Article

Not peer-reviewed version

It's written in the Clot: Rotational Thromboelastometry in Severe Burn Injury – A Holistic Coagulation Assessment

Eirini Nikolaidou *, Andriana Lazaridou, Christina Iasonidou, Alexandra Tsaroucha, Sophia Papadopoulou, Eleni Kaldoudi, Apostolos Sovatzidis, Despoina Kakagia

Posted Date: 26 July 2023

doi: 10.20944/preprints202307.1771.v1

Keywords: Severe Burn Injury; coagulation; Rotational Thromboelastometry; Viscoelastic Coagulation Assays; Conventional Coagulation Assays; Coagulation Factors

Article

It's Written in the Clot: Rotational Thromboelastometry in Severe Burn Injury—A Holistic Coagulation Assessment

Eirini Nikolaidou [1,*], Andriana Lazaridou [2], Christina Iasonidou [3], Alexandra Tsaroucha [4], Sophia Papadopoulou [5], Eleni Kaldoudi [6], Apostolos Sovatzidis [7] and Despoina Kakagia [7]

[1] Department of Plastic, Reconstructive and Hand Surgery & Burns ICU, General Hospital of Thessaloniki "G. Papanikolaou", 57010, Thessaloniki, Greece; eirininikolaidou&gmail.com
[2] Hematology Department-Hematopoietic Cell Transplantation Unit, Gene and Cell Therapy Center, "George Papanikolaou" Hospital, 57010, Thessaloniki, Greece; andrianalazaridou@gmail.com
[3] 2nd Department of Intensive Care Medicine, George Papanikolaou General Hospital, G. Papanikolaou Avenue, 57010, Thessaloniki, Greece; ciasonidou@gmail.com
[4] Second Department of Surgery and Laboratory of Experimental Surgery, Democritus University of Thrace,Alexandroupolis, Greece; atsarouc@med.duth.gr
[5] Department of Plastic, Reconstructive and Hand Surgery & Burns ICU, General Hospital of Thessaloniki "G. Papanikolaou", 57010, Thessaloniki, Greece; sophipap@hotmail.com
[6] Medical Physics and Medical Informatics, School of Medicine, Democritus University of Thrace, Greece; kaldoudi@med.duth.gr
[7] Department of Surgery, General Hospital of Giannitsa, 58100 Pella, Greece; t.sovatzidis@gmail.com
[8] Department of Plastic and Reconstructive Surgery, Democritus University Hospital, Alexandroupolis, Greece; despoinakakagia@yahoo.com
* Correspondence: eirininikolaidou7@gmail.com; 00302313307180

Abstract: The alterations of coagulation status in patients with Severe Burn Injury are associated with serious complications, increased morbidity and mortality. This study aims to compare Rotational Thromboelastometry(ROTEM)–a Viscoelastic Coagulation Assays(VCAs) with Conventional Coagulation Assays(CCAs) including Prothrombin time(PT), Activated Partial Thromboplastin time(aPTT), International normalized ratio(INR), Complete Blood Count(CBC) and Coagulation Factors during the early five post- burn days in Survivors and Non- Survivors with Severe Burn Injury in order to correlate these results with Burn Coagulopathy and Prognosis. Seventeen Survivors and ten Non- Survivors, with a mean Total Burn Surface Area(TBSA) of 33,78% were included in our study. Even though CCAs measurements were abnormal, they were unable to detect overall burn patients' coagulopathy. On the contrary, VCAs from day 2 to day 5 took pathological values, especially for Non- survivors. Those changes were underlined through pathological values of Coagulation Factors. As a result, CCAs were considered poor indicators of coagulation status in burn injury, whereas VCAs were more accurate in demonstrating coagulation alterations from the early post- burn period and detecting patients in greater risk of mortality. These advantages of VCAs could be used for timely intervention in high risk patients and for the guidance of blood product transfusions.

Keywords: severe burn injury; coagulation; rotational thromboelastometry; viscoelastic coagulation assays; conventional coagulation assays; coagulation factors

1. Introduction

Coagulation disorders are frequently seen in burn patients with Total Body Surface Area (TBSA) > 20%, defined as Severe Burn Injury [1]. These disorders appear immediately after the injury and are divided into two entities: traumatic coagulopathy due to systemic inflammatory response, hypothermia, platelet dysfunction and tissue injury, and iatrogenic coagulopathy due to resuscitation, hemodilution and blood loss after surgical excision [2]. The alterations of coagulation status in severe burn patients are associated with serious complications such as thromboembolic episodes [3], multiple organ failure [4], increased morbidity and mortality [5,6].

 doi:10.20944/preprints202307.1771.v1

Understanding the importance of early changes in coagulation status of burn patients, it is imperative to monitor coagulation and fibrinolytic system. Conventional coagulation assays (CCAs) such as Prothrombin time (PT), activated partial thromboplastin time (aPTT) and International normalized ratio (INR) are indirect indicators of extrinsic and intrinsic clotting pathway, but they do not take into account platelets and fibrinogen, which undergo significant changes during the early post-burn period, affecting the equilibrium of clot [7–10].

Furthermore, the dilution and consumption of coagulation factors is another aggravating factor in clotting disorders [11]. A series of processes start as body's physiologic response vascular endothelial injury due to trauma, which are summarized in 3 pathway that are part of the coagulation cascade: the intrinsic, the extrinsic, and the common pathway. The first responds to vascular endothelium damage whereas the second to external trauma. After their initial activation, they share a common cascade called the common pathway. Coagulation Factors XII, XI, IX, and VIII are responsible for the intrinsic pathway and Coagulation Factors VII, III are involved in the extrinsic pathway. The common pathway Coagulation Factors are X, V, II, I, and XIII [11]. Several studies have shown a decrease in coagulation factors for trauma patients but data about burn patients is sparse [1,12].

Viscoelastic coagulation assays (VCAs), such as Rotational Thromboelastometry (ROTEM), came to cover this weakness of CCAs by analyzing all stages of hemostasis in whole blood samples [10]. Throughout a graphical chart, ROTEM provides information about clot formation, clot progress and its lysis, qualitative platelet function and fibrinolysis [11]. In critically ill patients VCAs are considered the best perioperative monitoring tests, since they can detect both transfusion needs and bleeding disorders [11]. Furthermore, they are successfully used as predictors of mortality in patients with severe trauma [12]. However, despite the fact that these assays are well studied and applied in trauma and critical ill patients, little is known about their application in severe burn patients.

