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COMPARATIVE ANALYSIS OF RAPID DIAGNOSTIC METHODS OF EARLY POST-TRAUMATIC COAGULATION DISORDERS AND THEIR USE IN A THERAPEUTIC INTERVENTION GUIDE

SUMMARY OF THE DOCTORAL THESIS

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Table of contents

List of scientific papers

List of abbreviations and symbols

Introduction

General part - current state of knowledge 1

1. Physiopathology 1

1.1. The physiology of coagulation 1

1.2. Trauma-induced coagulopathy 5

1.3. Endotheliopathy of trauma 8

1.4. Trauma-induced platelet malfunction 15

1.5. Thrombin generation deficiency 23

1.6. Fibrinogen deficiency 25

1.7. Hyperfibrinolysis 27

1.8. Auto-heparinization 30

Original part - personal contribution 33

2. Work hypothesis and the general objectives 33

3. General methodology of the research 35

3.1. Study population 35

3.2. Materials and Methods 36

3.3. Statistical analysis 38

4. Thromboelastometric analysis of coagulopathy phenotypes in polytraumatized patients 40

4.1. Introduction 40

4.2. Technical methods 42

4.3. Applications in the diagnosis and treatment of trauma-induced coagulopathy ..43

4.4. Materials and Methods 47

4.5. Results 48

4.6. Discussions 74

4.7. Conclusions 77

5. Study on the prognostic value of markers of endothelial activation and platelet contribution in traumatic brain injury patients 78

5.1. Introduction 78

5.2. Materials and Methods 81

5.3. Results 84

5.4. Discussions 92

5.5. Conclusions 95

6. Study on the prognostic value of immune activation markers in the inflammation-coagulopathy in blunt chest trauma patients 97

6.1. Introduction 97

6.2. Materials and Methods 99

6.3. Results 102

6.4. Discussions 112

6.5. Conclusions 116

7. Conclusions and personal contributions 117

7.1. Conclusions 117

7.2. Personal contributions 118

8. Bibliography 121

LIST OF ABBREVIATIONS AND SYMBOLS

ACP- activated C protein

aPCC- activated prothrombin complex concentrate

aPTT- activated partial thrombolastin time

ARDS- acute respiratory distress syndrome

AT- antithrombin

CIRP- cold-inducible RNA-binding protein

DAMPs - damage-associated molecular patterns

EOT- endotheliopathy of trauma

EPCR- C protein endothelial receptor

HMGB1- high mobility group box 1 protein

INR- international normalised ratio

mtDNA – mithocondrial DNA

NETs- neutrophil extracellular traps

NO –nitric oxide

PAI-1 – plasminogen activator inhibutor

PAMPs - pathogen-associated molecular patterns

PCC- prothrombin complex concentrate

PLAs- platelet-leukocyte agggregates

PT- prothrombin time

RAGE- receptor for advanced glycation endproducts

ROTEM – rotational thromboelastometry

SHINE - shock induced endotheliopathy

TAFI- thrombin activatable fibrinolysis inhibitor

TBI- traumatic brain injury

TEG – thromboelastography

TF- tissue factor

TIC - trauma induced coagulopathy

TLR- toll-like receptor

t-PA – tissue-type plasminogen activator

u-PA- urokinase-type plasminogen activator

u-PAR- u-PA receptor

vWF- von Willebrand factor

INTRODUCTION

The most recent analysis of the health condition of the world population, objectived by the Global Burden of Diseases, Injuries, and Risk Factors (GBD) statistic from 2021, ranks post-traumatic injuries in third place both among the causes of mortality and morbidity. Worldwide, in 2021, 608 million new cases and 4 million deaths (6.4% of all deaths) from traumatic causes were registered (1). According to the World Health Organization, in 2019, in Romania, 9,546 patients died secondary to trauma, among them 7,423 men and 2,123 women, approximately 30% aged between 35 and 54 and approximately 38% between 55 and 74 years (2). Despite the favorable evolution of mortality and morbidity indicators in the last 20 years, traumatic injuries continue to represent an important public health problem at the national level.

The original description of the pathogenic mechanisms of trauma in relation to mortality highlighted a trimodal distribution in most health systems, represented by: immediate deaths - occurring in less than an hour after the traumatic event (results of major injuries, most frequently at the level of the central nervous system, the heart or the great vessels - formally incompatible with survival), rapid - which appear during the first hours (produced by severe injuries, but with potential for recovery; most often secondary to major hemorrhage) and late - which appear days, weeks or months after the trauma (due to septic, thromboembolic complications or multiple organic dysfunction) (3). Both the progress in the diagnosis and treatment of these patients, as well as the global evolution of the health systems, which allowed the improvement of access to health care of increased complexity, led to the concentration of the targeted population among the second category. Thus, the prompt and effective treatment of post-traumatic hemostatic disorders becomes mandatory for the reduction of immediate mortality, as well as the reduction of morbidity. It is estimated that 25% of patients with severe post-traumatic injuries develop a form of coagulopathy induced by trauma (trauma-induced coagulopathy - TIC), which associates a mortality between 35 and 50%. A significant part of these represent preventable or potentially preventable deaths, explaining the research team's interest in this topic (4).

Although extensive, the specialized literature has not yet managed to provide answers to all questions related to TIC. Moreover, a series of new questions appeared as a result of the deepening of the understanding of the physiopathological mechanisms, the increase in technical performance - translated into the increase in the detection and analysis capacity of previously

uncharacterized molecular elements, respectively the increase in the capacity to analyze information through techniques of artificial intelligence (5).

In the general part, this work aims to describe the pathophysiological mechanisms of TIC, the methods of diagnosis, screening, prevention and treatment, as they are known in the current specialized literature. Brief presentation of physiological coagulation mechanisms.

In the special part, management of TIC is presented using an algorithm guided by rotational thromboelastometric analysis of the clot (ROTEM), developed in the Anesthesia and Intensive Care Clinic of the Bucharest Clinical Emergency Hospital, a reference center for the care of polytrauma patients in Romania.

The objectives of the research were multiple: exhaustive demographic characterization of the polytraumatized adult population admitted to the aforementioned tertiary center, in the time period 2019-2023; comparative analysis of viscoelastic tests and classical coagulation tests in the target population; the presentation of the new algorithm and those previously used, taken from the literature; identification of phenotypes of TIC that benefit from dedicated treatment; the identification of inexpensive markers, easily accessible to the clinician, with predictive value, for the main hemostatic disorders specific to TIC; familiarization of the Clinic staff with viscoelastic techniques; optimization and standardization of TIC management through the routine use of thromboelastometric analysis in the Clinic.

This prospective, descriptive study has a number of limitations inherent in the type of analysis.

First of all, by excluding patients with pathologies and/or chronic treatments likely to influence the mechanisms of physiological coagulation and, implicitly, those of coagulation in the context of trauma, we ensured the homogeneity of the studied group and increased the probability that TIC is really responsible for the observed changes. However, through the prism of the increase in life expectancy - translated by the aging of the general population, a new profile of the polytraumatized patient is taking shape, which is not "in full apparent health" prior to the event. Unfortunately, this group was not included in our analysis, although it is increasingly present in our practice. Naturally, studies dedicated to the analysis of these patients are needed.

Secondly, the collected data will be used the usual analyzes available in the Bucharest Clinical Emergency Hospital, which are part of the standard of care for the polytraumatized

patient presenting with acute bleeding, but cannot be considered exhaustive in terms of the diagnosis, either he and classic, of the countless possible disorders of hemostasis. Moreover, a series of diagnostic elements, such as the extensive analysis of the immune profile by determining cytokine values or the analysis of endotheliopathy by determining glycocalyx degradation products, are either not available at all in current clinical practice, or are hardly available, due to the high costs of the long duration of processing or the particular technical conditions required for it. This limitation is embodied in the impossibility of a comparative analysis of known and validated cellular markers for different pathophysiological phenomena, in trauma or in other pathologies, with the derived markers that we analyzed, but which, in the end, only represent an extension of the physiopathological understanding , susceptible to error at several levels.

Thirdly, although the study assumed the follow-up of patients until discharge and collected relevant data about their evolution throughout the hospitalization, we believe that longer follow-up, after discharge, is necessary to assess the medical, psycho-social and economic impact of TIC. Unfortunately, I did not have the human or material resources to start a study of such magnitude, but extending the period of post-traumatic surveillance can only enrich the current understanding of some complex mechanisms.

Last but not least, the present analysis raised a series of questions, and in some cases it resulted in issuing some hypotheses that are worthy of being topics for future studies. Although it is not a limitation, according to the meaning of the term, but rather a personal regret of the author, the lack of a national trauma registry brings with it the impossibility of comparative analysis of the data obtained, of their validation in other populations of polytraumatized patients from Bucharest and Romania, as well as their use for further studies.

I. GENERAL PART- CURRENT STATE OF KNOWLEDGE

1. PHYSIOPATHOLOGY

1.1 The physiology of coagulation

Following a trauma, the vascular repair processes begin with the formation of a hemostatic clot rich in platelets and fibrin. Once the structural and functional integrity of the vessel is regained, the clot is processed by the fibrinolytic systems to allow normal blood flow to be restored. The sum of the local and systemic reactions carried out for this purpose describes physiological hemostasis. The complexity of TIC resides in the unique interactions between the mechanisms triggered by the trauma itself, the causes associated with resuscitation, and the patient's preexisting status (6). A brief presentation of the physiological processes will serve as a reference for understanding the changes occurring in TIC.

1.2. Trauma-induced coagulopathy

The latest editions of the " The European guideline on management of major bleeding and coagulopathy following trauma " reiterate the complexity of coagulopathy, resulting from the unique interactions between the patient, trauma and causes associated with resuscitation (6,7).

