"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

MEDICAL DOCTORAL SCHOOL

The importance of biochemical markers in the rapid diagnosis and treatment of crush syndrome

PhD THESIS SUMMARY

Doctoral supervisor:
PhD. GREABU MARIA

PhD candidate:
Creangă Constantin Iulian

2022

Contents

List of published scientific works

Articles published *in extenso* as a result of doctoral research

1. **Iulian Creangă**, Alexandra Totan, Olivera Lupescu, Iulia - Ioana Stănescu, Costin Dumitru, Maria Greabu. Aldolase - From Biochemistry to Laboratory Medicine, REV.CHIM. (Bucharest) 70, No. 2, p578-580, 2019.

https://www.revistadechimie.ro/pdf/44%20CREANGA%202%2019.pdf

2. **Iulian Constantin Creangă**, Alexandra Totan, Iulia-Ioana Stănescu, Daniela Miriicescu, Maria Greabu. The importance of the biochemical markers in rapid diagnosis and treatment of crushing syndrome. Romanian Journal of Medical Practice - Vol. XV, No. 1 (70), p 88-95, 2020.

https://rjmp.com.ro/articles/2020.1/RJMP_2020_1_Art-17.pdf

3. **Iulian Constantin Creangă**, Alexandra Totan, Iulia-Ioana Stănescu, Daniela Miricescu, Maria Greabu. Aldolase A and carbonic anhydrase III, two new candidate biomarkers versus acknowledged parameters in the context of crushing syndrome. Romanian Journal of Medical Practice - Vol. XVI, No. 3 (80), p380-386, 2021.

https://rjmp.com.ro/articles/2021.3/RJMP_2021_3_Art-12.pdf

4. Costin Dumitru, Stănescu Iulia-Ioana, Totan Alexandra, Miricescu Daniela, **Iulian Constantin Creangă**, Greabu Maria. Approach to the polytraumatized patient with pelvic fracture. Romanian Journal of Medical Practice, Vol. XV Issue 1, p37-45. 9p, 2020.

https://rjmp.com.rO/articles/2020.1/RJMP_2020_1_Art-08.pdf

List of abbreviations and symbols

1. Crush syndrome - (CS)
2. Radiography - (RX)
3. Computer tomography - (CT)
4. Nuclear magnetic resonance - (NMR)
5. Ultrasound - (ECO)
6. Aldolase - (ALD)
7. Aldolase A - (ALD A)
8. Creatine kinase - (CK)
9. Creatine kinase-MB - (CK-MB)
10. Creatine kinase-MM - (CK-MM)
11. Oxidative stress response protein 1 - (OXSR1)
12. Erythrocyte sedimentation rate - (ESR)
13. C-reactive protein - (CRP)
14. Lactate dehydrogenase - (LDH)
15. Fibroblast growth factor 2- (FGF)-2
16. Inhibitory growth factor 1 - (IGF-1)
17. Vitamin D - (Vit. D)
18. Growth hormone - (GH)
19. Matrix metalloproteinase-2 - (MMP-2)
20. Interleukin - (IL)
21. Family with sequence similarity 5, member C - (FAM5C)
22. Proteoglycans - (PGs)

23. Bone morphogenetic proteins - (BMPs)

24. Vascular endothelial growth factor - (VEGF)

25. Hepatocyte growth factor - (HGF)

26. Adenosine triphosphate - (ATP)

27. Acetyl coenzyme A - (acetyl-CoA)

28. Tricarboxylic acid cycle - (TCA)

29. Multiple organ failure - (MSOF)

30. Myoglobin - (Mb)

31. Disseminated intravascular coagulation - (DIC)

32. Acute renal failure - (IRA)

33. Ratio between ischemia and reperfusion - (I / R)

34. Troponin C - (TnC)

35. Troponin I - (TnI)

36. Troponin T - (TnT)

37. Aspartate aminotransferase - (AST)

38. Carbonic anhydrase III - (CAIII)

39. Glutathione peroxidase - (GPx)

40. 8-hydroxy -2'- deoxyguanosine - (8-OHdG)

41. Total antioxidant capacity - (TAC)

42. Intracompartmental pressure - (PIC)

43. Electrocardiogram - (EKG)

44. Interleukin 1 - (IL-1)

45. Interleukin 6 - (IL-6)

46. Superoxide dismutase - (SOD)

47. Hydroxylysine (HO-Lys)

48. Hydroxyproline (HO-Pro)

49. Delayed Onset Muscle Soreness - (DOMS)

50. Deficiency of carnityl palmitotransferase - (CPT)

51. Acute myocardial infarction - (AMI)

52. Nitric oxide - (NO)

53. Ferrous ion - (Fe^{2+})

54. Ferric ion - (Fe^{3+})

55. Fructose-1,6-bisphosphate - (F-1,6-P2)

56. Glyceraldehyde-3-phosphate - (GAP)

57. Dihydroxyacetone phosphate - (DHAP)

58. Triglycerides - (TG)

59. Pyruvic acid - ($C_3H_4O_3$)

60. Phosphfructokinase-1 - (FFK-1)

61. Glutathione peroxidase - (GPx)

62. Squamous cell carcinoma - (SCC)

63. Lung SCC - (LSCC)

64. C-reactive protein - (CRP)

65. Native CRP - (nCRP)

66. High sensitive CRP - (hsCRP)

67. Monomeric CRP - (mCRP)

68. Tumor necrosis factor α - (TNF-α)

69. Phosphocholine - (PCh)

70. Oral hormone replacement therapy - (HRT)

71. Proteins associated with carbonic anhydrase - CARPs

72. Human hepatocellular carcinoma - (HCC)

73. Rheumatoid arthritis - (RA)

74. Receptor expressed in lymphoid tissues – RELT

INTRODUCTION

Crush syndrome (CS) is a severe medical condition found in patients in emergency departments, who have suffered accidents of various causes, resulting in severe destruction of muscle tissue in a certain area. Such a trauma can lead to death even in the absence of an initial multi-organ involvement, if therapeutic intervention is not carried out in a fast and effective way.

In certain circumstances, a large number of people can be affected, for example in the case of natural disasters or terrorist attacks, which can generate not only immediate death, but also severe potentially irreversible complications.

Rapid assessment and emergency treatment (local and general) offer the patient increased chances of recovery, reduce the number of complications and facilitate rapid integration into society.

We emphasize "rapid diagnosis" because the patient who suffered a crush injury may initially have, upon emergency presentation to the emergency room, non-specific, modest signs and symptoms (especially in the case of muscle injuries that are not associated with bone injuries - fractures), which can be easily overlooked by a less experienced eye and frequently associated with contusion-type injuries. This often delays the establishment of specialized treatment, a fact that can produce ***irreversible local but also general damage, serious sequelae or death***.

The local/general clinical examination as well as the *monitoring of biochemical markers* have a very important role. *From our point of view, the screening of biochemical markers in CS and their dynamic monitoring provides an overview of the general prognosis*.