The aim of this study is to compare CCAs, Coagulation Factors and VCAs for Survivors and Non- survivors the early post- burn period of five days and to interpret those results as part of Burn Coagulopathy.

2. Materials and Methods

2.1. Study design and participants

A prospective observational study was conducted at the Burn Intensive Care Unit (BICU) of G. Papanikolaou General Hospital, Thessaloniki, Greece from February 2020 to February 2023. The inclusion criteria were adult patients with any type of burn injury with TBSA >20% admitted at the BICU within the first 24 hours. Patients were excluded if they had other concomitant trauma injuries or coagulation dysfunction, including liver damage, anticoagulant drugs, antiplatelet agents or long-term alcoholism. Those patients, who didn't survived up to the fifth post-burn day, were also excluded. Inhalation injury was defined broncoscopically as inflammatory reaction in the bronchial mucosa.

2.2. Blood sampling

Through radial or femoral arterial catheters with a continuous flush system, arterial blood samples were collected in vacutainer tubes, from day 1 to day 5. For the first five post burn days, patients had routine blood and coagulation analyses (PT, INR and aPTT, d-dimers, fibrinogen) and Viscoelastic coagulation analysis using Rotational Thromboelastometry (ROTEM; Pentapharm, Munich, Germany). For days 1, 3, 5 coagulation factors: Von Willebrand, V, VII, IX, X, XI, XII were analyzed.

2.3. CCAs and cell counting

Analysis of blood samples included the Complete Blood Cell count (CBC) (Red blood cell counts (RBCs), White Blood Cell counts (WBCs), platelet counts (PLTs)) and Conventional coagulation assays (CCAs) (PT, PTT, INR, D-dimers, fibrinogen) over the first five post-burn days.

 doi:10.20944/preprints202307.1771.v1

2.4. Coagulation Factors

Soluble clotting factors were analyzed for day 1, 3 and 5. These comprise the factors Von Willebrand, FV, FVII, FIX, FX, FXI, FXII. Factor activity was assessed and given as a percentage of standard activity by comparison of the samples with standard human plasma assays of clotting factors (SHP, Dade Behring Marburg GmbH, Marburg, Germany).

2.5. ROTEM Parameters

For ROTEM analysis, whole blood samples from day 1 to day 5 were collected. Every sample was placed into a cuvette. Through a cylindrical pin that is immersed and rotated into the blood, clot is formed. ROTEM evaluates the clot firmness via the restriction that appears at the moving pin, in an inverse proportional way by measuring kinetic changes of the clot elasticity.[14] A graphical chart is then extracted with different values: Clotting Time value (CT, in seconds) depicts the time from start to initial clot formation, depending almost on clotting factors; The Clot Formation Time (CFT, in seconds) is the time to achieve a certain level of clot strength, relating to fibrin polymerization and clot stabilization and finally propagation phase though alpha angle (α, in degrees) assesses the rate of clot formation.[13] Clot amplitude at 10 / 20 min (A10, A20) express the clot firmness at respective time points and refer to the fibrinolysis at 10 and 20 min CT. Maximum clot firmness (MCF, in millimeters) is defined as the highest amplitude reached before clot dissolving and estimates the firmness and the quality of the clot and Maximum Lysis (ML, described in percent of MCF) depicts the maximum lysis.

Modifications of ROTEM that were used: FIBTEM, evaluation of fibrinogen contribution to blood clot with tissue factor and cytochalasin D which blocks contribution of platelets to maximum clot firmness leaving the impact of fibrin formation and polymerization; EXTEM, evaluation of extrinsic coagulation pathways with tissue factor; INTEM, evaluation of intrinsic system of coagulation pathway.

2.6. Statistical analysis

Descriptive statistics were calculated using mean and standard deviation for continuous variables, frequencies and percentages for categorical variables. Data normality was tested using the Shapiro-Wilk test. Student's t-test was used for comparisons of means of continuous variables. Levene's Test assessed equality of variances. For non- parametric values, Mann-Whitney U test was used. For all tests, p value < 0.05 was considered to be statistically significant. Statistical analysis was performed using the IBM® SPSS® Statistics Version 29.

3. Results

3.1. Demographical Data

During the study period, 27 adults patients were enrolled, 15 males and 12 females. The mean cohort age was 58,04 (SD: 16,9). A total of 10 deaths occurred (37,04%). Based on the survival, two groups were formed (Survivors, Non-survivors). 10 males and 15 females survived, with a mean age of 51,88 (SD: 15,87). The mean age for non-survivors was 68,5 (12,99) and sex was equally distributed. All patients were admitted within the first 24 hours and received resuscitation according to Parkland Formula (4 x TBSA% x kg).

3.2. Type, TBSA and depth of burn injury

All burn injuries were thermal, apart from two chemicals and one electrical burn injury. Total Burn Surface Area (TBSA) ranged from 20% to 71%. Total mean TBSA was 33,78% (SD: 14,56), 32,65% (SD: 10,7) for survivors and 35,7% (SD: 19,28) for non-survivors. Regarding depth of burn injury, for survivors, 17,53% (SD: 11,19) of TBSA was second degree and 15% (SD: 12,26) was third degree, whereas for non-survivors 12,% (SD: 9,5) was second degree and 23,2% (SD: 18,38) was third degree.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2023 doi:10.20944/preprints202307.1771.v1

The incidence of inhalation injury in our Cohort was 25,9%. From the seven patients with inhalation injury, 71,43% died. The mortality rate for patients with inhalation injury was 18,5%. (Table 1)

Table 1. Demographics and burn characteristics. (TBSA = Total Burn Surface Area,*= result with statistical significance) .

