

Table X.1 Comparison of Investigated Parameters in Relation to the Reference Population Averages

Biomarker	**Mean reference**	**Mean ± SD**	**Mean diference (95% C.I.)**	**p***
IL-1 beta (p=0.642**)	3.31	14.19 ± 2.49	10.88 (9.71-12.04)	**<0.001**
IL-6 (p=0.081**)	2.5	8.96 ± 1.37	6.46 (5.81-7.10)	**<0.001**
TNF alfa (p=0.106**)	1.323	16.71 ± 2.30	15.39 (14.31-16.47)	**<0.001**
Hb (p=0.698**)	14.339	11.17 ± 1.98	-3.169 (-4.1 - -2.237)	**<0.001**
Leucocite (p=0.114**)	6.16	14.502 ± 6.313	8.342 (5.387-11.297)	**<0.001**
Trombocite (p=0.844**)	252.87	239.65 ± 128.49	-13.22 (-73.36 – 46.92)	0.651

Fibrinogen (p=0.117**)	334.2	359.2 ± 236.41	25 (-85.65 – 135.65)	0.642
GGT (p=0.866**)	24	49.7 ± 15.38	25.7 (18.5-32.9)	**<0.001**
Creatinină (p=0.082**)	0.98	1.329 ± 0.535	0.349 (0.098-0.6)	**0.009**

***One-Sample T-Test, **Shapiro-Wilk Test**

The data from Table X.1 represent the comparison of investigated parameters in relation to the reference population averages. The following results are observed for the tested group:

- Significantly higher IL-1 beta values compared to the reference population ($p<0.001$), with a mean difference of 10.88 (95% C.I.: 9.71-12.04);

- Significantly higher IL-6 values compared to the reference population ($p<0.001$), with a mean difference of 6.46 (95% C.I.: 5.81-7.10);

- Significantly higher TNF-alpha values compared to the reference population ($p<0.001$), with a mean difference of 15.39 (95% C.I.: 14.31-16.47);

- Significantly lower Hb values compared to the reference population ($p<0.001$), with a mean difference of -3.169 (95% C.I.: -4.1 - -2.237);

- Significantly higher leukocyte values compared to the reference population ($p<0.001$), with a mean difference of 8.342 (95% C.I.: 5.387-11.297);

- No significant differences in platelet values between the tested group and the reference population ($p=0.651$);

- No significant differences in fibrinogen values between the tested group and the reference population ($p=0.642$);

- Significantly higher GGT values compared to the reference population ($p<0.001$), with a mean difference of 25.7 (95% C.I.: 18.5-32.9);

- Significantly higher creatinine values compared to the reference population ($p=0.009$), with a mean difference of 0.349 (95% C.I.: 0.098-0.600).

Table X.3 Comparison of the Values of NT-proBNP, hsCRP and OXSR1 Relation to the Reference Population Averages

Group / NT-proBNP	**Mean ± SD**	**Median (IQR)**	**Mean Rank**	**p*** (p=0.264***)
Control (p=0.462**)	126.46 ± 46	121 (85.5-173.5)	-	0.180
Lot – Patients (p=0.235**)	154.6 ± 63.91	148.5 (93.25-203)	-	
Group / hsCRP	**Mean ± SD**	**Median (IQR)**	**Mean Rank**	**p**** (**p=0.003***)
Control (p=0.978**)	0.746 ± 0.29	0.7 (0.5-0.95)	-	**<0.001**
Lot – Patients (p=0.089**)	5.73 ± 1.87	5.5 (4.12-6.45)	-	
Group / OXSR1	**Mean ± SD**	**Median (IQR)**	**Mean Rank**	**p*****
Control (p=0.211**)	83.72 ± 32.07	72.1 (56.2-110.9)	24.54	**<0.001**
Lot – Patients (p=0.016)	39.9 ± 27.31	31.2 (21-55.22)	12.10	

***Student T-Test, **Shapiro-Wilk Test, ***Levene's Test for Equality of Variances, ****Welch T-Test, *****Mann-Whitney U Test**

Table X.4. Analyzed correlation between TNF-alpha and CK

- Correlation	p
TNF alfa (p=0.106***) **x CK (p<0.001***)**	**0.035, R= -0.474****