The development of this type of screening and the discovery of biochemical markers with increased specificity for crush syndrome - Aldolase A (ALD A), Carbonic Anhydrase III (CAIII), N-terminal prohormone of Cerebral Natriuretic Peptide (NT-pro-BNP), protein of oxidative stress response 1 - (OXSR1) together with the evaluation of markers already known and used in current medical practice - creatine kinase (CK), lactate dehydrogenase (LDH), fibrinogen, C reactive protein (CRP) - represents a priority for us and for our future project, in order to *improve the rapid diagnosis protocols of CS*.

In this context, this doctoral research paper *has as its main objective the determination of the levels of some biochemical makers (ALD A, CAIII, NT-proBNP, OXSR1*) which, at the present

time, are not determined in a current and usual way, but can have an important role in the future in the evaluation of muscle injuries of traumatic etiology.

The increased specificity of these markers for muscle injuries (of non-traumatic etiology) has been demonstrated in numerous studies, researchers being able to demonstrate a direct proportional relationship between their values (assessed dynamically) and the rate of muscle destruction in numerous pathologies involving muscle damage (rheumatoid arthritis, systemic lupus erythematosus, collagenosis, muscle stress in performance athletes).

To date, ***there are very few studies that place these markers in relation to traumatic injuries***.

I. GENERAL PART

The general part includes two chapters that contain relevant information for the topic addressed in the special part, as well as a descriptive analysis of the achievements in the field, based on the Romanian and international specialized literature.

In the **first chapter**, a detailed description of the changes occurring in the biochemistry and physiology of muscle and bone tissue in patients with crush syndrome was made. The particularities of the muscle and bone tissue and the effects of the crush syndrome both locally and systemically were described. Also, the biochemical changes of interest appearing in CS, the evaluation, monitoring and the special therapeutic conduct necessary for the treatment of these patients were described.

Chapter 2 contains detailed information about the main complication in CS: rhabdomyolysis. The causes of its occurrence, the risk factors and the main manifestations that make this complication an extremely important objective in the treatment and monitoring of CS patients are described.

II. PERSONAL CONTRIBUTION

Chapter 3. Biochemical markers showing changes with clinical significance in crush syndrome

Muscle injuries trigger physical, physiological and biochemical changes. Metabolic disorders in the injured muscle are illustrated by the release of specific biomarkers in the extracellular matrix:

1. used in the current diagnostic protocol:

- muscle proteins: myoglobin, troponin
- muscle lysis markers: creatine kinase, MM and MB isozymes
- inflammation markers: TNF-a, IL-6, IL-1β, hsCRP

2. less studied:

- aldolase (ALD A)
- carbonic anhydrase III (CAIII)
- N-terminal prohormone of brain natriuretic peptide (NT-pro-BNP)
- oxidative stress response protein 1 (OXSR1)

The quantification of these parameters can substantially contribute to the assessment of muscle injuries and the establishment of subsequent therapeutic behavior [47].

This chapter highlights the essential characteristics of these markers and the changes that have occurred in relation to CS.

Chapter 4. Working hypothesis and general objectives

The **motivation for choosing the topic** of this doctoral research is represented by the need to detect some biochemical biomarkers with increased sensitivity and specificity for CS, which facilitate an early diagnosis, in order to initiate the appropriate therapy as quickly as possible. Avoiding the occurrence of severe complications specific to this condition considerably reduces the risk of morbidity and mortality.

Through the research carried out during the doctoral internship, we tried to find the answer to the following questions:

"Can new biochemical parameters of interest be identified in CS?"

"How involved are ALD A and CAIII, NT-ptoBNP and OXSR1, in CS?"

Based on these questions, the following hypotheses were formulated that were the basis of the research undertaken:

- identifying the relationship between ALD A and CS
- identifying the relationship between CAIII and CS
- identifying the relationship between NT-proBNP and CS
- identifying the relationship between OXSRl and CS
- identification of a possible link between the established biochemical markers for muscle lesions and ALD A, CAIII, NT-proBNP and OXSR1

In order to confirm or deny these hypotheses, extensive bibliographic research was performed, as well as the quantitative and statistical analysis of some parameters of interest.

In this context, the research in this paper consists in performing a comparative study carried out on a number of 42 patients (22 patients who suffered a crush trauma and 20 healthy patients - who represent the control group), a study that compares the values ALD A, CAIII, NT-proBNP and OXSR1 with the values of the other established muscle destruction markers (CK, TNF-a, IL-6, IL-1β, hsCRP).

The aim of this study is to establish the degree of sensitivity and specificity of ALD A, CAIII, NT-proBNP and OXSR1 in crush injuries, starting from the information already existing in the literature (high specificity and sensitivity of ALD A, CAIII, NT-proBNP and OXSR1 for patients with chronic inflammatory diseases, people with muscle stress of non-traumatic etiology).

Analyzing the correlations between the values of the markers ALD A, CA III, NT-proBNP, OXSR1 and a series of clinical and paraclinical parameters aims to report the results of the studies in the clinical context of the patients.

Chapter 5. General methodology

The data from the specialized literature were corroborated with the study on biological products (serum) of the patients and volunteers who gave their written consent for the processing of these samples.

Patient samples were harvested, separated, stored and analyzed according to the reagent kit manufacturer's instructions. The selection of patients and volunteers was carried out in collaboration with orthopedic doctors as well as with the support of a medical analysis laboratory in Bucharest. The samples were collected, stored and analyzed within 4 years.

State-of-the-art automatic or semi-automatic equipment was used, checked by service engineers to ensure accuracy in their measurement and minimize the source of error. Where possible, control methods were used precisely to ensure that the results are as accurate as possible. Patients were selected based on predetermined inclusion and exclusion criteria.

The determination methods used were: wet chemistry, ELISA and chemiluminescence. 42 patients divided into two groups were included in the study:

1. patients who have suffered a crush injury
2. patients hospitalized for other pathologies, without pathology of traumatic etiology whose diagnosis was established by the specialist doctor according to specific diagnostic criteria.

All patients were hospitalized in the Bucharest Emergency Clinical Hospital between 2016-2021.

For each subject included in the study, a series of clinical and biological data were noted: age at the time of inclusion in the study, gender, associated comorbidities and a wide range of biochemical parameters.

The control groups in each study of the present research were made up of clinically and biologically healthy subjects, without other conditions with the potential for false positive or false negative results.

All subjects voluntarily participated in this research and signed the informed consent. They were informed that their data would be used for scientific purposes and that their anonymity would be preserved, and that the research would have no impact on the diagnostic or therapeutic management of patients.

All study participants had 2-5 mL of blood harvested. Sample collection was carried out in two stages: at admission and 24 hours after the first harvest, after the initiation of emergency therapy.

Serum collection for the determination of ALD A and CAIII, NT-proBNP and OXSR1 was carried out simultaneously with the determination of established markers of muscle necrosis (CK, TNF-a, IL-6, IL-1β, hsCRP) as well as with the usual analyzes (usually collected in the case of all patients admitted to the Emergency Department).

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 as means with standard deviations or medians with interpercentile ranges. The independent quantitative variables with non-parametric distribution were tested using the Mann-Whitney U test and the independent quantitative variables with parametric distribution were tested using the **Student** test (correlations between them were tested using the Pearson correlation coefficient).