The patient presents a series of particularities such as age, comorbidities, underlying medical treatment, genetic anomalies, etc. what is the basis of a pro or anti-coagulant profile pre-existing trauma. The actual trauma leads to tissue destruction, hemorrhage, shock, with the consequence of hypoperfusion, which is pathophysiologically translated by a systemic endotheliopathy. At this level, mechanisms such as activation of the sympathetic nervous system, damage to the glycocalyx, autoheparinization, inflammatory response, platelet dysfunction, decreased activity of coagulation factors, hyperfibrinolysis, etc. they will accumulate with the reduction of coagulation factors through loss and through consumption. Additionally, factors related to resuscitation measures such as dilution of coagulation factors, hypothermia and acidosis contribute to the mentioned dysfunctions. Each of these elements constitutes a potential stopping point in the vicious circle of trauma-induced coagulopathy, a direction of diagnostic research and a possible therapeutic target (4,6,7).

1.2.1. TIC – hypoperfusion and tissue damage

Hemorrhagic shock leads to the quantitative loss of blood elements and coagulation factors, as well as to the reduction of platelet margin as a result of the rheological changes induced by anemia. The acute reduction of circulating volume through post-traumatic hemorrhage not effectively controlled by physiological mechanisms will lead to a decrease in cardiac output and, finally, to a reduction in the volume of delivered oxygen, in conditions of increased tissue oxygen consumption. The redistribution of blood to the tissues with increased metabolic need and the increase in the tissue extraction rate of oxygen are rapidly exhausted mechanisms, so hypoperfusion and hypoxia cause the switch to anaerobic metabolism. The progressive oxygen debt leads to the accumulation of lactic acid, oxygen radicals and inorganic phosphates, the depletion of cellular energy reserves and the release of damage-associated molecular patterns (DAMPs) or alarmins, which stimulate the complement system, initiate and maintain the systemic inflammatory response syndrome. Isolated tissue damage, not accompanied by shock, is associated with a hypercoagulable phenotype, but together, tissue damage and shock cause maladaptive activation of endothelial cells, platelets and the immune system and induce the hypocoagulable phenotype of TIC (8).

1.2.2. TIC – cause associated with resuscitation

To the previously described endogenous elements are added a series of external factors involved in the evolution of TIC: hypothermia, hypocalcemia, acidosis and hemodilution (8). The prompt correction of these disorders is a prerequisite for the restoration of an effective hemostasis. Their persistence is associated with suboptimal resuscitation, which translates into increased levels of morbidity and mortality.

Hypoperfusion contributes to metabolic acidosis which is subsequently maintained by resuscitation with large volumes of crystalloid solutions, colloids or some preservatives and metabolites from blood products. Acidosis disrupts the normal hemostatic activity of coagulation factors, quantitatively and qualitatively affects platelets, stimulates hyperfibrinolysis, reduces thrombin generation, fibrin polymerization and finally clot firmness (9). Base excess is an independent predictor of mortality in patients with hemorrhagic shock (10).

Hypothermia (T<35°C) is an independent risk factor for bleeding and death in the trauma patient. The effects of hypothermia include platelet dysfunction, reduced function of coagulation factors, and fibrinolysis (6). Currently, in the absence of brain trauma (traumatic brain injury - TBI), active prevention of hypothermia, reaching and maintaining normothermia is recommended (7).

A low value of ionized serum calcium at the admission of a traumatized patient is a predictor of mortality, and the persistence of hypocalcemia at 24h predicts the need for transfusion better than the level of fibrinogen, platelets or pH (9). Calcium has an essential role in coagulation, contributing to platelet adhesion and the activation of vitamin K-dependent coagulation factors (4). Reduced levels of ionized serum calcium are due to the chelating effect of citrate present in blood products.

The injudicious administration of crystalloid solutions, colloids and even blood products can cause the aggravation of the coagulopathy through the dilution effect of the coagulation factors, as well as through the aggravation of the endotheliopathy. Advances in the understanding of vascular physiology have led to the development of the Michel-Weinbaum model or the revised Starling principle, which emphasizes the role of the glycocalyx in fluid balance and explains the phenomenon of autodilution or autotransfusion of approximately 500 ml of interstitial fluid, which may contribute to TIC (4,11,12).

„ The "lethal triad" from trauma brought together coagulopathy, acidosis and hypothermia, to which hypocalcemia was later added (13). As their effect became easier to control, research highlighted a new "lethal triad", which includes coagulopathy, complementopathy and endotheliopathy. Polytraumatized patients who show signs of simultaneous malfunction of these systems have a significantly increased mortality (14).

1.2.3. TIC – patient characteristics

Demographic, socio-economic, cultural or religious factors have a difficult to quantify influence on TIC production, but impossible to ignore in the reality of the clinical management of a traumatized patient.

The evolution of TIC is influenced by the patient's pre-existing health status, especially when there are pre-existing hemostasis defects due to genetic factors (von Willebrand disease, hemophilia A, hemophilia B, hemophilia C or other congenital deficiencies of coagulation

factors) or acquired (pregnancy, renal dysfunction, liver dysfunction, medicinal treatment with anticoagulant, antiaggregant, immunosuppressive substances), as well as extreme age (15).

Physiological excess estrogen in the female population is potentially responsible for the protective procoagulant status for TIC described in some studies, without imposing a gender-specific management of TIC (4).

1.2.4. TIC phenotypes – hypocoagulability and hypercoagulability

The mechanisms that determine the hypocoagulable phenotype are hyperfibrinolysis, platelet dysfunction - qualitative and quantitative, fibrinogen deficiency and thrombin generation deficiency. At the opposite pole, the hypercoagulable phenotype includes hypofibrinolysis or fibrinolysis shutdown, platelet activation, hyperfibrinogenemia and excess thrombin generation. In vivo, the phenomena can be entangled, giving rise to mixed phenotypes (4).

Activated protein C seems to have a dual role, initially in maintaining the hypocoagulable phenotype, and later the hypercoagulable one, associated with thromboembolic and septic complications. Low levels of PAI-1 have been documented as a result of increased ACP levels secondary to trauma-induced hypoperfusion. The practical consequence is the lack of t-PA inhibition, so hyperfibrinolysis. However, it is considered that the deficiency of PAI-1 is one of the responsible, but the main cause of the excess of t-PA is the massive release from the endothelial level, in response to the action of catecholamines, respectively vasopressin, in the context of SHINE (4,16,17).

The transition from the hypocoagulable to the hypercoagulable phenotype is documented in most patients who develop TIC and occurs most frequently within 6-24h from the time of trauma (16).

II. ORIGINAL PART- PERSONAL CONTRIBUTIONS

2. WORK HYPOTHESIS AND THE GENERAL OBJECTIVES

National and international epidemiological analyzes continue to indicate that traumas, and especially those whose extension, lesion severity and multi-organ consequences classify them among polytraumas, represent an important cause of preventable mortality and morbidity among the active population. At the same time, it is known that most of the early deaths recorded among these patients are directly related to hemorrhagic phenomena. These occur either as a result of massive bleeding, the control of which is not achieved, despite the prompt interventions of lay rescuers and then through the specialized ones of the pre-hospital crews, or through the initiation of trauma-induced coagulopathy mechanisms, the diagnosis and treatment of which rest with the hospital team , trauma team, which involves the anesthesiologist-resuscitator, most frequently from the position of case manager. With rare exceptions, survival of an initial major hemorrhagic event is followed by the onset of coagulopathy (2,18,19).

Thus, the main hypotheses of this work, made up of three complementary studies, are the following:

• The first step in reducing in-hospital mortality and morbidity is the identification of coagulopathy induced by trauma with speed and efficiency, in the form of a diagnostic algorithm;

• The different phenotypes of coagulopathy induced by trauma require dedicated therapeutic measures, which can be structured in the form of a therapeutic algorithm;

• The lesional pattern is closely related to the systemic consequences of the trauma, including the coagulopathy phenotype.

The testing of these hypotheses took the form of three studies, whose general objectives are represented by:

• Study I - Thromboelastometric analysis of coagulopathy phenotypes in polytraumatized patients: screening of coagulation disorders present at the time of admission among polytraumatized patients, through thromboelastometric analysis of a whole blood sample;

• Study II - Study of the prognostic value of markers of endothelial activation and platelet contribution in cranio-cerebral trauma: establishing the therapeutic indications for each element of the coagulopathy in the form of a decision tree, which ensures the treatment of

each patient with blood-derived products in the right doses, administered in the right sequence and at the right time;

• Study III - Study of the prognostic value of immune activation markers in the inflammation-coagulopathy continuum from thoracic trauma: identification of the phenotypic peculiarities of coagulopathy in cranio-cerebral trauma and in thoracic trauma.

Starting from the aformentioned objectives, this paper managed to complete the specialized literature with new theoretical elements, subject to external validation, but, above all, to bring the fundamental research closer to the clinical one, by developing, applying and optimizing diagnostic algorithms and treatment applicable in emergency situations, at the patient's bedside.

3. GENERAL METHODOLOGY OF THE RESEARCH

3.2. STUDY POPULATION

Historically, the concept of polytraumatism has undergone a series of changes in terms of definition, so that, historically, it meant the coexistence of at least two traumatic injuries in different anatomical regions, of which at least one or their combination would be threatening of life (20). In the absence of a satisfactory level of evidence and, above all, given the lack of quantifiable objective parameters of the severity of the lesion, the revision of the concept appeared as a necessity. Thus, starting from the analysis of data from the German National Trauma Registry, the most recent definition, the Berlin Definition, took shape. Patients will have been admitted if they have multiple post-traumatic injuries and have been admitted to intensive care units (20).

The particularity of the present analysis is given by the almost unaltered pathophysiological picture of the pathophysiological mechanisms of trauma, as the Bucharest Emergency Clinical Hospital is a reference center in the treatment of acute traumatic pathology. This aspect explains not only the wealth and diversity of the cases, but also the general tendency felt in the local pre-hospital emergency systems or in the regional hospitals that provide complex cases to not intervene therapeutically when it is not vital. By comparison with other medical systems, especially those in which civil emergency management was built on the basis of military experience, the limitation of pre-hospital access to a series of material resources, whose importance cannot be denied, was transformed into an opportunity to understanding of natural physiopathological evolution.