	Total	Survivors	Non- survivors
	27	17	10
Sex	Males: 15	Males: 10	Males: 5
	Females: 12	Females: 15	Females: 5
Mean Age (years)	58,04 (SD: 16,9)	51,88 (SD: 15,87)	68,5 (12,99)
	Thermal: 24	Thermal: 14	Thermal: 10
Burn type	Chemical: 2	Chemical: 2	Chemical: 0
	Electric: 1	Electric: 1	Electric: 0
Mean Total TBSA (%)	33,78 (SD: 14,56)	32,65 (SD: 10,7)	35,7 (SD: 19,28)
2nd degree TBSA (%)	15,67(SD: 10,87)	17,53 (SD: 11,19)	12,5 (SD: 9,5)
3rd degree TBSA (%)	18,04 (SD: 15,34)	15 (SD: 12,26)	23,2 (SD: 18,38)
Inhalation injury	7	2 (28,57%)	5 (71,43%)

3.3. Complete Blood Count results (CBCs)

From the five post–burn days CBCs results, we compared White Blood cells counts (WBC), hematocrit (HT), hemoglobin (HB) and Platelets (PLT) for Survivors and Non- survivors. For the total of patients, mean WBCs dropped each day from admission to a nadir at day 5. Comparing the two groups, WBCs were higher for Non-survivors during the 5 post- burn period, statistically significant for day 3 (Non- survivors; WBC_{DAY3} 13827 SD: 5630,89; Survivors; WBC_{DAY3} 9528,75 SD: 4369,88; p=0,04).

Mean HB on admission was 15,27 mg/ dl (SD: 2,40) for Non- survivors and 15,09 mg/ dl (SD: 2,19) for Survivors, and decreased every day. At the fifth post–burn day, mean HB was 9,25 mg/ dl (SD: 2,28 mg/ dl) for Non- survivors and 9,74 mg/dl (SD: 1,68 mg/ dl) for Survivors. As expected, HT followed a similar pattern as HB. For days one to three, Non- survivors had higher values of HT and HB than survivors, statistically significant for day 2 (Non- survivors; HT_{DAY2} 43,16 SD: 8,63; Survivors; HT_{DAY2} 37,29 SD:5,16; p=0,02 and Non- survivors; HB_{DAY2} 14,15 SD: 2,71; Survivors; HT_{DAY2} 12,31 SD:1,74; p=0,03).

PLTs had a downward trend from the first post-burn day to the fifth post-burn day, with greatest drop in values for Non–survivors (Non- survivors; PLT_{DAY1} 384,10 SD: 308,37; PLT_{DAY5} 138,70 SD: 88,87, Survivors; PLT_{DAY1} 236,88 SD: 80,58; PLT_{DAY5} 184,44 SD: 71,79, p=0,02, paired samples t-test analysis). (Table 2)

Table 2. Results of Complete Blood Cell counts for Survivors and Non-survivors for the first five post-burn days. (WBC= White Blood Cells counts, HT= hematocrit, HB= hemoglobin, PLT= Platelets, *= result with statistical significance).

	Survivors	Non-survivors	P-value
HT_{DAY1}	45,04 (6,15)	46,14 (6,7)	0,30
HT_{DAY2}	37,29 (5,16)	43,16 (8,63)	0,02*
HT_{DAY3}	33,52 (4,79)	35,14 (7,16)	0,32
HT_{DAY4}	30,20 (7,33)	32,76 (5,81)	0,18
HT_{DAY5}	30,11 (5,25)	28,61 (6,80)	0,27
HB_{DAY1}	15,09 (2,19)	15,27 (2,40)	0,4
HB_{DAY2}	12,31 (1,74)	14,15 (2,71)	0,03*
HB_{DAY3}	10,97 (1,57)	11,62 (2,29)	0,26
HB_{DAY4}	11,77 (5,86)	10,77 (1,85)	0,3
HB_{DAY5}	9,74 (1,68)	9,25 (2,28)	0,2
WBC_{DAY1}	18749,38 (8982,75)	22467 (6042,72)	0,21

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2023 doi:10.20944/preprints202307.1771.v1

WBC DAY2	11747,13 (5562,52)	16486,33 (8455,71)	0,08
WBC DAY3	9528,75 (4369,88)	13827 (5630,89)	0,04*
WBC DAY4	8651,2500 (3648,58)	13407 (8381,74)	0,12
WBC DAY5	8505 (3441,78)	10211 (6184,88)	0,44
PLT DAY1	236,88 (80,58)	384,10 (308,37)	0,6
PLT DAY2	188,81 (67,56)	293,30 (256,16)	0,22
PLT DAY3	161,25 (51,23)	207,90 (186,54)	0,44
PLT DAY4	161,68 (52,36)	162,00 (109,89)	0,99
PLT DAY5	184,44 (71,79)	138,70 (88,87)	0,16

3.4. Conventional Coagulation Assays (CCAs)

Regarding INR, even though baseline measurements of day 1 were the same between the two groups, for Non- survivors became pathological from day 2, without been normalized. This difference between the two groups was statistically significant for days 4 and 5 (Non- survivors; INR_{DAY4} 1,27 SD: 0,24; Survivors; INR_{DAY4} 1,05 SD: 0,11; p= 0,01 and Non- survivors; INR_{DAY5} 1,23 SD: 0,28; Survivors; INR_{DAY5} 1,05 SD: 0,09; p= 0,04). The same pattern, as INR, emerged for PT analysis (Non-survivors; PT_{DAY4} 15,17 SD: 2,46; Survivors; PT_{DAY4} 12,48 SD: 1,25; p= 0,04 and Non- survivors; PT_{DAY5} 14,70 SD: 3,28; Survivors; PT_{DAY5} 12,44 SD: 1,17; p= 0,001) whereas for PTT values no differences were observed for the two groups. Finally, both d-dimers and fibrinogen levels were augmented for Non-survivors the whole time period, statistically different for day 2 (FIBRINOGEN $_{DAY2}$; Non- survivors: 4,2 SD: 1,03; Survivors: 2,88 SD: 0,94 p=0,03). (Table 3)

Table 3. Results of Conventional Coagulation Assays for Survivors and Non-survivors for the first five post- burn days. (PT= Prothrombin Time, aPTT= activated Partial Thromboplastin Time, INR= International normalized ratio, *= result with statistical significance).