Chapter 6. Study 1: New possible biochemical markers with clinical significance in patients with crush syndrome-ALD A and CAIII

6.1. Introduction

The specific objectives of this study consisted in:

1. determination of ALD A activity in the blood of patients with crush syndrome and comparison with the level of ALD A in the blood of patients who constitute the control group
2. determination of CAIII activity in the blood of patients with crushing syndrome and comparison with the level of CAIII in the blood of patients who constitute the control group

6.2 Materials and methods

The patients were divided into two groups:

Group 1: consists of emergency hospitalized patients with the diagnosis of CRUSH SYNDROME, in the Bucharest Clinical Emergency Hospital, in the period 2016-2021.

Group 2: was made up of the control group - patients hospitalized for other pathologies, EXCEPT CRUSH SYNDROME.

Patients in study group 1 benefited from the collection of two blood samples and implicitly two determinations of ALD A and CAIII values, the first performed at emergency admission, the second 24 hours after admission (after emergency treatment and balancing patient's primary care).

Working protocol for the determination of ALD A

ALD A determination was performed by the sandwich ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 0.31-20 ng/mL, and the sensitivity 0.19 ng/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

Working protocol for the determination of CAIII

CAIII determination was performed by the competitive ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 0.5-10 ng/mL, and the sensitivity 0.1 ng/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

6.3 Results

The distribution of ALD A values in both groups is parametric, according to the **Shapiro-Wilk** test ($p>0.05$).

According to **Student's** test, patients in the study group had significantly higher ALD A values (3105 ± 849.81 pg/mL) compared to patients in the control group (961.82 ± 165.7 pg/mL) ($p<0.001$)

Table VI.1. Comparison of ALD A values between batches

Group	Average ± SD	Median (IQR)	p* (p<0.001***)
Control group (p=0.664**)	961.82 ± 165.7	938.9 (824.1-1100.57)	<0.001
Study group (p=0.414**)	3105 ± 849.81	3051.25 (2479.02-4021.17)	

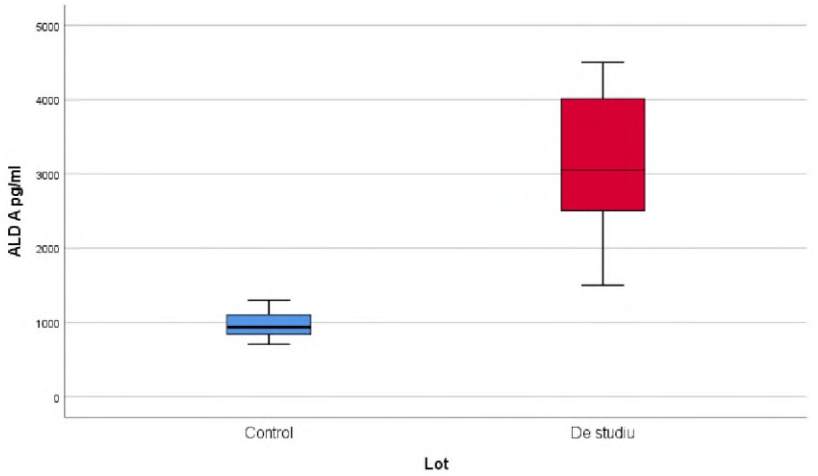

Fig. 6.1. Comparison of ALD A values between groups

The distribution of CAIII values in the control group is non-parametric, according to the **Shapiro-Wilk test (p=0.001).** According to the **Mann-Whitney U** test, patients in the study group had significantly higher CAIII values (154.06 ± 18.97 ng/mL) compared to patients in the control group (37.48 ± 19.31 ng/mL) (**p<0.001**).

Table VI.2. Comparison of CAIII values between groups

Group	Average ± SD	Median (IQR)	Average rank	p*
Control group (p=0.001**)	37.48 ± 19.31	33.15 (23.3-41.42)	10.50	<0.001
Study group (p=0.316**)	154.06 ± 18.97	152.65 (137.92-167.97)	31.50	

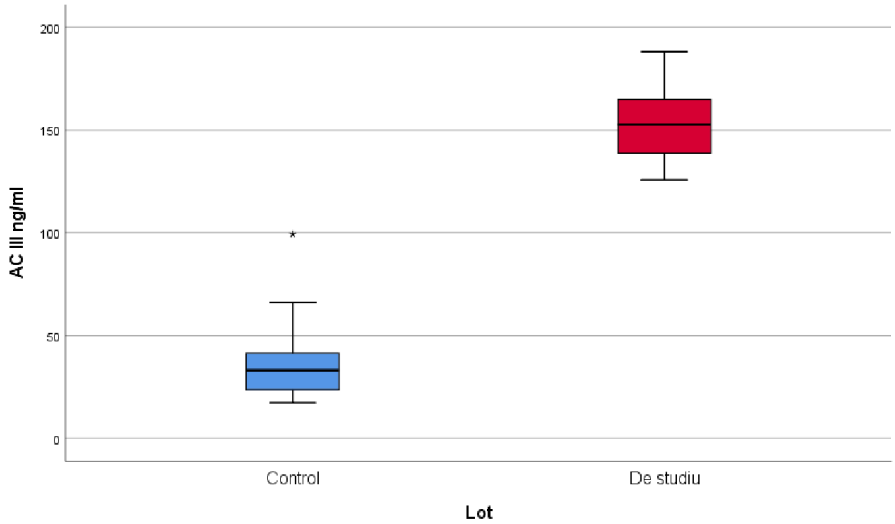

Fig. 6.2. Comparison of CAIII values between groups

6.4. Discussions

Currently, serum CK is considered the most sensitive indicator of crush injury assessment. ALD A and CAIII are not routinely determined and the monitoring of these markers is not included in any CS diagnosis and monitoring protocol.

In this first study, it was observed that ALD A has increased concentrations in patients with crush syndrome, the differences between the 2 groups being statistically significant ($p<0.001$).

Situations are described in clinical practice when, although there is CS, the CK values are not significantly increased or the CK increase is not directly proportional to the severity of the lesions. For these purposes, Nozaki and Pestronk emphasized the usefulness of serum ALD A measurement in all patients with muscle injuries who present normal CK values [21].

The molecular mechanism by which ALD A could be released from muscle cells without a parallel increase in serum CK has not yet been explained. In order to provide an explanation for these clinical data, Casciola-Rosen and his collaborators analyzed the level of gene and protein expression of ALD A and CK during the in vitro differentiation of muscle cells [22, 23]. In vitro experimental results showed that during the muscle regeneration phase ALD A is expressed at higher levels compared to CK [22, 23]. They also identified a phase during muscle cell differentiation in which cells exclusively express ALD A [22, 23].

The activation or inhibition of CAIII by various compounds is a topic of current and future research for the treatment of pathologies related to CAIII expression and functions. Like ALD A, CAIII is not routinely determined in CS patients. CAIII is released into the circulation following muscle damage. Currently, there is a special interest in the *ratio of Mb/CAIII in the serum as a possible diagnostic indicator in myocardial infarction* [128, 129, 130]. This ratio could show increased values because CA III is not found in the myocardium in the same proportions as Mb.