3.3. MATERIAL AND METHODS

The present studies did not need a randomization strategy, as no compound or drug was tested on a specific population. After establishing both the three study hypotheses and the data to be collected, the approval of the Ethics Council of the Bucharest Emergency Clinical Hospital was obtained for the research in accordance with the Declaration of Helsinki.

Thus, the three component studies of this doctoral thesis are prospective and analytical, carried out in the period 2019-2023.

Immediately, after the admission of the patients to the Bucharest Emergency Clinical Hospital, the demographic data (sex, age, weight, body mass index), imaging data obtained during the computer tomography evaluation thus establishing a lesional pattern, specific laboratory analyzes were recorded : hemogram, usual biochemical profile and classic coagulogram. Also, since the patient's admission to the Intensive Care Unit, a whole blood sample was processed with the rotary thrombelastrometry device (ROTEM Sigma). Thus, a dynamic image of the coagulation disorders specific to the polytraumatized patient was obtained, which could not have been objectified by a classic coagulogram.

In the viscoelastic analysis of the blood clot, we focused both on problems frequently cited in the literature such as: hyperfibrinolysis, thrombin generation deficiency, fibrinogen deficiency and thrombocytopathy, but we also tried to objectify detailed aspects: the fibrinolytic patterns and auto-heparinization.

Because we definitely wanted a pathophysiological picture that accurately reflects the polytraumatized patient, the three studies also took into account integral aspects of the other devices and systems, focusing on cardio-circulatory dysfunction, neurological dysfunction, blood transfusion requirements and derivatives.

Polytraumatized patients were included in the first study. A screening of all coagulation disorders constituting coagulopathy induced by multiple trauma and their impact on transfusion requirements and general prognostic elements was performed on them.

The second study included only patients who had sustained moderate-severe TBI, and we were able to describe specific coagulation problems that occurred contextually. In this study we focused on the platelet contribution (PLTEM) to clot formation and used an endothelial inflammation and injury score (EASIX) for the first time. Both parameters were tested for prognostic purposes.

The third study focused on patients who suffered chest trauma brings together two axes of the body's response: inflammation and the consequent coagulopathy. In this case, such a response was actively sought to bring about survival benefits.

Each study has its methodology described in detail throughout the thesis.

3.4. STATISTICAL ANALYSIS

The statistical program used was GraphPad Prism 10.3.

After establishing the main objectives for each individual study, a statistical methodology was determined to be able to calculate the required sample size. This was done so that we managed to avoid a type I error (alpha) by selecting a significance level of the tests of 0.05, with a power of 80% (type II error- beta=0.2).

To estimate the type of data distribution (normal/non-normal) we used the D'Agostino-Pearson test. The subsequent univariate analysis of the data always took into account the observance of the Gaussian distribution. To compare the difference between two means, of some variables with normal distribution, we used the t-test, and for non-parametric data, we compared the medians using the Mann-Whitney test. The presentation of the comparative results of the parametric data was done by highlighting the averages with the corresponding standard deviations. For non-parametric data, the median and 25% and 75% quartiles were presented.

The Ordinary One-Way Anova test was used to compare the difference between three means of normally distributed data, and the results were presented as mean and standard deviation. Whenever necessary, a multiple comparison was performed, in this case using an adjusted significance level value. Kruskal-Wallis analysis was used to compare the difference between three medians. The multiple comparison in this case of the "ranks" was performed using Dunn's test.

Correlation analyzes used r coefficients (Pearson or Spearman) depending on whether normal distribution was observed. An r coefficient value of 0 excludes any unidirectional relationship between two studied parameters. A value tending to 1 shows the direct relationship, and towards -1 an inverse relationship. Whenever it was necessary, in order to make the analysis more efficient, we used correlation matrices in the form of a "heat map".

ROC curves were used to determine the predictive power of a dependent variable with respect to a sought event. Cut-off points were established according to the Youden index, and their presentation was accompanied by the determined sensitivity and specificity.

Analysis of survival curves (Kaplan-Meier) was performed using the Mantel-Cox (log-rank) test. The influence of survival by several parameters acting simultaneously was estimated using the Cox-proportional hazard ratio analysis.

Multiple linear regression was used to estimate the relationship between quantitative dependent variables and two or more independent variables.

The statistical analyzes used for each individual study are detailed in the thesis text.

4. THROMBOELASTOMETRIC ANALYSIS OF COAGULOPATHY PHENOTYPES IN POLYTRAUMATIZED PATIENTS

The ROTEM analysis is intended to answer the question "Why is this patient bleeding?", so that the administration of unnecessary blood products is prevented, and the necessary ones are carried out in the right dose, at the right time and in the right order. A surgical cause of the bleeding must be reevaluated in the face of a bleeding patient with a normal thromboelastogram; in the case of all bleeding patients, hypoperfusion, anemia, hypothermia, hypocalcemia, and acidosis will be corrected as soon as possible, according to the most recent guidelines for the management of massive hemorrhage and coagulopathy secondary to trauma (Figure IV.1.)(7)

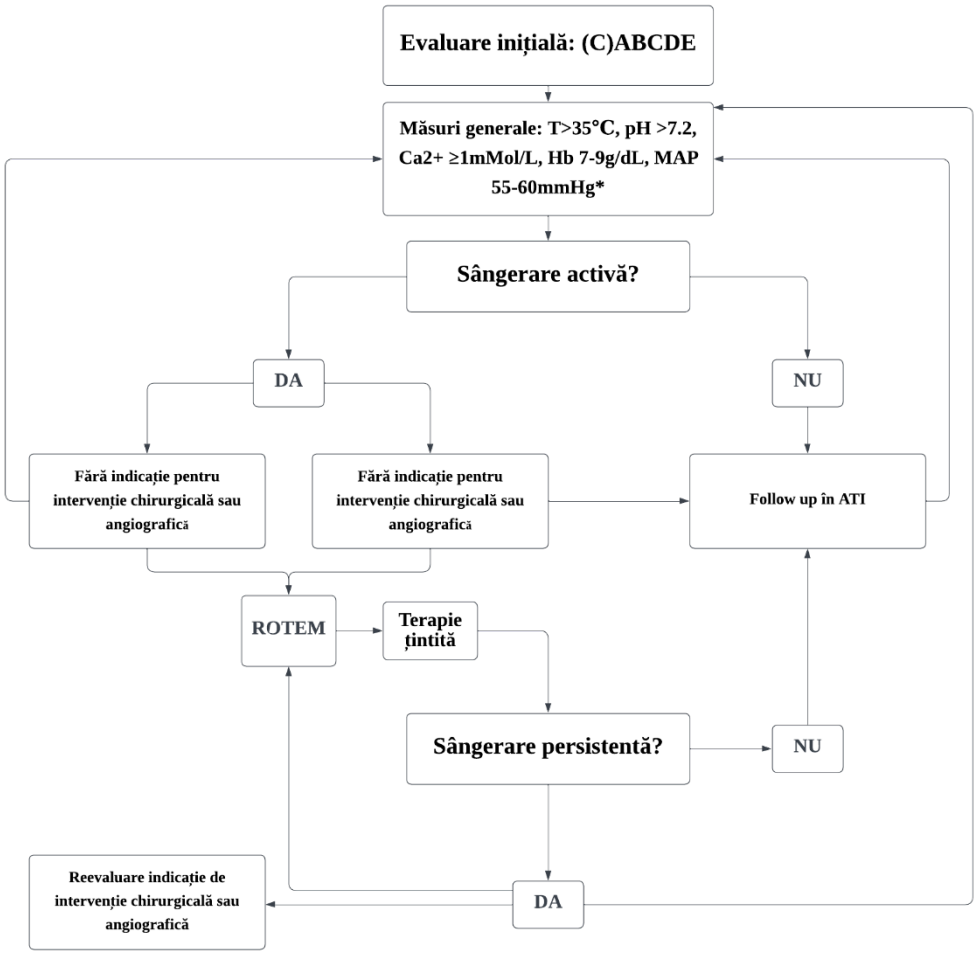

Figure IV.1. Algorithm for the management of the polytraumatized patient with major bleeding. Adapted from Spahn et al. (6)

4.2. MATERIAL AND METHODS

Population and study design

The present study is prospective, observational and includes patients admitted to the Bucharest Clinical Emergency Hospital with the diagnosis of polytraumatism, according to the Berlin criteria in the period 2019 - 2023.

The inclusion criteria used were the enrollment of only adult patients (>18 years), who arrived at the Bucharest Emergency Clinical Hospital within the first six hours after the traumatic event.

The main objective of this study was to identify TIC among polytraumatized patients by highlighting phenotypes, using ROTEM analysis. The secondary objectives included: the comparative analysis of the hemostasis profile highlighted by the classical and ROTEM tests; analysis of TIC phenotypes according to demographic data, production mechanisms and diagnostic or prognostic elements specific to trauma, respectively paraclinical data. The achievement of these objectives involved the analysis of blood samples collected at admission, processed in the shortest possible time, reflecting the same moment in the evolution of the traumatized patient.

4.3. RESULTS

This study included patients (n=217, 129 men and 88 women) with a mean age of 43.43 years and a standard deviation (95% CI) of 15.45 years. Even though the inclusion criteria were based on the Berlin definition, the magnitude of the trauma was estimated using the lesion severity index (ISS). The mean ISS value was 36.98 ± 1.875. The physical mechanisms that led to multiple traumas were mostly road accidents (58.25%, n = 127), followed by falls from a height (19.40%, n = 42), work accidents (16.35 %, n = 35) and sports accidents (6%, n = 13).

The fibrinolysis profile was not limited to the identification of hyperfibrinolysis (ML EXTEM > 15%), but was completed by the objective of physiological fibrinolysis (ML EXTEM 3-15%) and fibrinolysis deficit - fibrinolysis shutdown (ML EXTEM < 3%). We analyzed a series of demographic and paraclinical data in the three groups described according to the fibrinolysis profile (Table IV.2).