	Survivors	Non-survivors	P-value
INRDAY1	1,12 (0,16)	1,11 (0,28)	0,41
INRDAY2	1,16 (0,16	1,22 (0,33)	0,16
INRDAY3	1,10 (0,14)	1,32 (0,42)	0,10
INRDAY4	1,05 (0,11)	1,27 (0,24)	0,01*
INRDAY5	1,05 (0,09)	1,23 (0,28)	0,04*
PT DAY1	13,25 (1,83)	13,27 (2,98)	0,46
PT DAY2	13,76 (1,95)	14,51 (3,64)	0,91
PT DAY3	13,10 (1,64)	15,69 (4,54)	0,07
PT DAY4	12,48 (1,25)	15,17 (2,46)	0,04*
PT DAY5	12,44 (1,17)	14,70 (3,28)	0,001*
PTT DAY1	27,69 (4,77)	27,06 (4,38)	0,75
PTT DAY2	29,38 (5,65)	33,03 (4,51)	0,19
PTT DAY3	33,48 (4,33)	36,07 (7,44)	0,42
PTT DAY4	32,19 (4,24)	33,49 (6,27)	0,91
PTT DAY5	32,06 (4,02)	36,99 (9,66)	0,28
DDIMERS DAY1	1,15 (0,89)	1,85 (2,16)	0,35
DDIMERS DAY2	1,11 (0,99)	1,80 (2,48)	0,42
DDIMERS DAY3	0,93 (0,89)	0,98 (0,63)	0,88
DDIMERS DAY4	0,46 (0,17)	0,97 (0,68)	0,82
DDIMERS DAY5	1,14 (1,02)	1,5 (1,17)	0,42
FIBRINOGEN DAY1	3,06 (0,98)	3,08 (0,88)	0,96
FIBRINOGEN DAY2	2,88 (0,94)	4,2 (1,03)	0,03*
FIBRINOGEN DAY3	3,74 (0,88)	4,93 (0,94)	0,07
FIBRINOGEN DAY4	4,74 (1,87)	5,78 (1,68)	0,33
FIBRINOGEN DAY5	4,65 (1,26)	5,37 (1,69)	0,26

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2023 doi:10.20944/preprints202307.1771.v1

3.5. Coagulation Factors

VonWillebrand Factor was elevated in both groups, being higher in Non-survivors than in Survivors on the early post-burn period. No critical differences were noticed for Factor IX. In contrast, for all other Coagulation Factors (V, VII, X, XI, XII) Non- survivors' levels were lower than Survivors, beyond normal ranges, with statistically significant results for day 5 (Non- survivors; $Factor V_{DAY5}$ 29,97 SD: 17,10; Survivors; $Factor V_{DAY5}$ 99,22 SD: 33,04; p= <0,001; Non- survivors; $Factor VII_{DAY5}$ 50,78 SD: 30,69; Survivors; $Factor VII_{DAY5}$ 107,24 SD: 30; p= 0,05; Non- survivors; $Factor X_{DAY5}$ 45,88 SD: 17,13; Survivors; $Factor X_{DAY5}$ 112,32 SD: 87,27; p= 0,05; Non- survivors; $Factor XI_{DAY5}$ 50,71 SD: 34,33; Survivors; $Factor XI_{DAY5}$ 91,02 SD: 19,50; p= 0,01; Non- survivors; $Factor XII_{DAY5}$ 27,94 SD: 9,27; Survivors; $Factor XII_{DAY5}$ 49,19 SD: 31,96; p= 0,05). (Table 4)

Table 4. Coagulation Factors measurements for Survivors and Non-survivors during the first five post- burn days. (*= result with statistical significance).

	Survivors	Non-survivors	P-value
VonWillebrand$_{DAY1}$	258,45 (121,91)	295,50 (109,24)	0,46
VonWillebrand$_{DAY2}$	243,31 (63,1)	333,94 (174,8)	0,2
VonWillebrand$_{DAY3}$	266,54 (132,7)	323,73 (102,01)	0,35
FactorV $_{DAY1}$	60,12 (25,78)	41,45 (15,89)	0,55
FactorV $_{DAY3}$	80,14 (36,28)	49,12 (20,23)	0,42
FactorV $_{DAY5}$	99,22 (33,04)	29,97 (17,10)	<0,001*
FactorVII $_{DAY1}$	68,91 (27,13)	56,75 (33,71)	0,34
FactorVII $_{DAY3}$	72,89 (32,92)	61,17 (25,16)	0,4
FactorVII $_{DAY5}$	107,24 (30)	50,78 (30,69)	0,05*
FactorIX $_{DAY1}$	97,91 (42,67)	80,36 (50,14)	0,36
FactorIX $_{DAY3}$	106,2 (33,48)	112,46 (55,61)	0,79
FactorIX $_{DAY5}$	145,95 (40,85)	119,4 (70,44)	0,33
FactorX $_{DAY1}$	67,90 (21,80)	55,48 (28,06)	0,24
FactorX $_{DAY3}$	73,84 (42,18)	54,35 (28,16)	0,27
FactorX $_{DAY5}$	112,32 (87,27)	45,88 (17,13)	0,05*
FactorXI $_{DAY1}$	78,62 (27,93)	62,94 (31,17)	0,22
FactorXI $_{DAY3}$	67,89 (21,13)	53,06 (28,11)	0,22
FactorXI $_{DAY5}$	91,02 (19,50)	50,71 (34,33)	0,01*
FactorXII $_{DAY1}$	53,77 (33,10)	43,87 (21,54)	0,42
FactorXII $_{DAY3}$	36,37 (24,74)	34,82 (16,24)	0,88
FactorXII $_{DAY5}$	49,19 (31,96)	27,94 (9,27)	0,05*

3.6. ROTEM Parameters

FIBTEM MCF at day 2 was increased for Non-survivors and statistically different between the two groups (p=0,04). This parameter remained augmented for Non- survivors for the whole post-burn period. Furthermore, for the same day, FIBTEM A10 and A20 was prolonged for the same group (p=0,01 and p=0,01). Regarding day 5, EXTEM A10, EXTEM A20, EXTEM MCF and EXTEM CFT were pathologic for Non- survivors (p=0,02, p=0,02, p=0,02, p=0,05 respectively). As long as clot amplitude is concerned at 10 and 20 minutes, both in FIBTEM and EXTEM, it increases at the 5 days period for Non- survivors.