The study carried out by us highlights the fact that the distribution of values in the control group is non-parametric and the patients in the study group had significantly higher CA III values compared to the patients in the control group.

These results reinforce the idea presented by Brancaccio, who states that ALD A and CAIII must be added to the already known list of biochemical markers in the case of crush injuries [16].

Chapter 7. Biochemical markers of inflammation in patients with crush syndrome

7.1 Introduction

Some of the most important biomarkers associated with the inflammatory syndrome are: IL-1 beta, IL-6, TNF-alpha, hsCRP.

The specific objectives of this study consisted in:

1. determining the level of TNF-a in the blood of patients with crush syndrome and comparing these values with the reference values
2. evaluation of IL-1 beta level in blood samples of patients with crush syndrome and comparison of these values with reference values
3. evaluation of the IL-6 level in the blood samples of patients with crush syndrome and their comparison with the reference values
4. determination of the hsCRP level in the blood of patients with crush syndrome and in patients who constitute the control group and comparison of the two values

7.2 Materials and methods

The patients were divided into two groups:

Group 1: patients hospitalized in the EMERGENCY ROOM with the diagnosis of CRUSH SYNDROME, in the Bucharest Clinical Emergency Hospital, in the period 2016-2021

Group 2: was made up of the control group - patients hospitalized for other pathologies, WITH THE EXCEPTION OF CRUSH SYNDROME

Working protocol for the determination of TNF-α

Determination of TNF-α was performed by the sandwich ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 15.6-1000 pg/mL, and the sensitivity <1pg/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

Working protocol for the determination of IL-6

IL-6 determination was performed by the sandwich ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 7.8-500 pg/mL, and the sensitivity <1pg/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

Working protocol for the determination of IL-1β

Determination of IL-1β was performed by the sandwich ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 3.9-250 pg/mL, and the sensitivity <0.15 pg/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

Working protocol for the determination of hsCRP

hsCRP determination was performed by the sandwich ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 0.78-50 ng/mL, and the sensitivity 0.3 ng/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers

7.3 Results

Table VII .1. Distribution of patients related to the analyzed groups

Group	No.	Percentage
Control	10	39.4%
Study	12	60.6%

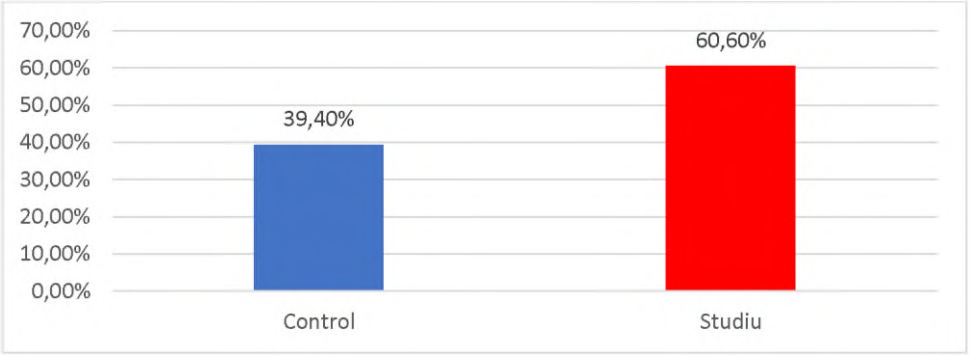

Fig. 7.1. The distribution of patients related to the analyzed groups

hsCRP values have a normal distribution in both groups according to the **Shapiro-Wilk** test (p>0.05). The differences between the groups are significant according to the **Student** test (**p<0.001**), with significantly higher hsCRP values being observed in the study group (6.02 ± 1.86 mg/L) compared to the control group (0.75 ± 0.29 mg/L).

Table VII.2. Comparison of hsCRP values between the analyzed groups

Group	Average ± SD	Median (IQR)	p* (p=0.005***)
Control (p=0.978**)	0.75 ± 0.29	0.7 (0.5-0.95)	<0.001
Study (p=0.083**)	6.02 ± 1.86	5.95 (4.40-6.80)	

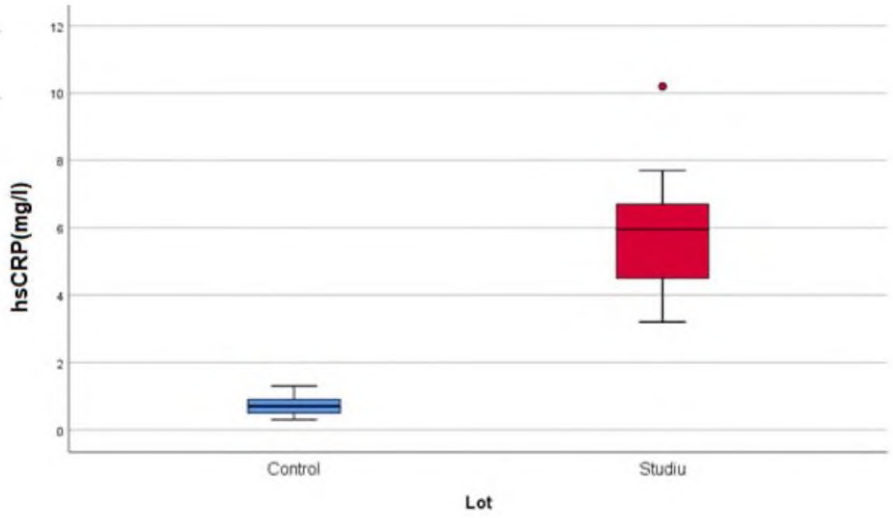

Fig. 7.2. Comparison of hsCRP values between the analyzed groups

Table VII.3. Comparison of IL-1 beta, IL-6, TNF-alpha values between the analyzed groups

Parameter	N	Average ± SD	Median (IQR)	Min	Max
IL-1 beta	22	13.16 ± 2.49	12.7 (11.7-15.3)	9	19
IL-6	22	8.53 ± 1.35	8.45 (7.28-9.4)	7	12
TNF-alfa	22	16.55 ± 2.24	16.8 (14.93-18.5)	12	19

IL-1 beta, IL-6, TNF-alpha, associated and established biomarkers of the inflammatory syndrome, have significantly increased values in the group of patients with crushing syndrome compared to the reference values (in the case of IL-1 beta, IL-6 and TNF -alpha).