Table IV.2. Comparative analysis of demographic, clinical and paraclinical data for the three studied groups

Parameter	**Group A - physiologic fibrinolysis (*n*=90)**	**Group B - fibrinolysis shutdown (*n*=79)**	**Group C- hyperfibrinolysis (*n*=48)**	**P value (two-tailed)**
Age (eyars)	45.33±16.06	42.11±13.19	43.12±18.03	0.42
ISS (pct.)	31.53±10.69	37.97±11.91	38.41±9.15	<0.0001
BMI (kg/m^2)	26.11±4.20	27.11±3.90	27.04±3.40	0.23
Temperature (°C)	35.62±0.74	35.52 ±0.72	35.31±0.91	0.11
pH*	7.32 (7.29-7.39)	7.28 (7.16-7.35)	7.21 (7.11-7.30)	0.03
Serum lactate (mmol/L)*	1.10 (0.60-2.10)	2.75 (1.50-4.40)	4.80 (3.20-6.10)	<0.0001
Base excess (mmol/L)*	-4.0 (-5.9- 2.4)	-11.45 (-14.00- -10.00)	-21.00 (-22.40- -18.90)	<0.0001
Serum bicarbonate (mmol/L)*	22.00 (21.00-24.00)	19.00 (15.25-22.00)	18.00 (14.00-21.00)	<0.0001
Hemoglobin(g/dL)*	10.50 (9.10-12.20)	8.40 (6.80-10.50)	8.30 (6.10-9.20)	<0.0001
Platelets (x10^9/L)*	217.00 (141.00-281.00)	123.50 (92.25-195.30)	107.00 (88.50-127.00)	<0.0001
INR*	1.32 (1.21-1.72)	1.48 (1.32-2.24)	2.30 (1.60-3.80)	<0.0001
aPTT (s)*	35.40 (28.80-41.20)	33.50 (27.70-43.10)	48.90 (38.40-63.75)	<0.0001
Fibrinogen (mg/dL)*	241.00 (223.00-277.00)	193.00 (152.80-251.00)	112.00 (96.00-138.00)	<0.0001
MCF/EXTEM (mm)	59.17±10.19	48.78±15.43	43.83±10.59	<0.0001
MCE/EXTEM*	138.10 (112.80-170.30)	94.20 (65.32-143.90)	81.82 (61.29-104.10)	<0.0001

aPTT - activated partial thromboplastin time, BMI – body mass index, INR - international normalized ratio, ISS – injury severity score, MCF/EXTEM – maximum clot firmness in EXTEM channel, MCE/EXTEM - maximum clot elasticity in EXTEM channel
* Differences between groups were identified with the Kruskal-Wallis test for non-parametrical data

Considered many times to be in fact coagulopathy induced by multiple trauma, the thrombin generation deficit is equally either the result of active bleeding following the traumatic event (called consumption coagulopathy) or the result of an aggressive initial volume resuscitation that makes it to become dilutional coagulopathy.

Diagnosed with ROTEM, the presence of a thrombin generation deficit is detrimental to patient survival. Analysis of survival curves (Kaplan-Meier), log-rank Mante-Cox test highlights both early and late massive hemorrhage deaths. However, even if the difference between the survival curves is statistically significant (Chi Square 3.97, p=0.04), it would be at

least irresponsible to draw a direct causal link between this coagulation phenotype and mortality from any cause. (Figure IV.9.)

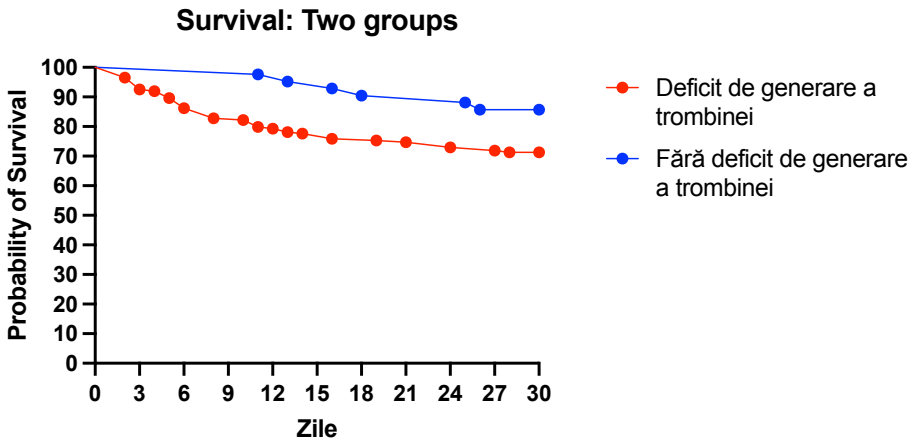

Figure IV.9. Analysis of survival curves of the entire study group divided according to the presence/absence of thrombin generation deficiency

Fibrinogen deficiency at admission the most important predictor of mortality in TIC (4). Low levels of fibrinogen on admission are associated with the degree of lesion severity, the degree of hypoperfusion, increased transfusion requirements, and increased mortality at both 24 hours and 28 days.

Even though they basically mean the same thing, fibrinogen level and maximum clot firmness on the FIBTEM channel do not correlate perfectly in the context of major trauma. MCF/FIBTEM was and is often referred to as "functional or accessible fibrinogen" while the level of fibrinogen dosed in the laboratory is total fibrinogen. Reporting the difference between laboratory objective total fibrinogen and functional fibrinogen (MCF/FIBTEM) to the lesion severity score aims to improve the prognostic capacity in terms of predicting mortality.

To begin with, there is a statistically significant difference between the group of surviving patients and the group of deceased patients (7.125 vs 4.146, $p<0.0001$). (Figure IV.18).

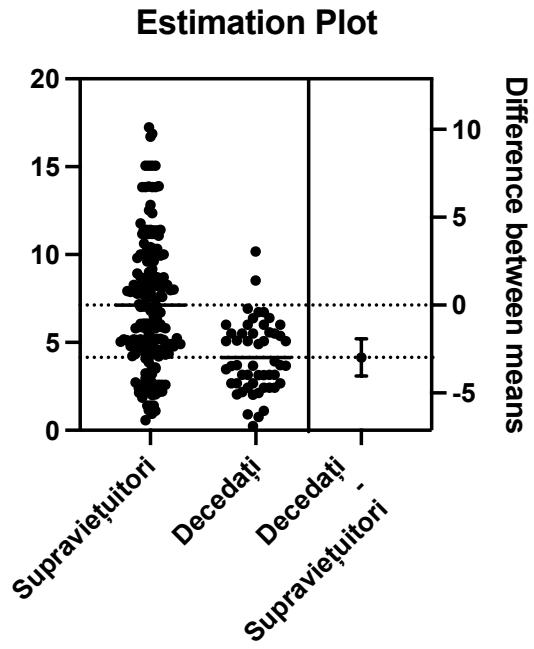

Figure IV.18. Estimate plot of the mean values of the difference between total fibrinogen and MCF/FIBTEM relative to the lesion severity score.

In the ROC analysis of this ratio, the area under the curve is higher than for the values taken individually (AUC=0.84, p<0.0001). The cut-off point chosen according to the Youden index is 5.5. At a lower value, it can predict the probability of death at 30 days with a sensitivity of 80% and a specificity of 78.61%. (Figure IV.19). Practically, but imperfectly, this derived formula emphasizes a patient's ability to access their functional fibrinogen according to the severity of polytrauma. (21,22). From the analysis of variance of the studied parameters, serum lactate (with an estimate of 0.02, p = 0.0098) and noradrenaline requirement to maintain MAP (with an estimate of 0.03, p = 0.0053) were the only variables that influences the CT ratio (INTEM/HEPTEM).

4.4. CONCLUSIONS

The present study was able to objectify both the most important coagulation disorders constituting coagulopathy induced by multiple trauma and their impact on prognosis. Direct links of these point coagulation problems and acid-base parameters have also been demonstrated, easy to interpret, use and cost effective. Beyond initial volume and acid-base

resuscitation, there is a small proportion of patients who have some form of subsequent coagulopathy, interpreted as a high suspicion of autoheparinization (21).

5. STUDY ON THE PROGNOSTIC VALUE OF MARKERS OF ENDOTHELIAL ACTIVATION AND PLATELET CONTRIBUTION IN TRAUMATIC BRAIN INJURY PATIENTS

Traumatic brain injury presents physiopathological peculiarities from which specific diagnostic and therapeutic measures derive, so they benefit from a distinct approach among polytraumatisms. At the same time, the socio-economic impact of TBI is major, both as a result of mortality and significant morbidity - in 2019 there were over 7 million YLDs (years lost to disability) associated with TBI worldwide (23). The analysis of the predictors of mortality in TBI led to the identification of five traditional markers: the Glasgow coma score (GCS), the craniocerebral component of the ISS, the presence of subdural bleeding, age and the photopupillary reflex; relatively recently the predictive value of coagulopathy in TBI has been demonstrated (24–26).

Following an isolated brain injury, a particular systemic response occurs, as a result of the cumulative effects of shock and direct brain tissue injury, which constitutes TBI-induced coagulopathy (TBI-IC – TBI-induced coagulopathy). TBI-CI is characterized by an exaggerated and short-lived cerebral hypercoagulability, which triggers hyperfibrinolysis and thrombocytopathy, and, finally, systemic hypocoagulability. Later, similar to TIC, local and systemic hypercoagulability was described, possibly through fibrinolysis shutdown (27,28). In the context of polytraumas, brain lesions can be secondary to TIC, through the known mechanisms. Also, the two distinct pathophysiological subtypes of coagulopathy can coexist.