4. Discussion

4.1. Demographical Data, Type, TBSA and depth of burn injury

The results of this prospective observational study regarding Demographical Data, the type, TBSA and depth of burn injury of 27 severe burn patients reinforced the existing knowledge that age and deep burn area are predictive factors of mortality, since Non- survivors had higher mean age

 doi:10.20944/preprints202307.1771.v1

(Non- survivors: 68,5 SD: 12,99; Survivors: 58,04 SD: 16,9) and 3rd degree TBSA (Non- survivors: 23,2 SD: 18,38; Survivors: 15 SD: 12,26) compared to Survivors [15]. Furthermore, the incidence of inhalation injury in our study was 25,9% and mortality rate for patients with inhalation injury was 18,5%. Our results are comparable with those reported in other studies, where incidence rates range between 10-20% [16–19] and mortality rate is 25,6% [20]. Through a systematic review of prognostic factors in adults with burn injury, Colohan reported a mortality rate of 27,6% [21] and concluded that the best predictors of mortality among the current published literature on burn prognosis are increased TBSA, increased age and the presence of inhalation injury [21].

4.2. Complete Blood Count results (CBCs)

Similar trends and several differences were noticed among Survivors and Non- survivors regarding CBCs results. The initial systemic inflammation, known as part of the pathophysiology of burn injury, leads to increased WBCs on admission for both Survivors and Non- survivors. Indeed, WBCs levels of the first day are the highest among the early post–burn period [22]. Non- survivors' response was greater than Survivors, suggesting that more severe burn patients trigger greater immune response to burn induced inflammation, leading to higher levels of WBCs [23]. Over the next few days, WBCs in both groups are decreasing. Two are the main pathological mechanisms for this deflation: the migration of the leukocytes from plasma to the burn areas and the suppression of the bone marrow following severe burn injury [24,25]. Comparing the two groups, on day 3, Non- survivors had statistically significant higher levels of WBCs (Non- survivors: 13827 SD: 5630; Survivors: 9528,75 SD: 4369,88, p=0,04), coming in accordance with results from several studies that correlated mortality with reduced WBCs [26,27].

Both HB and HT of the two groups were augmented on admission and showed decreasing values the first five post–burn days. Between groups, there was a statistically significant difference for day 2 for both HT, HB. (HT; Non- survivors: 43,16, SD: 8,63; Survivors: 37,29 SD: 5,16, p=0.02; HB; Non- survivors: 14,15, SD: 2,71; Survivors: 12,31 SD: 1,74, p=0.03). Soman S. et al. examined trends in the components of the CBC in severely burned patients over the first week after injury and concluded that survivors had significantly lower measurements of HGB, HCT every day from admission to day 7 [27]. The depression of bone marrow production, due to inflammation, in combination with hemolysis of red blood cells explains the decline [28,29]. Furthermore, the hypovolemic status on admission followed by the aggressive fluid resuscitation leads to dilution [26].

Non- survivors PLTs on admission were higher than Survivors (PLT; Non- survivors: 384,10, SD: 308,37; Survivors: 236,88 SD: 80,58). For the first group, they declined and reached their lowest values at day 5, whereas Survivors' nadir was on day 4 and then started recover. Our findings agree with Osuka a. et al. results, with the only difference that the lowest point of the PLTs for Survivors was reached at 3 days [26]. PLT consumption due to burn injury is mainly responsible for this decline [30]. Undeniably, thrombocytopenia in the early post- burn period is associated with increased mortality [26,27,31].

4.3. Conventional Coagulation Assays (CCAs)

The major drawback in the investigation of coagulopathy in burn injury is the absence of a clear definition and defined thresholds. In our study, even though INR started from the same baseline for both Survivors and Non- survivors, it increased for Non- survivors and remained higher compaired to Survivors for the whole 5 days period, but not within coagulopathic range. The same pattern was noticed for PT, whereas PTT was within normal ranges for both groups the first five post–burn days. Beyond studies, the thresholds for INR differ [2]. One study borrowed the term "acute traumatic coagulopathy" from trauma patients in order to describe coagulation disorders in major burn injury with thresholds: INR greater than 1.3 and aPTT ratio >1.5 times mean normal [32]. No patient met these criteria concluded that major injury is not associated with acute traumatic coagulopathy [32]. On the other hand, Mitra et al. [33] modified the thresholds of INR>1.5 and aPTT>60s according to recent studies of acute traumatic coagulopathies [34]. The same rigorous thresholds were used by Kaita et al. [6] Sherren et al., [35,36] Kang et al. [37] and Muthukumar et al.'s [38] study used the same

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2023 doi:10.20944/preprints202307.1771.v1

cut-off points: INR>1.2, PT>14.6s, aPTT>45s for acute burn induced coagulopathy (ABIC). These cut-off values were consistent with the International Definition of Acute Traumatic Coagulopathy (ATC) by Davenport et al. [39] The incidence was 39.3% and 31% respectively. For Tejiram et al. [40] abnormal coagulation was defined as: INR>1.5 and PTT>45s, with 0% incidence on admission, 22% after. In our study, even though there was a statistically significant difference of INR between the two groups for days 4 and 5 (INR_{DAY4}; Survivors: 1,05 SD: 0,11; Non- survivors 1,27 SD: 0,24 p=0,01; INR_{DAY5}; Survivors: 1,05 SD: 0,09; Non- survivors 1,23 SD: 0,28 p=0,04), values were lower than any of the above definitions of coagulopathy except Muthukumar et al.'s study [38]. When the ABIC criteria were applied, both for INR and PT using the cut- off points of 1,2 and 14,6s respectively, for days 3,4 and 5, Non- Survivors were coagulopathic (PT_{DAY3}; Survivors: 13,10 SD: 1,64; Non- survivors 15,69 SD: 4,54; PT_{DAY4}; Survivors: 12,48 SD: 1,25; Non- survivors 15,17 SD: 2,46 p=0,04; PT_{DAY5}; Survivors: 12,44 SD: 1,17; Non- survivors 14,7 SD: 3,28 p=0,001;). It is obvious that different thresholds in the diagnostic criteria are responsible for the inconsistency of the results.