****Spearman's rho Correlation Coefficient,**

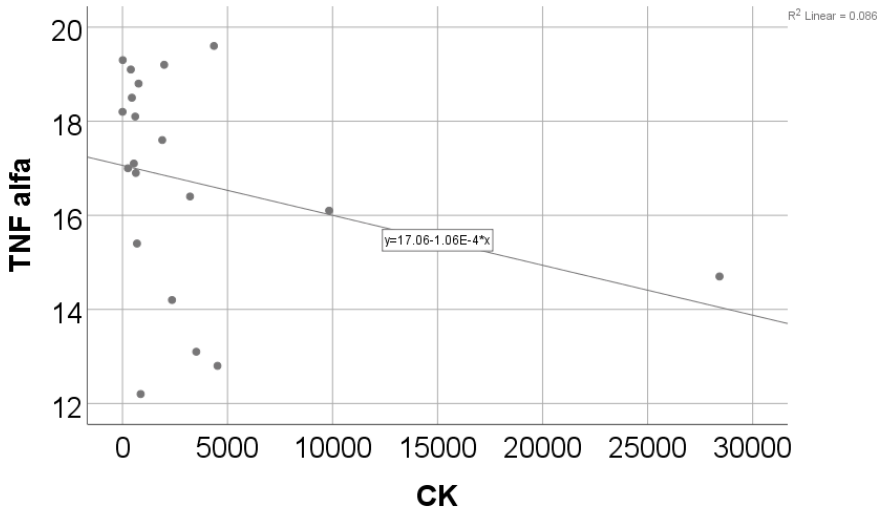

Fig.10.2. Correlation between TNF-alpha and CK

The data from Table X.4 and Fig. 10.2 represent the correlation between TNF-alpha and CK, which was observed to be a significant and moderate negative correlation (p=0.035, R= -0.474). This indicates that patients with higher values of TNF-alpha were significantly more likely to have lower values of CK and vice versa.

This negative correlation might seem surprising at first glance, considering that TNF-alpha is known for its pro-inflammatory effects. However, TNF-alpha has also been described as having anti-inflammatory properties, potentially limiting T-cell mediated responses directly through apoptosis or indirectly by regulating IL-12, a major mediator in type 1 inflammatory responses [167].

Another explanation for this negative correlation could be the very short half-life of TNF-alpha, which is only 5-8 minutes [168]. This leads to a pronounced variability in the levels of this substance. By comparison, the half-life of CK-MB is 10-12 hours, which can result in discrepancies in the plasma persistence of these two compounds [169].

Table X.5. Analyzed correlation between IL-6 and OXSR1

Correlation	**p**
IL-6 (p=0.081***) **x OXSR1 (p=0.016***)**	**0.005, R= 0.601****

****Spearman's rho Correlation Coefficient,**

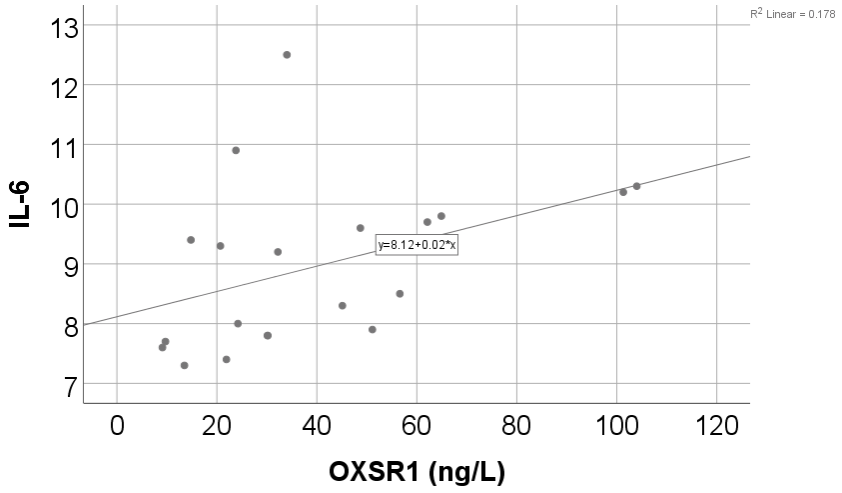

Fig. 10.3. Correlation between IL-6 and OXSR1

The data from Table X.5 and Figure 10.3 represent the correlation between IL-6 and OXSR1, which was observed to be a significant and positive high-strength correlation (p=0.005, R= 0.601). This indicates that patients with higher values for IL-6 also had significantly more often higher values for OXSR1, and vice versa.

OXSR1, a member of the Serine/Threonine kinase family, acts as a regulator in response to environmental stress, which is crucial for its phosphatase activity and tumor suppressor function [170, 171]. It has been reported that OXSR1 and the inflammation marker IL-6 are significantly elevated in serum samples from patients with diabetes undergoing hemodialysis compared to healthy participants [172,173].

Although the specific mechanisms of OXSR1 activation remain poorly defined, largely due to its extremely low basal activity [174], the correlation between OXSR1 and oxidative stress is well-known. This could also explain the positive correlation with IL-6, a well-established marker of inflammation. It is recognized that there is a direct proportional link between oxidative stress and inflammation.

12: Conclusions and Personal Contributions

12.1 Conclusions

This work represents the outcome of research focused on a socio-economically impactful issue in current medicine: polytrauma in general, and its associations with significant mortality and morbidity that negatively and long-term affect the quality of life. This has implications not only at the individual level but also on a societal scale, considering the indirect economic impact of the population's health on the global economy.