The effects of the direct tissue injury are the damage to the microvascularization and the blood-brain barrier (BHE), with the progression of the hemorrhagic injury. BHE damage is translated by the interruption of the continuity of the endothelium, the exposure of TF and subendothelially arranged vWF to platelets and circulating coagulation factors, triggering the known mechanisms of coagulation. The extent of the formation of extrinsic tenase through the TF-VIIa reaction is disproportionate, which generates the conditions for the exaggerated activation of platelets, followed by their exhaustion and the formation of an unstable clot, subject to hyperfibrinolysis (24).

5.2. MATERIAL AND METHODS

Population and study design

In this study, we performed a prospective, observational analysis of a group of 63 patients admitted to the Bucharest Emergency Clinical Hospital in the last four years, with the diagnosis of moderate (GCS 9-12p) or severe (GCS 3-8p) isolated brain trauma, in whom the ROTEM analysis was performed at admission.

The following data were retrieved and analyzed:

• Demographics - gender, age, height, body mass index - BMI;

• Paraclinical, derived from usual analyzes - pH, base excess - BE, serum lactate, hemoglobin- Hb, platelet count- Plt, INR, aPTT, lactate dehydrogenase- LDH, creatinine;

• Paraclinical, derived from ROTEM analysis (EXTEM channel- A5, A10, A20, MCF, MCE), FIBTEM channel (A5, A10, A20, MCF, MCE);

• Survival at 30 days.

To determine the MCE, the formula MCE = (MCF × 100)/(100 – MCF) was applied; to determine PLTEM the formula PLTEM = (EXTEM MCE - FIBTEM MCE) was applied, and to determine EASIX the formula EASIX = lactate dehydrogenase (U/L) × creatinine (mg/dL) / platelets (10^9/L) was applied (29,30).

5.3. RESULTS

The present study includes 63 patients, of which 39 are men and 24 are women. The average age of the entire study group is 42.32 years, with a standard deviation of 16.40 years. The TBI production mechanism was mainly represented by road accidents (n=34, 53.97%), followed by assaults (n=19, 30.16%) and falls from a height, in 10 cases (15, 87%).

Analysis of acid-base balance reveals a statistically significant difference between the two groups in terms of base excess - survivors and non-survivors (-7.03±6.99 vs. -11.90±10.85 p=0.03) . However, there are no differences in serum pH, lactate and bicarbonate. Although no differences were detected between hemoglobin or aPTT values, patients who died (median PLT=108,000/μL) were more thrombocytopenic than those who survived (median PLT=189,000/μL), p<0.0001. Also, INR was more prolonged in deceased patients (median INR=2.4) compared to survivors (median INR=1.76) p=0.02, as shown in Table V.1.

Table V.1. Comparative analysis of paraclinical data between survivors and non-survivors			
Parameter	Survivors (n=44)	Non- survivors (n=19)	p (two tailed)
Age (years)*	40,64±15,04	44,95±15,84	0,30
BMI (kg/m²)*	26,11±4,20	25,12±3,90	0,38
Severe TBI ***	n=34(77,27%)	n=16(84,21%)	0,73
Moderate TBI ***	n=10(22,73%)	n=3(15,79%)	
Epidural hematoma ***	7 (15,91%)	2 (10,53%)	0,71
Subdural hematoma***	10 (22,73%)	8(42,11%)	0,13
Subarachnoid hemorrhage ***	26 (52,63%)	10 (59,09%)	0,78
pH*	7,28±0,10	7,22±0,15	0,07
Base excess (mmol/L)*	-7,03±6,99	-11,90±10,85	0,03
Serum lactate** (mmol/L)	2,10 (1,1-3,4)	2,60(0,6-6)	0,29
Serum bicarbonate (mmol/L)*	19,70±4,11	20,00±5,73	0,81
Hemoglobin (g/dL)**	9,60 (8,7-11,60)	9,30 (8,8-10,70)	0,72
Platelets/μL**	189000 (143000-298000)	108000 (89000-151000)	<0.0001
INR**	1,76 (1,11-2,46)	2,40 (1,22-3,56)	0,02
aPTT (sec)*	36,16±7,40	39,11±7,80	0,15
EXTEM A5 (mm)*	38,2±3,18	28,95±5,89	<0.0001
EXTEM A10 (mm)*	47,59±3,28	38,11±6,02	<0.0001
EXTEM A20 (mm)*	56,14±3,40	45,37±6,68	<0.0001
EXTEM MCF (mm)*	59,86±2,80	50,05±5,99	<0.0001
FIBTEM A5 (mm)*	9,61±1,79	6,78±2,41	<0.0001
FIBTEM A10 (mm)*	11,11±1,94	7,94±2,65	<0.0001
FIBTEM A20 (mm)*	13,52±2,17	10,16±2,63	<0.0001
FIBTEM MCF (mm)*	15,20±2,25	11,53±2,93	<0.0001
LDH (U/L)**	348,50 (303,00-423,5)	484,00 (411.00-624.00)	<0.0001
Serum creatinin (mg/dL)**	0,64 (0,60-0,73)	0,82 (0,74-0,82)	<0.0001
* Unpaired t-test for parametrical data (the means and standard deviations are presented in the table); **Mann-Whitney test for non-parametrical data (the medians and interquartile ranges are presented in the table); BMI – body mass index, INR - international normalized ratio, aPTT - ac-tivated partial thromboplastin time, EXTEM A5, A10, A20 – clot amplitude at 5, 10, 20 minutes in EXTEM channel, EXTEM MCF – maximum clot firmness in EXTEM channel, FIBTEM A5, A1, A20 – clot amplitude at 5, 10, 20 minutes in FIBTEM channel, FIBTEM MCF – maximum clot firmness in FIBTEM channel, LDH - lactate dehydrogenase.***Fisher's exact test			

Considering the very high AUC values and high predictive powers for 30-day mortality for both PLTEM and EASIX, we performed a Hanley and McNeil analysis to compare them. The

difference between the areas under the curve is 0.015 (PLTEM AUC=0.93 and EASIX AUC=0.95), z=0.31, p=0.75 (Figure V.9.).

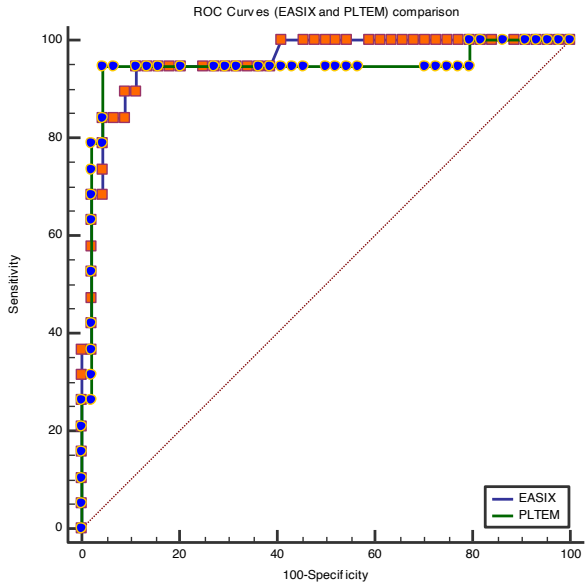

Figure V.9. Comparison of ROC-PLTEM curves vs. EASIX- Classification variable: death at 30 days. Hanley and McNeil analysis

Spearman's analysis revealed an inverse correlation between platelet contribution (PLTEM) and endothelial activation and stress index (EASIX), as evidenced by a correlation coefficient r value of -0.57 (95% CI -0.72 to - 0.37), p<0.0001. Basically, the greater the platelet contribution, the lower the endothelial stress (Figure V.10.).

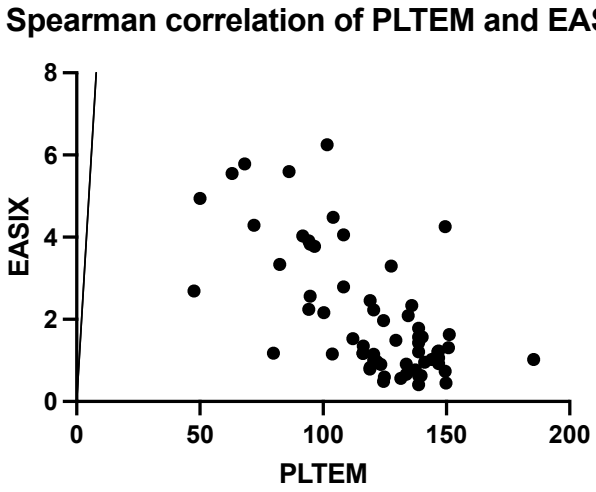

Figure V.10. Spearman correlation of PLTEM and EASIX

5.4. CONCLUSIONS

Endothelial dysfunction and platelet dysfunction represent two major mechanisms of TBI-induced coagulopathy, which can be assessed by determining PLTEM and EASIX. They proved their increased predictive value as prognostic markers of mortality, reflecting TBI- induced coagulopathy in patients with moderate and severe TBI. Further prospective studies are needed to validate their independent prognostic value and to analyze their combined prognostic value. Currently, there are no formal recommendations regarding specific assessment methods for TBI- induced coagulopathy.

6. STUDY ON THE PROGNOSTIC VALUE OF IMMUNE ACTIVATION MARKERS IN THE INFLAMMATION-COAGULOPATHY IN BLUNT CHEST TRAUMA PATIENTS

Patients who suffer preventable and potentially preventable deaths from non-penetrating chest trauma constitute a group whose early identification, followed by optimization of management, must be a goal in itself. High-performance trauma centers report a bimodal distribution of deaths from traumatic injuries, with a decrease in those attributable to late complications, but our center maintains the classic pattern described by Trunkey, with three peaks of incidence. According to the trimodal distribution, deaths caused by traumatic injuries are classified as immediate - caused by severe brain injuries or severe injuries to the heart and large vessels; early - caused mainly by uncontrolled massive hemorrhage; late - due to septic, thromboembolic complications, respectively multiple organic dysfunction (3,31).