As long as d-dimers and fibrinogen levels are concerned, they reflect fibrinolytic activity. Both d-dimers and fibrinogen concentrations were increased for both groups, but remained elevated for Non- survivors. Our results agree with several studies where hypercoagulable status and increased clot formation was found through augmentation of these two parameters [10,40,41]. Martini et al. making isotope infusion of 1-^{13}C-phenylalanine and d_5-phenylalanine in severe burn patients to quantify fibrinogen production and consumption, proved that both fibrinogen synthesis and breakdown were increased after burn, but the magnitude of synthesis was larger than that of fibrinogen breakdown, resulting in the increase of fibrinogen availability after burn injury [42].

Fibrinogen levels were significantly increased when compared to the control group throughout the observation period, statistically significant for day 2 (Non- survivors: 4,2 SD: 1,03; Survivors: 2,88 SD: 0,94; p=0,03). This has been described previously and corresponds to increased fibrinogen synthesis and hyper coagulable status [40].

4.4. Coagulation Factors

Primary hemostasis initiates through platelet adhesion via Von Willebrand Factor (vWF) and proceeds the intrinsic coagulation cascade, as it stabilizes VIII coagulation factor [43]. This glycoprotein is also released from the injured endothelium and increases in inflammatory situations [44,45]. In our study, vWF levels were increased in both groups from day 1, with an upward trend for days 3 and 5. Considering that burn injury involves major endothelial damage and activation of the cytokine cascade, enhancing the inflammation response, this augmentation is rational. Furthermore, Non- survivors had higher values of vWF for the whole post–burn period. This fact can be associated with greater TBSA%, deeper burn injury and worse prognosis. vWF has been associated with inflammatory situation, such as diagnosis of Acute Respiratory Distress Syndrome, in which increased levels were associated with bad outcomes- long mechanical ventilation time and mortality, [46] but not with burn injury [46]. Our study is the first depicting the great impact of severe burn injury in vWF levels.

Coagulation Factor V levels were lower in admission for Non- survivors, compared to Survivors whose values were within normal range, and remained decreased for the 5 days post–burn period, statistically significant for day 5 (Non- survivors: 29,97 SD: 17,10; Survivors: 99,22 SD: 33,04; p<0,001). The present study identifies an association between mortality and low levels of Factor V. This association is already known for trauma patients, whose increasing traumatic injury severity is associated with decreased FV antigen levels [47]. Regarding burn patients and Factor V, our knowledge is limited. Tejiram et al. found normal range FV activity in all burn patients on admission, but there was no follow-up for the next post- burn days [40]. Keyloun et al. evaluated Factor V dynamics following burn and nonburn trauma, found that burn severity was not associated with Factor's levels, but no correlation for mortality was studied. Since data on Factor V in burn patients are limited, indirect conclusions can be drawn from studies of Protein C in this population. The endogenous anticoagulant activated Protein C (aPC) is a serine protease that inactivates several factors in the coagulation cascade, including factor V. Activated protein C increases from day 3

 doi:10.20944/preprints202307.1771.v1

following a severe burn injury [48]. Based on these findings, we would expect a decrease in Factor V levels which is consistent with our results.

Factor VII was within normal values for both groups but at the lower normal for Non- survivors. Our results are consistent with Tejiram et al. who found that admission Factor VII remained within normal range and severely burned patients demonstrated decreased mean activity compared with the low burn size group VII (96.2 ± 27.4% versus 70.5 ± 11.9%) [40].

Factors IX, X, XI for the study period were within normal values for both groups, but Non-survivors levels were lower compared to Survivors. Again, these Factors have not been studied in relation to burn injury. A recent narrative review evaluating coagulation alterations in Major Burn Patients summarized burn Coagulation Factors over time and concluded that Factor IX is usually within normal range of high during the first 4 post–burn days, with no data for Factors X, XI [49].

Finally, Factor XII was deficiency was noticed in both groups, higher for Non- Survivors. Once more, little are known for this Factor in burn patients. For decades, Factor XII was considered to have no function for coagulation in vivo, explaining the lack of study interest [50]. Nowadays, we do know that even though it does not contribute to "physiologic" hemostatic fibrin formation at sites of vessel injury, Factor XII has a crucial role in fibrin formation during "pathologic" thrombosis. Low levels of this Coagulation Factor are associated with thrombosis [51].

4.5. ROTEM Parameters

In our study, FIBTEM MCF was increased for Non- survivors, indicating an increased fibrinogen concentration. Indeed, for day 2, both FIBTEM MCF and fibrinogen levels were statistically significant different between the two groups. FIBTEM isolates fibrinogen function, by using a platelet inhibitor -cytochalasin D, blocking the platelet contribution to clot formation. A FIBTEM MCF in excess of 25mm suggests that there is an excess of fibrinogen and a procoagulable state [51]. These results are constant with Schaden et al. results, in which mean FIBTEM MCF was within the reference range until 24h after burn trauma but increased significantly 48h after [51]. At the same study, the same pattern was observed for fibrinogen levels, concluded that FIBTEM MCF could be used as a point- of- care assay sensitive for fibrinogen function, in turn patients at risk of hypercoagulability [51]. The limitations of this study is that patients are investigated only for the first 48hours after burn injury.

Clot amplitude at 10 and 20 minutes is a measure of clot strength, with higher values depicting a strengthen clot, a hypercoagulable status. In our case, according to results, Non- survivors were more coagulopathic compared to Survivors at the 5 days post- burn. Wiegele et al. performing a 7 day study on burn patients, concluded that there is a 20% increase in clot strength, assessed through ROTEM [10].