Consequently, polytrauma represents an area of broad interest both economically and medically, with the significant efforts aimed at increasing the survival of these patients and reducing the number of post-traumatic complications and sequelae.

The study indicates that the most affected age group by polytrauma with associated pelvic ring fractures is 30-40 years, followed by the 18-30 age group, thus 80% of the patients in the study group are within the 18-40 age range. Practically, the socio-economically active age group is most often affected by this type of trauma, making rapid social and economic reintegration of these patients imperative. This can only be achieved through rapid and appropriate diagnostic and treatment methods.

The purpose of this scientific research was to integrate biochemical correlations into the treatment algorithm of polytraumatized patients with pelvic fractures to reduce mortality and the incidence of complications. In this regard, the concrete objectives that this scientific research proposed and achieved were:

1. Defining the biochemical profile of the polytraumatized patient, consisting of identifying the optimal biochemical parameters for efficient patient monitoring, and

2. Identifying new biochemical markers with diagnostic, therapeutic, and prognostic value as those compounds that can indicate when a polytraumatized patient with a pelvic ring fracture is fit for a certain treatment and the optimal type of treatment applicable, and can be used for monitoring the evolution and assessing the patient's prognosis.

This work demonstrates the interdisciplinarity of approaching the polytraumatized patient with a pelvic ring fracture, adding elements of biochemical research to aspects characteristic of intensive therapy and orthopedics.

Polytrauma is a rare event compared to the total number of patients admitted following trauma, making the selection of patients for studies sometimes difficult. Therefore, there are few scientific papers published on this topic, making it difficult to compare the obtained results with the specialized literature.

From the conducted studies, illustrated cases, and specialized literature, it is evident that the evolution of a polytraumatized patient cannot be confined to a predefined pattern but can only have directions in which it can evolve that must be well known and monitored to ensure the best chance of survival and socio-professional reintegration of the patient.

A very important conclusion, considering the peculiarities mentioned regarding patients with polytrauma and their evolution, is that all the determined biochemical parameters, IL-1 beta, IL-6, TNF-alpha, hsCRP, CK, GGT, and OXSR1, showed statistically significant changes, which reflect and reinforce the clinical significance of these parameters.

Another important and clinically significant mention is their evolution and the correlations of these parameters, although, for the reasons I have already explained, they were not statistically significant but consistent with the patient's evolution and the complex pathological processes characteristic of polytrauma that excel in multiple lesion associations. Therefore, it is essential to address all injuries, have an integrative vision of the patient, and implicitly an interdisciplinary team whose main goal is the patient's survival.

The analysis of the obtained data revealed an important finding, namely that the majority of the analyzed parameters and all those that constituted the objectives of this scientific research are outside the reference interval, demonstrating the significant and multifaceted impact of polytrauma on the body (Fig. 10.1). In the case of GGT, analyzed as an oxidative stress marker, 95% of patients had pathological values.

The above statements support, on the one hand, the clinical significance of these parameters, and on the other hand, supports the desire for decisions regarding the evaluation of the polytraumatized patient to be based on objective parameters that reflect the patient's condition.

I consider that through doctoral research, I have analyzed a series of biochemical parameters that reflect the exacerbation of inflammation and other pathological processes in polytrauma and that meet the criteria mentioned earlier and the obtained results that fully responded to the formulated research hypotheses.

12.2 Personal Contributions

In the specialized literature, there are very few studies regarding molecular diagnosis in polytraumatized patients with pelvic ring fractures, thus these determinations give this work an innovative and original character.

Another personal contribution I consider is the idea of determining and using these biochemical markers not only for diagnostic purposes but also as a prognostic of the patients' evolution and monitoring of treatment, reducing morbidity and mortality in these particularly fragile patients with uncertain evolution.

Another novel and innovative element of this personal research is the highlighting of oxidative stress in the complex pathological processes in polytraumatized patients. Oxidative stress was highlighted by determining a new biomarker of oxidative stress, OXSR1, and a classic biomarker, GGT. The findings of the studies suggest the need for molecular diagnostic methods that could be associated with clinical data for the elaboration of a definitive diagnosis, ideally as early as possible, and the establishment of protocols for precise diagnosis. By corroborating clinical data with molecular methods, the optimal treatment modality can be chosen.

This doctoral research highlighted several aspects that require further research, namely, the analysis of each type of complication and the identification of specific biomarkers, establishing threshold values for certain biochemical parameters, extending research on biochemical markers considering the complexity of the pathophysiological processes, multidisciplinary approach to polytrauma, and increasing the involvement of fundamental research in current medical practice.

The results of the doctoral research have demonstrated that the analyzed biochemical parameters can be correlated with the evolution of the polytraumatized patient, being useful tools for prognosis, diagnosis, and monitoring of treatment.

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