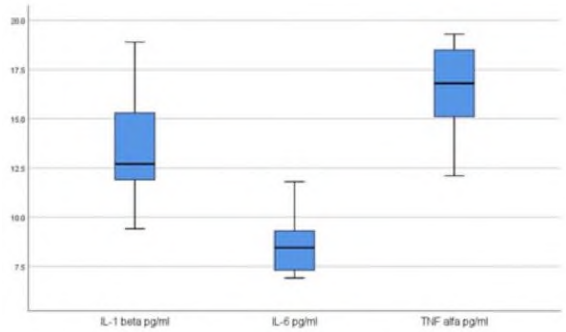

Fig. 7.3. Box-plot representation of the IL-1 beta, IL-6 and TNF-alpha values

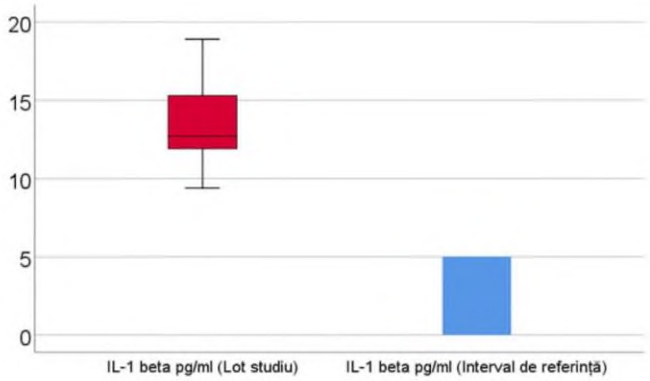

Fig. 7.4. IL-1 beta value in the study group illustrated next to the reference range

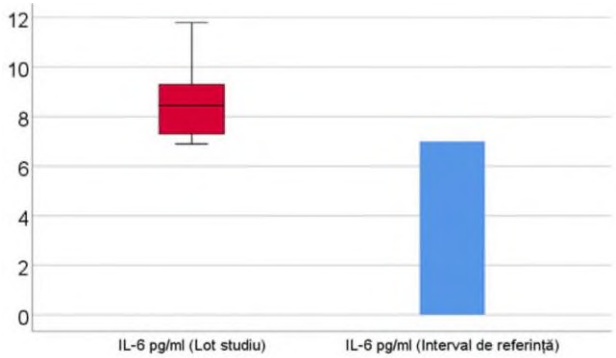

Fig. 7.5. IL-6 value in the study group illustrated next to the reference interval

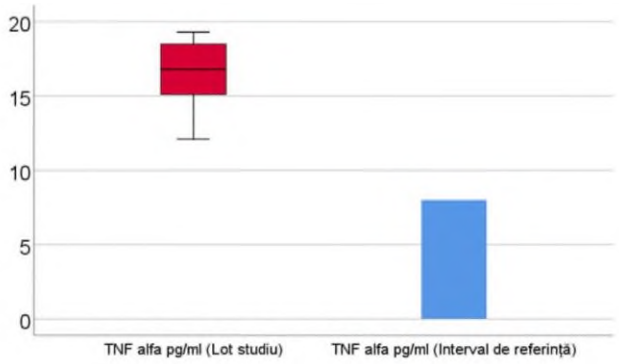

Fig. 7.6. TNF-alpha value in the study group illustrated next to the reference interval

7.4 Discussions

It is known that CRP levels increase dramatically in response to trauma, infection and inflammation. CRP is mainly classified as an acute inflammatory marker and is the main mediator of the acute phase response following an inflammatory event. CRP is mainly synthesized by IL-6-dependent hepatic biosynthesis [153,154]. Also IL-6 is the main inducer of CRP gene expression, IL-1 increasing its effect [142].

The dynamic monitoring and analysis of these markers together with the recently studied biochemical markers (ALD A, CAIII, NT-proBNP, OXSR1) is very useful because they represent a starting point as a reference and control model in our study.

A directly proportional change between the biochemical markers already established and used (hS CRP, IL-1 beta, IL-6, TNF-alpha) and the markers with increased specificity studied (ALD A, CAIII, NT-proBNP, OXSR1) highlights their increased utility and motivates us to continue the current research in the future.

Chapter 8. Biochemical markers of muscle lysis in patients with crush syndrome

8.1 Introduction

Ventricular natriuretic peptide or brain natriuretic peptide (BNP), also known as type B natriuretic peptide, is a hormone secreted by cardiomyocytes at the ventricular level in response to the elongation produced by the increase in blood volume.

The total serum CK level increases in cardiac, cerebral or skeletal muscle diseases (Duchenne muscular dystrophy, polymyositis, dermatomyositis, muscle trauma, rhabdomyolysis).

The specific objectives of this study consisted in:

1. determining the level of NT-proBNP in the blood of patients with crush syndrome and in patients who constitute the control group and comparing the two values.
2. evaluation of the CK level in the blood of patients with crush syndrome and comparison with the reference values.

8.2 Materials and methods

The patients were divided into two groups.

Group 1 (patients): admitted to the EMERGENCY ROOM with the diagnosis of **CRUSH SYNDROME**, in the Bucharest Clinical Emergency Hospital, in the period 2016-2021.

Group 2 (patients): was made up of the control group - patients hospitalized for other pathologies, EXCEPT CRUSH SYNDROME.

Working protocol for the determination of NT-proBNP

Determination of NT-proBNP was performed by the competitive ELISA method, using kits and a Stat Fax 300+ plate reader. The detection range of the kit was 50-1000 pg/mL, and the sensitivity 1 pg/mL. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

Woking protocol for the determination of CK

The determination of CK was carried out by the spectrophotometric method. The detection limit of the kit was 9.2U/L. The detection method is colorimetric. The work procedure is carried out according to the instructions of the kit manufacturers.

8.3 Results

NT-proBNP values have a normal distribution in both groups according to the **Shapiro-Wilk** test (p>0.05). The differences between the groups are not significant according to the **Student** test (p=0.202), so in the analyzed patients the NT-proBNP values were not significantly different between the control group (126.46 ± 46 pg/mL) and the study group (153.25 ± 64 pg/mL). mL).

Table VIII.1. Comparison of NT-proBNP values between the groups analyzed

Group	Average ± SD	Median (IQR)	p* (p=0.265***)
Control (p=0.462**)	126.46 ± 46	121 (85.5-173.5)	0.202
Study (p=0.307**)	153.25 ± 64	147.5 (95.75-202.75)	