In conclusion, fibrinogen levels augmented from day 1 to day 5 and by the end of the 5 day post-burn period, a hypercoagulable status is shown for Non- survivors. A recent systematic review on ROTEM values for the diagnosis of coagulopathy, prediction and guidance of blood transfusion and prediction of mortality in trauma patients, after evaluation of 13 observational studies involving 2835 adult trauma, concluded that abnormal FIBTEM clot amplitude and MCF are capable of diagnosing acute trauma coagulopathy predict the need for massive transfusion, and predict mortality [52]. Furthermore, in a randomized control study evaluating ROTEM versus clinical judgment in burn patients undergoing excisional surgery, the use of ROTEM was associated with decreased blood product transfusions. Unfortunately, there are no studies evaluating blood product transfusions according to ROTEM values during the early post-burn period.

5. Conclusions

Several changes occur within the first 5 post- burn days. Our study is the first assessing those changes throughout CCAs, VCAs, Coagulation Factors and CBCs. Even though changes in CCAs exist, they are unable to detect burn patients' coagulopathy. On the contrary, VCAs from day 2 to day 5 take pathological values, especially for Non- survivors. Those changes are underlined through pathological values of Coagulation Factors and fibrinogen products. As a result, CCAs are considered

poor indicators of coagulation status in burn injury, whereas VCAs are more sensitive tests in demonstrating coagulation alterations from the early post- burn period and detecting patients who are in greater risk of mortality. These advantages of VCAs could be used for timely intervention in high risk patients and for the guidance of blood product transfusions. Further interventional randomized studies are required, demonstrating the superiority of either approach in the treatment of severe burn injury patients.

Author Contributions: Conceptualization, E.N. and D.K.; methodology, D.K..; software, E.N. and A.S.; validation, S.P. and C.I..; formal analysis, A.L.; investigation, A.T.; resources, E.K.; data curation, A.T.; writing—original draft preparation, E.N., A.S. and D.K.; writing—review and editing, E.N. and D.K.; visualization, S.P.; supervision, D.K.; project administration, A.L., C.I., A.T., E.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of G. Papanikolaou General Hospital of Thessaloniki, Greece (8/08.01.2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: We are indebted to the invaluable collaboration of the nursing staff of Papanikolaou Burning Unit.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lavrentieva A, Kontakiotis T, Bitzani M, Papaioannou-Gaki G, Parlapani A, Thomareis O, Tsotsolis N, Giala MA. Early coagulation disorders after severe burn injury: impact on mortality. Intensive Care Med 2008; 34: 700–6.
2. Nikolaidou E, Kakagia D, Kaldoudi E, Stouras J, Sovatzidis A, Tsaroucha A. Coagulation Disorders And Mortality In Burn Injury: A Systematic Review. Ann Burns Fire Disasters. 2022 Jun 30;35(2):103-115.
3. Lippi G, Ippolito L, Cervellin G. Disseminated intravascular coagulation in burn injury. Semin Thromb Hemost 2010; 36: 429– 36
4. Garcia-Avello A, Lorente JA, Cesar-Perez J, Garcia-Frade LJ, Alvarado R, Arevalo JM, Navarro JL, Esteban A. Degree of hypercoagulability and hyperfibrinolysis is related to organ failure and prognosis after burn trauma. Thromb Res 1998; 89: 59–64.
5. Tie-ning Zhang, Te Ba, Fang Li, Qiang Chen, Zhi-peng Chen, Biao Zhou, Zeng-qiang Yan, Quan Li, Sheng-jun Cao, Ling-feng Wang. Coagulation dysfunction of severe burn patients: A potential cause of death. Burns. 2022
6. Kaita Y, Nishimura H, Tanaka Y, Suzuki J et al.: Effect of acute coagulopathy before fluid administration in mortality for burned patients. Burns, 47(4): 805-811, 2020.
7. Larsson A, Tynngård N, Kander T, Bonnevier J, Schött U. Comparison of point-of-care hemostatic assays, routine coagulation tests, and outcome scores in critically ill patients. J Crit Care. 2015
8. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, et al. The coagulopathy of trauma: a review of mechanisms. J Trauma. 2008;65(4):748–754.
9. Rizoli SB, Scarpelini S, Callum J, Nascimento B, Mann KG, Pinto R, et al. Clotting factor deficiency in early trauma-associated coagulopathy. J Trauma. 2011;71(5 Suppl 1):S427–S434.
10. Wiegele M, Kozek-Langenecker S, Schaden E: Point-of-care testing in burn patients. Semin Thromb Hemost, 43(4): 433-438, 2017.
11. Barmore W, Bajwa T, Burns B. Biochemistry, Clotting Factors. 2022 May 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–.
12. Bolliger D, Seeberger MD, Tanaka KA: Principles and practice of thromboelastography in clinical coagulation management and transfusion practice. Transfus Med Rev, 26: 1-13, 2012.
13. Alaina R. Smith, Saleema A. Karim, Rebecca R. Reif, William C. Beck, John R. Taylor, Benjamin L. Davis, Avi V. Bhavaraju, Hanna K. Jensen, Mary K. Kimbrough, Kevin W. Sexton. ROTEM as a Predictor of Mortality in Patients with Severe Trauma. Journal of Surgical Research. Vol. 251. 2020.
14. H. Schochl, W. Voelckel, A. Grassetto, C.J. Schlimp. Practical application of point-of-care coagulation testing to guide treatment decisions in trauma. J Trauma Acute Care Surg, 74 (2013), pp. 1587-1598
15. Griffe O, Gartner R, Captier G, Brabet M, Baro B, Selloumi D, Otman S. Evaluation des facteurs pronostiques chez le patient brûlé [Evaluation of prognostic factors in the burned patient]. Ann Chir Plast Esthet. 2001 Jun;46(3):167-72.