This study starts from the hypothesis that patients with thoracic trauma present different phenotypes of coagulopathy and abnormalities of the immune system from the early stages of the pathophysiological process, and markers derived from the hemogram, easily accessible and inexpensive, could promptly reflect this. The study aims to investigate whether there might be a subgroup of patients among whom inflammation-induced coagulation might confer a survival benefit and whether this could form the basis for future personalized therapeutic approaches (32).

emogram-derived ratios such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and neutrophil-to-lymphocyte x platelet ratio (NLPR) – defined as neutrophils/(number of platelets x lymphocytes) have been studied as indicators of dysfunction immune and survival including multiple trauma, TBI, TBI-IC and ARDS (33–37). Studies investigating NLR on admission among nonpenetrating chest trauma patients have evaluated its predictive value for delaying the development of ARDS or pneumothorax, but at present no study has investigated the role of any of these hemogram-derived markers in predicting specific TIC phenotypes in patients with chest trauma (38,39).

6.2. MATERIAL AND METHODS

Population and study design

In this prospective, observational study, all patients admitted to the Bucharest Emergency Clinical Hospital between 2019 and 2023 with the diagnosis of isolated non-penetrating chest trauma were included. The study was carried out in accordance with the Declaration of Helsinki and following approval by the Ethics Committee of the Bucharest Emergency Clinical Hospital for data collection and analysis and publication of the results (protocol code no. 11098/03.12.2019).

We determined the values of immune dysfunction markers derived from the hemogram, as follows:

- NLR= Neu (x 10^9 celule/L)/ Lym (x 10^9 celule/L);
- PLR= Plt (x 10^9 celule/L)/ Lym (x 10^9 celule/L).)
- NLPR = Neu (x 10^9 celule/L)/ (Lym (x 10^9 celule/L) x Plt (x 10^9 celule/L)).

Reference ranges for ROTEM Sigma parameters were defined according to thromboelastometry-guided trauma management algorithms as follows (22):

• hypercoagulability:
  - an increased generation of thrombin: EXTEM CT < 45s or EXTEM MCF > 68 mm;
  - a hyperfibrinogenemia: FIBTEM MCF > 22 mm;
  - fibrinolysis deficit – fibrinolysis shutdown: EXTEM ML<3%;

• hypocoagulability:
  - a hyperfibrinolysis: EXTEM ML ≥ 15% or FIBTEM ML ≥ 10%;
  - fibrinogen deficiency: EXTEM A5 < 35mm or FIBTEM MCF < 12mm or FIBTEM A5 < 9 mm;
  - thrombin generation deficiency - EXTEM CT >80 s.

PLTEM requires measurement of maximum clot elasticity (MCE) and is calculated as follows: PLTEM = EXTEM MCE – FIBTEM MCE. MCE is defined as (MCFx100)/(100-MCF) (30).

The main objective of the study was to evaluate the predictive capacity of mortality in patients with non-penetrating chest trauma using NLR, PLR, NLPR, CRP. Secondary objectives included description of immune response phenotypes and prediction of non-fatal adverse events.

6.3. RESULTS

The values of all acid-base parameters of major importance showed statistically significant differences, except for serum bicarbonate. Classical coagulation tests showed a prolonged INR in deceased patients (p=0.003), the difference not being supported by a corresponding increase in aPTT value (p=0.059). Considering the tendency to hypocoagulability, obtaining a similar mean value of coagulation time on the EXTEM channel (63.25±21.11 vs. 66.27±14.95) is surprising. However, although coagulation initiation appears similar over time, the lower clot amplitude at 5 minutes and maximum clot firmness in deceased patients appears to indicate a clot of poorer mechanical quality (Table VI.1.).

Table VI.1. Comparative analysis of paraclinical data between survivors and non-survivors

Parameter	Survivors (n=62)	Non-survivors (n=24)	p (two tailed)
Age (years)*	41.36±12.86	44.86±9.60	0.23
BMI (kg/m²)*	25.15±4.21	26.11±3.90	0.33
Surgical intervention***	n=16 (25.81%)	n=8 (33.33%)	0.48
CRP (mg/dL)*	2.90 (1.37 to 4.67)	3.75 (1.75 to 10,60)	0.11
NLR*	7.86±3.48	10.78±5.61	0.004
PLR*	0.14 (0.10 to 0.19)	0.19 (0.13 to 0.29)	0.03
NLPR*	4.31±2.51	10.66±6.63	<0.0001
pH*	7.31 (7.25 to 7.40)	7,18 (7.09 to 7.31)	<0.0001
Base excess (mmol/L)**	-6.05(-11.00 to -3.15)	-12.10 (-16.95 to -8.70)	0.0006
Serum lactate (mmol/L) **	2.30 (1.10 to 4.20)	4.1 (2.45 to 5.65)	0.01
Serum bicarbonat (mmol/L)*	20.23±3.83	19.21±5.05	0.31
Hemoglobin (g/dL)**	9.40 (7.10 to 11.10)	9.15 (6.60 to 9.55)	0.02
Platelets /mL**	188.50 (161.00 to 224.00)	103.50 (86.00 to 149.50)	<0.0001
INR**	1.66 (1.29 to 1.82)	1.95 (1.47 to 2.60)	0.003
aPTT (s)*	36.18±7.23	39.46±6.90	0.059
EXTEM CT (s)*	66.27±14.95	63.25±21.11	0.45
EXTEM A5 (mm)**	38.00 (36.00 to 40.00)	30.00 (28.00 to 33.00)	<0.0001
EXTEM MCF (mm)**	61.00 (58.00 to 62.00)	51.00 (46.00 to 54.00)	<0.0001
EXTEM ML (%)**	3.00 (0.00 to 6.00)	8.00 (2.00 to 16.00)	0.02
FIBTEM A5 (mm)**	10.00 (8.00 to 11.00)	7.00 (5.00 to 9.00)	<0.0001
FIBTEM MCF (mm)**	14.00 (12.00 to 16.00)	11.00 (7.00 to 12.00)	0.0003
FIBTEM ML (%)**	0.00 (0.00 to 3.00)	0.00 (0.00 to 7.00)	0.31
PLTEM**	138.80 (123.80 to 147.50)	94.45 (74.56 to 103.80)	<0.0001

*Unpaired t-test - parametrical data; **Mann-Whitney test - non-parametrical data; ***Fischer's exact test; the medians, means, interquartile ranges, and standard deviations are presented in the table; BMI – body mass index, CRP- C-reactive protein, NLR - neutrophil- to- lymphocyte ratio, PLR – platelet- to- lymphocyte ratio, NLPR - neutrophil-to- lymphocyte x platelet ratio, INR - international normalized ratio, aPTT - activated partial thromboplastin time, EXTEM CT – clotting time in EXTEM channel, EXTEM A5 – clot amplitude at 5 minutes / EXTEM, EXTEM MCF – maximum clot firmness / EXTEM, EXTEM ML – maximum lysis / EXTEM, FIBTEM A5 – clot amplitude at 5 minutes / FIBTEM, FIBTEM MCF – maximum clot firmness / FIBTEM, FIBTEM ML – maximum lysis / FIBTEM, PLTEM- qualitative platelet contribution to clot formation.

All three hemogram-derived markers of immune dysfunction have statistically significantly lower mean/median values in the non-survivor group (NLR 7.86±3.48 vs. 10.78±5.61, p=0.004 , PLR 0.14 IQR (0.10 to 0.19) IQ (0.191) vs. to 0.29), p=0.03, NLPR 4.31±2.51 vs. 10.66±6.63, p<0.0001).

Even though analysis of means or medians showed the greatest difference for NLPR, an analysis comparing ROC curves for all markers of immune dysfunction derived from the hemogram, using death 30 days after the traumatic event as the dichotomous variable, is needed. NLPR proves to be the most reliable predictive marker of 30-day mortality, followed by PLR, NLR and CRP. (Table VI.2. and Figure VI.1.). This necessitated further analysis of its potential value in predicting TIC phenotypes and non-fatal adverse events in blunt chest trauma, as well as analysis of its performance as a predictor of mortality in patients with blunt chest trauma.

Table VI.1. ROC curves of NLPR for predicting mortality in chest trauma patients

Parameter	AUC	Standard error	95% CI	p
NLPR	0.71	0.06	0.60 to 0.80	0.002
NLR	0.65	0.07	0.54 to 0.75	0.04
PLR	0.64	0.06	0.53 to 0.74	0.01
CRP	0.61	0.07	0.50 to 0.71	0.13

AUC= area under curve, CI-confidence interval, CRP- C-reactive protein, NLR - neutrophil- to- lymphocyte ratio, PLR – platelet- to- lymphocyte ratio, NLPR - neutrophil-to- lymphocyte x platelet ratio.

We identified a bimodal distribution of NLPR values in deceased patients. As can be seen, there is a group of patients with extremely poor survival but low NLPR (Table VI.1, Figure VI.2).

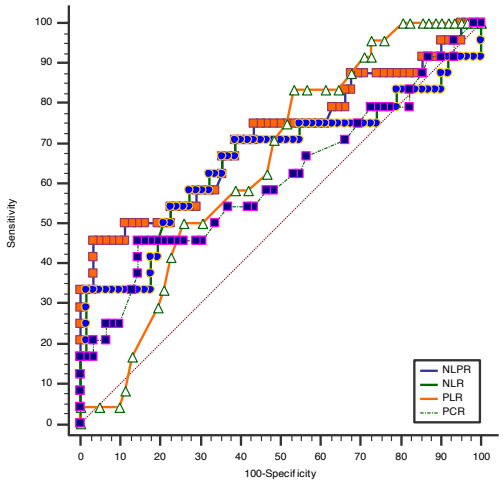

Figure VI.1. ROC curves of NLPR for predicting mortality in patients with thoracic trauma, CRP- C-reactive protein, NLR - neutrophil-lymphocyte ratio, PLR - platelet-lymphocyte ratio, NLPR - neutrophil-lymphocyte x platelet ratio.