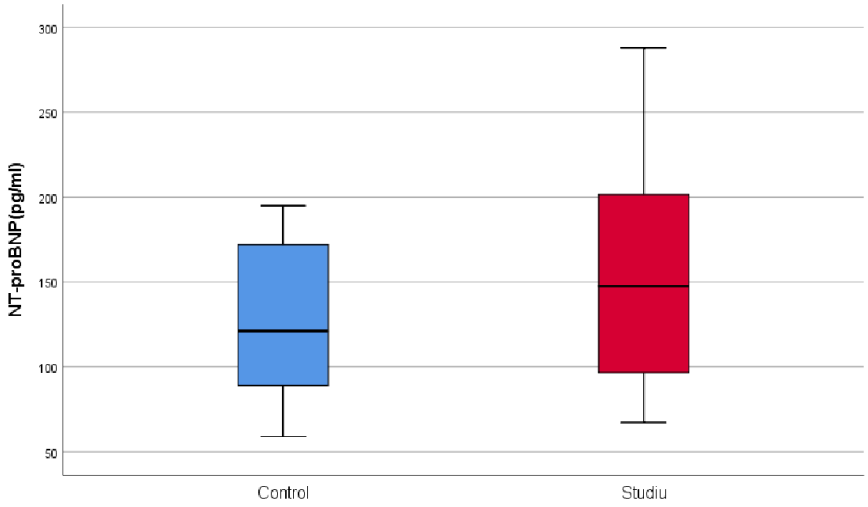

Fig. 8.1. Comparison of NT-proBNP values between the analyzed groups

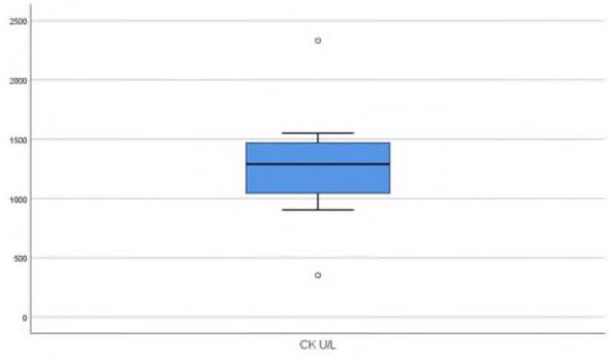

Fig. 8.2. Box-plot representation of CK values

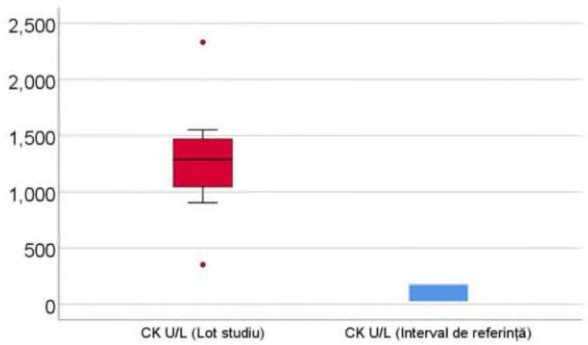

Fig. 8.3. CK value in the study group illustrated next to the reference range

For the group of patients with crush syndrome, the CK value was 1286.5 ± 561.6 U/L compared to the reference interval for CK 38-174 U/L men and 26-140 U/L women.

8.4 Discussions

Currently NT-proBNP is used in the evaluation of the diagnosis of heart failure (HF). Globally, it is used as a useful and cost-effective biomarker for the diagnosis, risk stratification and monitoring of acute and chronic myocardial infarction therapy [112].

The extension of the domain in the traumatic spectrum does not highlight significant changes according to the Student test (p=0.202), between the control group and the study group.

CK variations are a marker of the patient's evolution. The decrease of CK by 30% daily signifies the effectiveness of the treatment and the persistence of the increased values may signify the fact that the medical/surgical treatment is not effective or the muscle lesions were only partially treated [48, 52].

In the present research, the evolution of CK was parallel to the evolution of the patient, proving that an important component in the context of polytrauma was represented by muscle damage.

Chapter 9. Biochemical markers of oxidative stress in patients with crush syndrome-OXSRl

9.1. Introduction

In the specialized literature, there are studies on polytraumatized patients, which highlight the occurrence of oxidative stress. Therefore, our objective was to determine the newest biomarker of oxidative stress, OXSR1, and highlight its possible correlation with the values of the other determined biomarkers, depending on the evolution of patients with crush syndrome.

The specific objective of this study consisted in:

Determination of the OXSR1 level in the blood of patients with crush syndrome and in patients who constitute the control group and the comparison of the two values.

9.2 Materials and methods

The patients were divided into two groups:

Group 1 (patients): admitted to the EMERGENCY with a diagnosis of CRUSHING SYNDROME, in the Bucharest Clinical Emergency Hospital, in the period 2016-2021

Group 2 (patients): was made up of the control group - patients hospitalized for other pathologies, EXCEPT CRUSH SYNDROME.

Working protocol for the determination of OXSR1

The determination of OXSR1 was carried out by the ELISA method, using kits and a Stat Fax 300 plate reader. The detection method is colorimetric.

9.3 Results

OXSR1 values have a non-parametric distribution in the study group according to the **Shapiro-Wilk** test (p=0.014). The differences between the groups are significant according to the **Mann-Whitney U test (p<0.001**), observing significantly lower values in the study group of OXSR1 (41 ± 28.4 ng/L) compared to the control group (83.72 ± 32.07 ng/L).

Table IX.1. Comparison of OXSR1 values between the groups analyzed

Group	Average ± SD	Median (IQR)	Average rank	p*
Control (p=0.211**)	83.72 ± 32.07	72.1 (56.2-110.9)	24.38	<0.001
Study (p=0.014**)	41 ± 28.4	32.1 (21.83-56.78)	12.20	

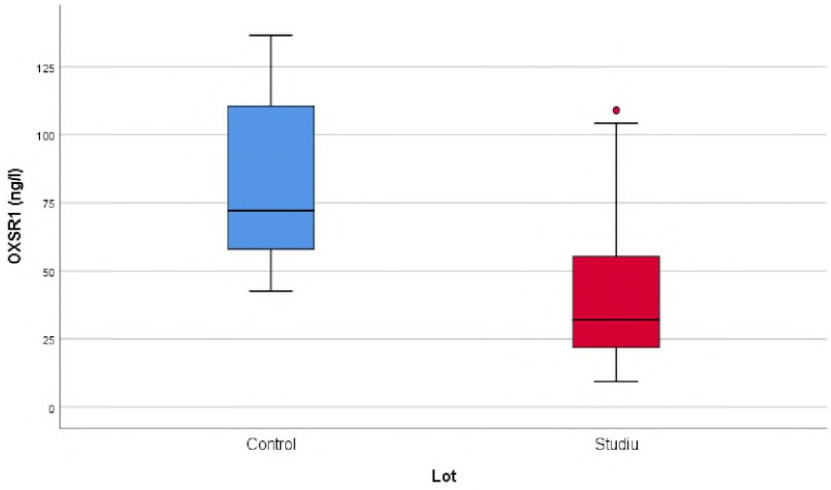

Fig. 9.1. Comparison of OXSR1 values between the groups analyzed

9.4 Discussions

In the specialized literature, there are studies that highlight the occurrence of oxidative stress in polytraumatized patients. This should be seen as a starting point in researching the role that oxidative stress has in the evolution of patients.

The results obtained in the present research, showed a significant decrease in the levels of OXSR1 in the study group compared to the control group, can lead to the idea that, under these conditions, protective effects appear, probably through a molecular pathway that results in the improvement of OXSR1.

Chapter 10. Possible correlations between different types of biochemical markers in patients with crush syndrome

10.1 Introduction

Responding to an acute current need, this doctoral research aims to be a contribution to the effort of modern medicine to improve the survival and quality of life of patients with crush

syndrome. For this purpose, we wanted to identify a potential correlation that would allow the use of the patient's biochemical profile to monitor and stage the treatment of crush syndrome.

The method of obtaining the data required for this analysis was complex, including as documentation sources: the patients' medical documents - medical records, laboratory analyses, specialist consultations, medication, and evolution, as well as all imaging and paraclinical investigations.

10.2 Materials and methods

The 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. The correlations established between them were performed using the Spearman's rho correlation coefficient, related to the non-parametric distribution of the variables.

10.3 Results

Table X.1. Correlation between aldolase A values and IL-1 beta values in the study group

Correlation	p*
ALD A (p=0.414**) x IL-1 beta (p=0.505**)	0.409, R= -0.185

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between ALD A and IL-1 beta was observed to be insignificant (p=0.409, R= -0.185), so in this study group, no significant association was found between these two variables.

Table X.2. Correlation between ALD A values and IL-6 values in the study group

Correlation	p*
ALD A (p=0.414**) x IL-6 (p=0.081**)	0.513, R=0.147

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between ALD A and IL-6 was observed to be insignificant (p=0.513, R=0.147), so in this study group, no significant association was found between these two variables.

Table X.3. Correlation between ALD A values and TNF-alpha values in the study group

Correlation	p*
ALD A (p=0.414**) x TNF-alfa (p=0.080**)	0.150, R=0.317

***Pearson Correlation Coefficient, **Shapiro-Wilk** Test

The distribution of the variables is parametric, the correlation between ALD A and TNF-alpha was observed to be insignificant (p=0.150, R=0.317), so in this study group, no significant association was found between these two variables.

Table X.4. Correlation between ALD A values and CK values in the study group

Correlation	p*
ALD A (p=0.414****) x CK (**p=0.649****)**	0.829, R=0.092

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between ALD A and CK was observed to be insignificant (p=0.829, R=0.092), so in this study group, no significant association was found between these two variables.

Table X.5. Correlation between CAIII values and aldolase A values in the study group

Correlation	P*
CAIII (p=0.316****) x ALD A (**p=0.414****)**	0.198, R=0.285

***Pearson Correlation Coefficient,** **Shapiro-Wilk** Test

The distribution of the variables is parametric, the correlation between CAIII and ALD A was observed to be insignificant (p=0.198, R=0.285), so in this study group, no significant association was found between these two variables.

Table X.6. Correlation between CA III values and IL-1 beta values in the study group

Correlation	p*
CA III (p=0.316****) x IL-1 beta (**p=0.505****)**	0.053, R=0.418

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between CAIII and IL-1 beta was observed to be insignificant (p=0.053, R=0.418), so in this study group, no significant association was found between these two variables.

Table X.7. Correlation between CAIII values and IL-6 values in the study group

Correlation	p*
CAIII (p=0.316****) x IL-6 (**p=0.081****)**	0.701, R= -0.087

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the observed correlation between CA III and IL-6 was observed to be insignificant (p=0.701, R= -0.087), so in this study group, no significant association was found between these two variables.

Table X.8. Correlation between CAIII values and TNF-alpha values in the study group

Correlation	p*
CAIII (p=0.316****) x TNF-alfa (**p=0.080****)**	0.477, R=0.160

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the observed correlation between CA III and TNF-alpha was observed to be insignificant (p=0.477, R=0.160), so in this study group, no significant association was found between these two variables.

Table X.9. Correlation between CAIII values and CK values in the study group

Correlation	p*
CAIII (p=0.316****) x CK (**p=0.649****)**	0.654, R=0.189

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the observed correlation between CA III and CK was observed to be insignificant (p=0.654, R=0.189), so in this study group, no significant association was found between these two variables.

Table X.10. Correlation between NT-proBNP and hsCRP

Correlation	p*
NT-proBNP (p=0.159**) x **hsCRP (p=0.004**)**	0.419, R=0.145

***Spearman's rho Correlation Coefficient, **Shapiro-Wilk Test**

The hsCRP value is distributed non-parametrically, the observed correlation is not statistically significant (p=0.419), so in the analyzed patients there was no significant association between NT-proBNP and hsCRP.

Table X.11. Correlation between NT-proBNP and OXSR1

Correlation	p*
NT-proBNP (p=0.159**) x **OXSR1 (p=0.030**)**	0.770, R=0.053

***Spearman's rho Correlation Coefficient, **Shapiro-Wilk Test**

The OXSR1 value is distributed non-parametrically, the observed correlation is not statistically significant (p=0.770), so in the analyzed patients there was no significant association between NT-proBNP and OXSR1.

Table X.12. Correlation between hsCRP and OXSR1

Correlation	p*
hsCRP (p=0.004) x **OXSR1 (p=0.030**)**	**0.002, R= -0.517**

***Spearman's rho Correlation Coefficient, **Shapiro-Wilk Test**

Both variables are non-parametrically distributed, the observed correlation is significant and negative, of a high degree (p=0.002, R= -0.517), so that **in the analyzed patients, high values for hsCRP are significantly more frequently associated with low values for OXSR1**.

Table X.13. Correlation between IL-1 beta values and IL-6 values in the study group

Correlation	p*
IL-1 beta (p=0.505**) x IL-6 (p=0.081**)	0.589, R= -0.122

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between IL-1 beta and IL-6 was observed to be insignificant (p=0.589, R= -0.122), so in this study group, no significant association was found between these two variables.

Table X.14. Correlation between IL-1 beta values and TNF-alpha values in the study group

Correlation	p*
IL-1 beta (p=0.505) x TNF-alfa (p=0.080**)**	**0.879, R=0.034**

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between IL-1 beta and TNF-alpha was observed to be insignificant (p=0.879, R=0.034), so in this study group, no significant association was found between these two variables.

Table X.15. Correlation between IL-1 beta values and CK values in the study group

Correlation	p*
IL-1 beta (p=0.505**) x CK (p=0.649**)	0.725, R=0.149

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between IL-1 beta and CK was observed to be insignificant (p=0.725, R=0.149), so in this study group, no significant association was found between these two variables.

Table X.16. Correlation between IL-6 values and TNF-alpha values in the study group

Correlation	p*
IL-6 (p=0.081**) x TNF-alfa (p=0.080**)	0.453, R=0.169

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between IL-6 and TNF-alpha was observed to be insignificant (p=0.453, R=0.169), so in this study group, no significant association was found between these two variables.

Table X.17. Correlation between IL-6 values and CK values in the study group

Correlation	p*
IL-6 (p=0.081**) x CK (p=0.649**)	0.553, R=0.248

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between IL-6 and CK was observed to be insignificant (p=0.553, R=0.248), so in this study group, no significant association was found between these two variables.

Table X.18. Correlation between TNF-alpha values and CK values in the study group

Correlation	p*
TNF-alfa (p=0.080**) x CK (p=0.649**)	0.779, R= -0.119

***Pearson Correlation Coefficient, **Shapiro-Wilk Test**

The distribution of the variables is parametric, the correlation between TNF-alpha and CK was observed to be insignificant (p=0.779, R= -0.119), so in this study group, no significant association was found between these two variables.

Chapter 11. Perspectives in the assessment and rapid diagnosis of CS

Table XI.2. Description of the parameters evaluated in the study

Parameter	N	Average ± SD	Median (IQR)	Min	Max
AC III	42	98.55 ± 61.89	127 (33.3-153.7)	17	188
ALD A	42	2084.44 ± 1247.5	1530 (946.9-3104.6)	706	4503
IL-1 beta	22	13.16 ± 2.49	12.7 (11.7-15.3)	9	19
IL-6	22	8.53 ± 1.35	8.45 (7.28-9.4)	7	12
TNF-alfa	22	16.55 ± 2.24	16.8 (14.93-18.5)	12	19
CK	8	1286.5 ± 561.6	1290 (974-1510.7)	352	2332
NT-proBNP	20	142.7 ± 58.32	137 (92-184)	59	288
hsCRP	20	3.94 ± 3	4.2 (0.85-6.1)	0.3	10.2
OXSR1	20	57.82 ± 36.25	52.5 (27.4-74.95)	9.4	136.5

Considering the seriousness of the consequences and evolution of CS, we believe that monitoring these markers in dynamics will be an important pawn in preventing the occurrence of local and general complications and will increase the life expectancy of patients with CS.