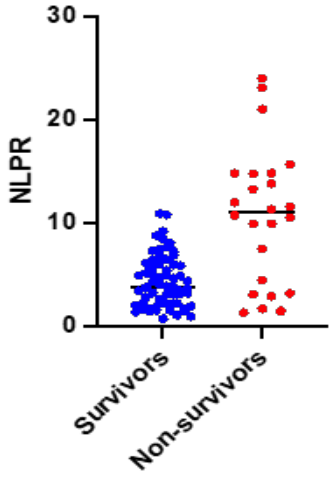

Figure VI.3. NLPR estimation plot (survivors vs non-survivors - t-test).

A comparison of survival curves shows the highest mortality in patients presenting with a hyperinflammatory status. Patients with NLPR between 3.1 and 9.5 have the best survival, followed by patients with NLPR less than 3.1. Patients with non-penetrating chest trauma and with an NLPR score greater than 9.5 had the highest probability of death (Log-rank Mantel-Cox test)-Chi-Squared=30.49, $p<0.0001$ (Figure VI .6.).

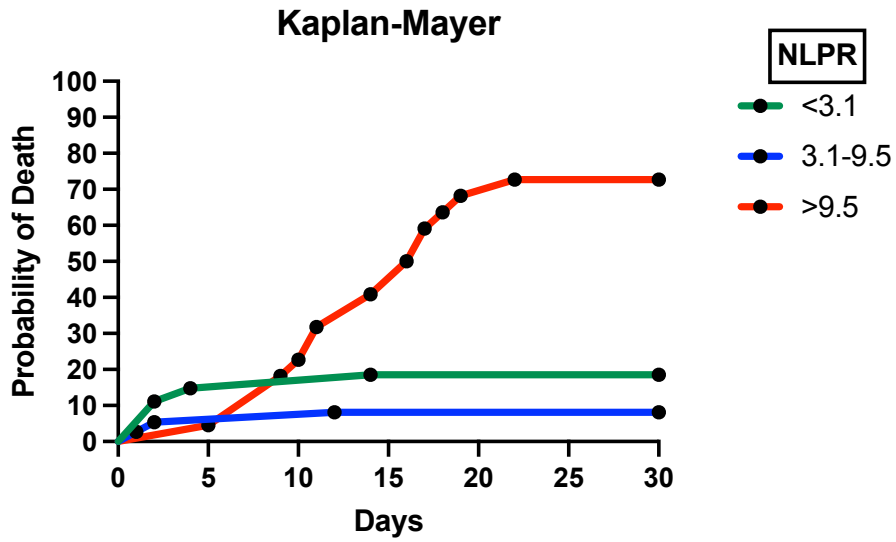

Figure VII.6. Survival curve of different inflammatory status phenotypes based on NLPR.

Patients with a hypoinflammatory status (NLPR<3.1) have the highest probability of death in the first days after sustaining chest trauma. Patients with NLPR between 3.1 and 9.5 closely follow this curve without exceeding it at any point. Patients with hyperinflammatory status (NLPR > 9.5) appear to have a steadily increasing mortality during hospitalization. From day eight onwards, the mortality curve diverges from the other two groups. At day 30, patients with hyperinflammatory status have an almost 80% probability of death. Based on the inflammatory response, we considered patients with an NLPR score < 3.1 as having a low inflammatory response, those with NLPR scores between 3.1 and 9.5 as intermediate inflammatory response, and those with NLPR > 9.5, respectively with an increased inflammatory response. Analysis of the number of patients who survived in each group reinforces the results of the survival curve analysis and reveals a U-shaped mortality distribution (Figure VI.7).

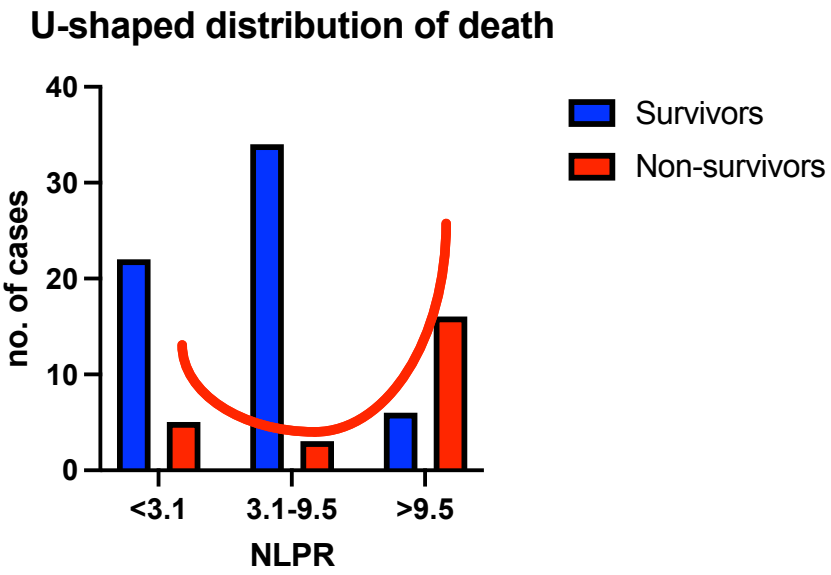

Figure VI.7. U-shaped distribution of 30-day mortality by inflammatory status based on NLPR.

Viscoelastic analysis highlighted all TIC phenotypes in all three groups, ranging from hypocoagulopathy (hyperfibrinolysis, fibrinogen deficiency, thrombin generation deficiency), to hypercoagulability (fibrinolysis shutdown, hyperfibrinogenemia and increased thrombin generation). The low inflammatory response group was mainly characterized by hypocoagulability (hyperfibrinolysis n=10, 37.04% and fibrinogen deficiency n=19, 70.37%). The high inflammatory response group mostly showed hypercoagulable features (fibrinolysis shutdown n=14, 63.64% and increased thrombin generation n=11, 50.00%). However, we could identify patients with high inflammation and hypocoagulability. Patients with an intermediate inflammatory response showed both hyper- and hypocoagulable phenotypes.

However, a comparative analysis of the distribution frequencies shows an intermediate response pattern in terms of coagulation, being less hypocoagulable than the low-inflammation group and less hypercoagulable than the high-inflammation group. Tranexamic acid was the least used in the high inflammatory response group. However, the analysis of the distribution frequency for the three studied lots does not reveal a statistically significant difference (Table VI.4.).

Table VI.4. Comparative analysis of distribution frequencies of the main TIC phenotypes between NLPR objectified inflammation groups.					
	Parameter	NLPR			
		<3.1 (n=27)	3.1-9.5 (n=37)	>9.5 (n=22)	p two tailed
Hypo-coagulability	Prehospital TxA	n=12 (44.44%)	n=17 (47.22%)	n=7 (19.44%)	0.57
	Hyperfibrinolysis *	n=10 (37.04%)	n=4 (10.81%)	n=3 (13.64%)	0.03
	Fibrinogen deficiency *	n=19 (70.37%)	n=9 (24.32%)	n=8 (36.36%)	0.001
	Thrombin generation deficiency*	n=8 (29.63%)	n=6 (16.22%)	n=5 (22.73%)	0.43
Hyper – coagulability	Fibrinolysis shutdown	n=5 (18.52%)	n=11 (29.73%)	n=14 (63.64%)	0.003
	Hyper-fibrinogenemia	n=2 (7.41%)	n=4 (10.81%)	n=5 (22.73%)	0.29
	Increased thrombin generation	n=2 (7.41%)	n=7 (18.92%)	n=11 (50.00%)	0.002
Platelet contribution (PLTEM)**		102.70 (85.25 - 172.90)	132.80 (103.80 - 149.50)	138.80 (118.90 - 146.80)	0.12
*Differences between distribution frequencies were identified with the Fischer's exact test. ** Differences between groups were identified with the Kruskal-Wallis test for non-parametrical data, Hypocoagulopathy: hyperfibrinolysis is defined as ML/EXTEM>15% or ML/FIBTEM>10%; fibrinogen deficit is defined as A5/EXTEM<35 mm or A5/FIBTEM<9 mm or MCF/FIBTEM<12 mm; thrombin generation deficit is defined as CT/EXTEM>80s or CT/INTEM>240s; and hypercoagulability: fibrinolysis shutdown is defined as ML/EXTEM<3%, Hyperfibrinogenemia is defined as MCF/FIBTEM>22 mmm and increased thrombin generation is defined as CT/EXTEM<45s or MCF/EXTEM>68mm; CT – clotting time, A5 – maximum clot amplitude at 5 minutes, MCF – maximum clot firmness, ML – maximum lysis.					

6.4. CONCLUSIONS

Blunt thoracic trauma presents unique pathophysiological features due to the complex interaction between coagulation and immunity, both at the level of healthy lung tissue and secondary to changes resulting from thoracic trauma.

Hemogram-derived NLPR biomarker has increased predictive value for mortality in patients with severe inflammatory response secondary to chest trauma. Both insufficient and exaggerated immune responses are shown to be maladaptive. Patients expressing a moderate

inflammatory response, as measured by an NLPR between 3.1 and 9.5, appear to have a survival benefit.

Early identification of specific subgroups of the inflammatory response could be useful in the further development of specific interventions to reduce the number of preventable deaths caused by chest trauma through immune modulation.

7. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

7.2. Conclusions

- The global evolution of the performance of health systems has led to the improvement of the prognosis of polytraumatized patients, but the persistence of high rates of preventable mortality and morbidity among them indicates the potential to optimize management, both from a diagnostic and therapeutic point of view;
- Trauma-induced coagulopathy represents one of the main causes of preventable mortality and morbidity, which means that it also represents a potential therapeutic target in the future;
- The complete analysis of trauma-induced coagulopathy phenotypes is possible only using viscoelastic tests, but the prediction of some of these phenotypes, with an increased degree of sensitivity and specificity, is possible using markers derived from common, inexpensive tests accessible to the clinician, such as acid balance analysis. Their validation may have implications in the management of trauma-induced coagulopathy in places with limited resources;
- All hypocoagulable and hypercoagulable phenotypes of trauma-induced coagulopathy were identified in the studied population; In the early post-traumatic period, death is associated with hypocoagulant phenotypes (hyperfibrinolysis, platelet dysfunction - qualitative and quantitative, fibrinogen deficiency and thrombin generation deficiency) and hemorrhagic manifestations, and in the late stage with hypercoagulant phenotypes (hypofibrinolysis or fibrinolysis shutdown, platelet activation, hyperfibrinogenemia and excess thrombin generation) and thromboembolic and immune manifestations;
- Early fibrinolysis deficit - fibrinolysis shutdown and the phenomenon of autoheparinization represent relatively new physiopathological aspects discussed in the context of trauma-induced coagulopathy. They present diagnostic, therapeutic and prognostic interest;
- The lesional pattern is closely related to the systemic consequences of trauma and the phenotype of coagulopathy induced by trauma, as we were able to demonstrate for cerebral trauma and thoracic trauma;

- Isolated traumatic brain injuries associate coagulopathy phenomena even when classical causal factors for hemorrhagic shock or trauma-induced coagulopathy do not coexist, differentiating it from other coagulopathy models through the intrinsic characteristics of brain tissue. Endothelial dysfunction and platelet dysfunction represent two major mechanisms of coagulopathy associated with brain trauma; these can be estimated using PLTEM and EASIX. This study demonstrated the value of PLTEM and EASIX as prognostic markers of mortality in moderate and severe traumatic brain injuries;
- Patients with blunt thoracic trauma show different phenotypes of coagulopathy and abnormalities of the immune system from the early stages of the pathophysiological process. This study demonstrated the increased predictive value for mortality of the hemogram-derived biomarker NLPR in patients with severe inflammatory response secondary to blunt chest trauma. Patients who express a moderate inflammatory response appear to have a survival benefit;
- Trauma-induced coagulopathy can be treated using decision trees that ensure the treatment of each patient with blood products in the right doses, administered in the right sequence and at the right time.

7.3. Personal contributions

The use of viscoelastic tests for the management of bleeding in the polytraumatized patient was seen as a major opportunity to improve the quality of the medical act in the Bucharest Clinical Emergency Hospital. Initially, we applied previously validated algorithms in other populations of polytrauma patients. Later, with the careful follow-up of a sufficiently large number of patients, with the acquisition of an increased level of experience and with the in-depth understanding not only of the utility, but also of the limits of the technique, I managed to mathematically formulate the questions that repeatedly appeared in clinical practice and to follow through on their solution. The result of this approach took the form of a guideline, which we named the Algorithm of the Bucharest Clinical Emergency Hospital for the management of bleeding in trauma and is available in two forms (Table VII.1. and Figure VII.1.).

The use of ROTEM algorithms to guide TIC treatment may be limited by the increase in hospitalization costs. Sometimes there is a discordance between the patient's clinical picture and the established cut-off values, which suggests the correct identification of the coagulation

disorder, but not its impact. This discrepancy can lead to multiple repetitions of the analysis until a balance is achieved. In this sense, we demonstrated that it is not enough to identify the specific disorders of clot formation, but it is also necessary to assess its magnitude by identifying cut-off values for "major deficits", respectively "minor deficits", to be translated by adjusting doses of blood products and increasing the success rate of establishing hemostasis through a single ROTEM analysis.

Table VII.1. Algorithm of the Bucharest Clinical Emergency Hospital for the management of bleeding in trauma

<table>
<tr><td rowspan="4">FIBRINOGEN DEFICIENCY</td><td colspan="2">MAJOR CRITERION</td><td colspan="2">MINOR CRITERION</td></tr>
<tr><td>MCF/FIBTEM < 7 mm</td><td>FBG 3-4g</td><td rowspan="2">A10/EXTEM < 45 mm and A10/FIBTEM < 10 mm</td><td rowspan="2">FBG 1g</td></tr>
<tr><td>MCF/FIBTEM 7 – 12 mm</td><td>FBG 2g</td></tr>
<tr><td>MCF/ EXTEM < 55mm</td><td>FBG 2g</td><td>A5/EXTEM < 35 mm and A10/FIBTEM < 9 mm</td><td>FBG 1g</td></tr>
<tr><td rowspan="4">HYPERFIBRINOLYSIS</td><td>A10/EXTEM < 45 mm</td><td colspan="3" rowspan="4">TxA 1g/100mL/15min</td></tr>
<tr><td>A5/EXTEM < 35 mm</td></tr>
<tr><td>CT/FIBTEM > 60 s</td></tr>
<tr><td>ML/EXTEM > 15%</td></tr>
<tr><td rowspan="3">THROMBIN GENERATION DEFICIENCY</td><td colspan="2" rowspan="2">MAJOR CRITERION</td><td colspan="2">MINOR CRITERION</td></tr>
<tr><td>CT/INTEM > 240s and A10/EXTEM > 45 mm and A10/FIBTEM >10 mm</td><td rowspan="3">PCC 10 UI/kgc or PPC 20mL/kgc</td></tr>
<tr><td>CT/EXTEM > 80 s</td><td>PCC 30 UI/kgc</td><td>A5/EXTEM>35mm and A5/FIBTEM>9mm
MCF/EXTEM>55mm and MCF/FIBTEM>12mm</td></tr>
<tr><td>THROMBOCITOPENIA</td><td colspan="2">A10 EXTEM <45mm and A10/FIBTEM >10mm
A5 EXTEM <35mm and A5/FIBTEM >9mm
MCF/EXTEM <55mm and MCF/FIBTEM>12mm
A10/EXTEM – A10/FIBTEM < 30 mm
MCF/EXTEM – MCF/FIBTEM <35mm</td><td colspan="2">PLT 1U/10kgc</td></tr>
</table>

CT – clotting time, MCF – maximum clot firmness, A5, 10 – clot amplitude at 5, respectively 10 minutes; ML – maximum lysis; FBg fibrinogen concentrate; PCC- prothrombin complex concentrate; PLT- platelet concentrate; TxA- tranexamic acid

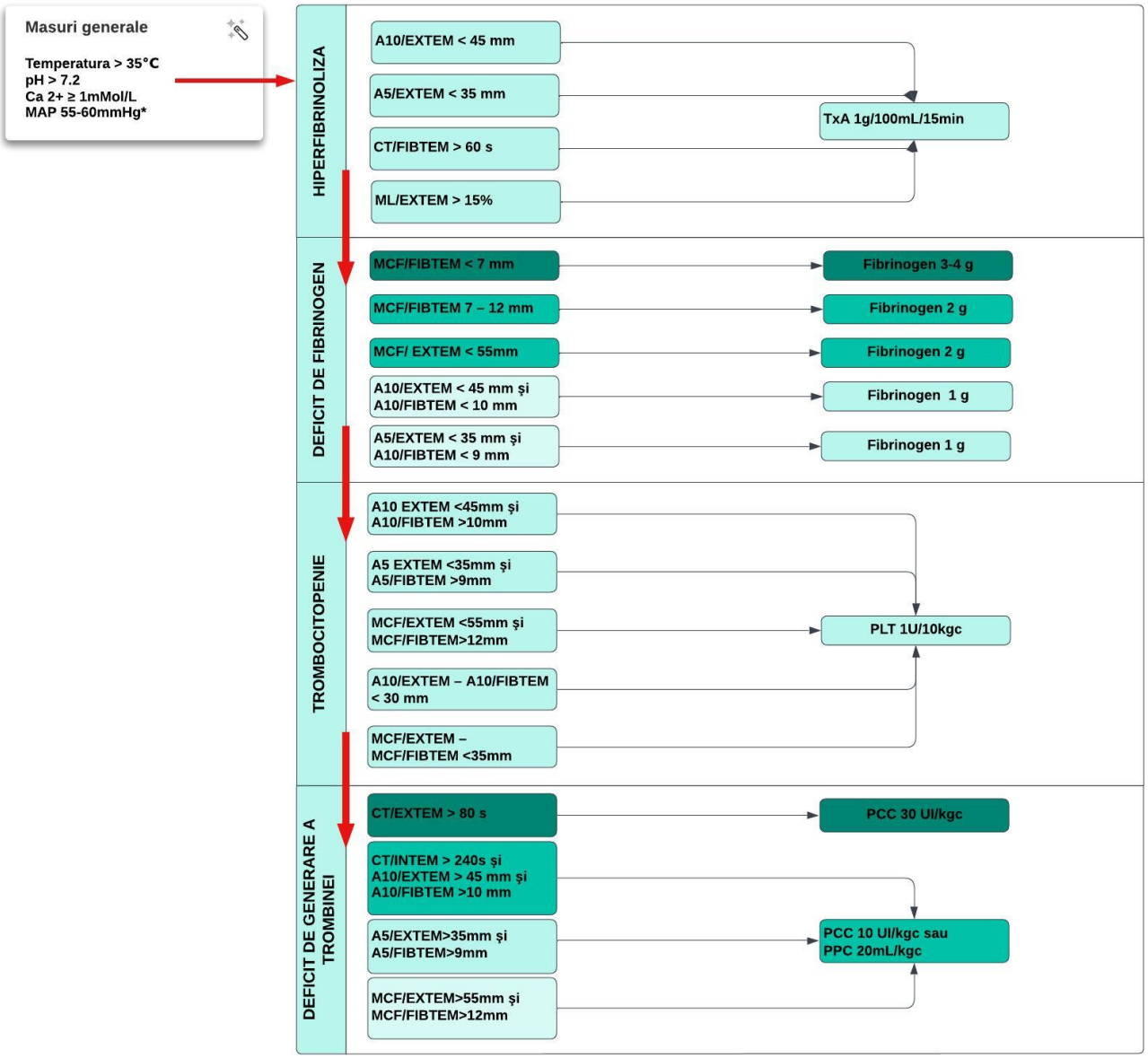

Figure VII.1. Algorithm of the Bucharest Clinical Emergency Hospital for the management of bleeding in trauma

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