Starting from this idea, I and the study team want to continue the research of these markers (ALD A, CAIII, NT-proBNP, OXSR1) in the future as well, in the hope that their rapid determination and their increased specificity for muscle lesions will decrease the duration of time required for making the diagnosis and instituting specialized treatment.

Chapter 12. Conclusions and personal contributions

This doctoral research aims to be a contribution to the effort of modern medicine to improve the survival and quality of life of patients with crush syndrome, confirming the need for an interdisciplinary approach to the pathology and highlighting the importance of fundamental research in improving current therapeutic algorithms, by introducing new and specific biochemical parameters for the evolution of patients with crush syndrome.

The purpose of this doctoral research is precisely an interdisciplinary approach to the treatment of these patients with a particularly weak state, which results in a decrease in mortality and morbidity.

These are only possible by approaching the treatment algorithms and by integrating these elements of biochemistry, in order to optimize the treatment.

The concrete objectives that the present scientific research proposed and fulfilled were:

- *definition of the biochemical profile of the patient with crush syndrome (consisting in the identification of the biochemical parameters with increased specificity necessary for the efficient monitoring of the patient)*

-*identification of new biochemical markers*

Biomarkers ALD A and CAIII, biochemical markers with particular clinical significance and which are not routinely determined in patients with crush syndrome, may have a particularly important role in their monitoring. ALD A activity correlates positively with CAIII activity, with inflammation markers (IL-6, TNF-a) and with CK activity, biomarker of muscle lysis, providing a molecular picture consistent with the clinical one.

Patients with crush syndrome most often present a systemic inflammatory response. The present study is consistent with these data and highlights the presence of inflammation in the analyzed patients with crush syndrome. Thus, these biomarkers can be considered indicators of evolution and prognosis in crush syndrome.

IL-1 beta, IL-6, TNF-alpha, hsCRP, biomarkers associated and well-known to the inflammatory syndrome, have significantly increased values in the group of patients with crush syndrome compared to the control group (in the case of hsCRP) or to the reference values (in the case IL-1 beta, IL-6 and TNF-alpha).

Patients with crush syndrome have a disturbed redox status of the body. The conclusion of this study is consistent with other studies in the specialized literature, which highlighted the

occurrence of oxidative stress in polytraumatized patients. The results obtained in the present research showed a significant decrease in the levels of OXSR1 in the study group compared to the control group and can lead to the idea that, under these conditions, protective effects appear, probably through a molecular pathway that results in the improvement of OXSR1.

Currently, the main use of the NT-proBNP marker is in the evaluation of the diagnosis of heart failure and the diagnosis, risk stratification and monitoring of the therapy of acute and chronic myocardial infarction.

Although there are specialist studies outlining a possible contribution of this marker in the case of crush injuries, the results obtained by us support the idea that the expansion of the research field in the traumatic spectrum does not highlight significant changes between the control group and the study group.

CK is an important prognostic marker in the evolution of patients with CS. The decrease of CK by 30% daily signifies the effectiveness of the treatment, while the persistence of increased values of CK signifies the fact that the medical/surgical treatment is not effective or the muscle lesions were only partially treated. In the group of patients monitored by us, the evolution of CK was parallel to the evolution of the patient, proving that muscle damage was an important component in the context of polytrauma.

Despite the fact that CK does not present a specificity dedicated to the crush syndrome, it represents an important component in the evaluation of CS, its monitoring being a model role in the dynamic evaluation of new biochemical markers.

Selective Bibliography

16. Brancaccio P, Lippi G, Maffulli N; 2010; Biochemical markers of muscular damage, *Clin Chem Lab Med*; 48(6) p 757-767, 2010.

21. Nozaki,K., Pestronk, *A.J. Neurol. Neurosurg. Psychiatry, 80,* p. 904-908, 2009.

22. Casciola-Rosen, L., Hall, J.C., Mammen, A.L., Christopherstine, L., Rosen, A. *Clin. Exp. Rheumatol., 30, no. 4,* p. 548-553, 2012.

23. Casciola-Rosen, L., Nagaraju, K., Plotz, P., Wang, K., Levine, S., Gabrielson, E., Corse, A., Rosen, *A. JExp Med. 201,* p.591-601, 2005.

47. **Iulian Constantin Creangă**, Alexandra Totan, Iulia-Ioana Stănescu,, Daniela Miricescu, Maria Greabu, Aldolase A and carbonic anhydrase III, two new candidate biomarkers versus

acknowledged parameters în the context of crushing syndrome. *Romanian Journal of Medical Practice — Vol. XVI, No. 3 (80),* p380-386, 2021.
48. McLeish, MJ., Kenyon, GL. Relating structure to mechanism in creatine kinase. *Critical Reviews in Biochemistry and Molecular Biology, 40,* p 1-20, 2005.
52. P. M. Clarkson, A. K. Kearns, P. Rouzier, R. Rubin, and P. D. Thompson, "Serum creatine kinase levels and renal function measures in exertional muscle damage," *Medicine and Science in Sports andExercise, vol. 38, no. 4,* p. 623-627, 2006.
53. Apple FS, Quist HE, Doyle PJ, et al: Plasma 99th percentile reference limits for cardiac troponin and creatine kinase MB mass for use with European Society of Cardiology/American College of Cardiology consensus recommendations. *Clin Chem Aug;49(8).p* 1331-1336, 2003.
112. Semenov AG, Katrukha AG. Analytical Issues with Natriuretic Peptides - has this been Overly Simplified? *EJIFCC 2016 Aug 1;27(3):* p 189-207, 2016.
128. Vaananen HK, Syrjala H, Rahkila P, Vuori J, Melamies LM, Myllyla V et al. Serum carbonic anhydrase III and myoglobin concentrations in acute myocardial infarction. *Clin Chem; 36:* p 635-638, 1990.
129. Beuerle JR, Azzazy HM, Styba G, Duh SH, Christenson RH. Characteristics of myoglobin, carbonic anhydrase III and the myoglobin/carbonic anhydrase III ratio in trauma, exercise, and myocardial infarction patients. *Clin Chim Acta 294:* p 115-128, 2000.
130. Vuotikka P, Uusimaa P, Niemela M, Vaananen K, Vuori J, Peuhkurinen K. Serum myoglobin/carbonic anhydrase III ratio as a marker of reperfusion after myocardial infarction. *Int J Cardiol;91:* p 137-144, 2003.
142. Ciubotaru I, Potempa LA, Wander RC. Production of modified C-reactive protein in U937-derived macrophages. *Exp Biol Med 230(10):* p 762-70, 2005.
153. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, inetleukin-6 and tumor necrosis factor-a. *Mol Psychiatry 21:* p 642-649, 2016.
154. Du Clos TW. Function of C-reactive protein. *Ann Med 32(4):* p 274-8, 2000.