16. Dries DJ, Endorf FW. Inhalation injury: epidemiology, pathology, treatment strategies. Scandinavian journal of trauma, resuscitation and emergency medicine. 2013;21:31. Epub 2013/04/20. pmid:23597126
17. Palmieri TL. Inhalation injury: research progress and needs. J Burn Care Res. 2007;28(4):549–54. Epub 2007/05/16. pmid:17502839.
18. Walker PF, Buehner MF, Wood LA, Boyer NL, Driscoll IR, Lundy JB, et al. Diagnosis and management of inhalation injury: an updated review. Crit Care. 2015;19:351. Epub 2015/10/29. pmid:26507130
19. Endorf FW, Gamelli RL. Inhalation injury, pulmonary perturbations, and fluid resuscitation. J Burn Care Res. 2007;28(1):80–3. Epub 2007/01/11. pmid:17211205.
20. Kim Y, Kym D, Hur J, Yoon J, Yim H, Cho YS, Chun W. Does inhalation injury predict mortality in burns patients or require redefinition? PLoS One. 2017 Sep 27;12(9):e0185195.
21. Colohan SM. Predicting prognosis in thermal burns with associated inhalational injury: a systematic review of prognostic factors in adult burn victims. J Burn Care Res. 2010;31(4):529–39.
22. P.D. Thomson, N.P. Moore, T.L. Rice, J.K. Prasad. Leukopenia in acute thermal injury: evidence against topical silver sulfadiazine as the causative agent. J Burn Care Rehabil. 1989:10(5):418-420
23. Laggner, M., Lingitz, MT., Copic, D. et al. Severity of thermal burn injury is associated with systemic neutrophil activation. *Sci Rep* **12**, 1654 (2022).
24. D. Hirth, S.A. McClain, A.J. Singer, R.A. Clark. Endothelial necrosis at 1 hour postburn predicts progression of tissue injury. Wound Repair Regen, 21 (2013), pp. 563-570
25. D.H. Livingston, D. Anjaria, J. Wu, C.J. Hauser, V. Chang, E.A. Deitch, et al. Bone marrow failure following severe injury in humans. Ann Surg, 238 (2003), pp. 748-753
26. Osuka A, Ishihara T, Shimizu K, Shintani A, Ogura H, Ueyama M. Natural kinetics of blood cells following major burn: Impact of early decreases in white blood cells and platelets as prognostic markers of mortality. Burns. 2019 Dec;45(8):1901-1907.
27. Soman Sen, Luke Hsei, Nam Tran, Kathleen Romanowski, Tina Palmieri, David Greenhalgh, Kiho Cho. Early clinical complete blood count changes in severe burn injuries, Burns, Volume 45, Issue 1, 2019, Pages 97-102
28. C. Lawrence, B. Atac. Hematologic changes in massive burn injury. Crit Care Med, 20 (9) (1992), pp. 1284-1288
29. J.A. Posluszny Jr., R.L. Gamelli. Anemia of thermal injury: combined acute blood loss anemia and anemia of critical illness. J Burn Care Res, 31 (2) (2010), pp. 229-242
30. Levin GY, Egorihina MN. The role of fibrinogen in aggregation of platelets in burn injury. *Burns* 36 (6):806–810, 2010.
31. Lin JC, Wu GH, Zheng JJ, Chen ZH, Chen XD. Prognostic Values of Platelet Distribution Width and Platelet Distribution Width-to-Platelet Ratio in Severe Burns. Shock. 2022 Apr 1;57(4):494-500.
32. Lu RP, Ni A, Lin FC, Ortiz-Pujols SM. Major injury is not associated with acute traumatic coagulopathy. J Trauma Acute Care Surg. 2013;74(6):1474–1479.
33. Mitra B, Wasiak J, Cameron P, O'Reilly G. Early coagulopathy of major burns. Injury. 2013;44:40–43
34. Muthukumar V, Karki D, Jatin B. Concept of lethal triad in critical care of severe burn injury. Indian J Crit Care Med. 2019;23(5):206–209
35. Sherren PB, Hussey J, Martin R, Kundishora T. Acute burn induced coagulopathy. Burns. 2013;39:1157–1161.
36. Sherren PB, Hussey J, Martin R, Kundishora T. Lethal triad in severe burns. Burns. 2014;40(8):1492–1496.
37. Kang G, Yonglin L, Yuting Y, Xiaobin D. Incidence and prognostic value of acute coagulopathy after extensive severe burns. J Burn Care Res. 2020;41(3):544–549.
38. Muthukumar V, Karki D, Jatin B. Concept of lethal triad in critical care of severe burn injury. Indian J Crit Care Med. 2019;23(5):206–209.
39. Davenport R, Manson J, De'Arth H, Platton S. Functional definition and characterization of acute traumatic coagulopathy. Crit Care Med. 2011;39(12):2652–2658.
40. Tejiram S, Brummel-Ziedins KE, Orfeo T, Mete M. Indepth analysis of clotting dynamics in burn patients. J Surg Res. 2016;202(2):341–351.
41. Zhang P, Huang K, SHI Z. Changes of fibrinogen and D-dimer in patients with severe burns and their relationship with prognosis. Chinese Journal of Primary Medicine and Pharmacy. 2018; (12): 988-990
42. Wenjun Z Martini, PhD, John B Holcomb, MD, Yong-Ming Yu, MD, PhD, Steven E Wolf, MD, Leopoldo C Cancio, MD, Anthony E Pusateri, PhD, Michael A Dubick, PhD, Hypercoagulation and Hypermetabolism of Fibrinogen in Severely Burned Adults, *Journal of Burn Care & Research*, Volume 41, Issue 1, January/February 2020, Pages 23–29
43. Lenting PJ, Christophe OD, Denis CV. von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. Blood. 2015 Mar 26;125(13):2019-28.
44. Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 7:678–689

45. Horvath B, Hegedus D, Szapary L, Marton Z, Alexy T, Koltai K et al. (2004) Measurement of von Willebrand factor as the marker of endothelial dys-function in vascular diseases. Exp Clin Cardiol. 9:31–34
46. ELfawy, D.M., Elkalek, M.A., Hamed, E. et al. Evaluation of von Willebrand factor as a marker for early diagnosis of Acute Respiratory Distress Syndrome in comparison to Interleukin-6. *Ain-Shams J Anesthesiol* **13**, 28 (2021)
47. Keyloun JW, Le TD, Orfeo T, Brummel-Ziedins KE, Bravo MC, Kaye MD, Bourne DE, Carney BC, Freeman K, Mann KG, Pusateri AE, Shupp JW; SYSCOT Study Group. Assessing Factor V Antigen and Degradation Products in Burn and Trauma Patients. J Surg Res. 2022 Jun;274:169-177.
48. Zhao R, Lang TC, Kim A, Wijewardena A, Vandervord J, McGrath R, Fulcher G, Xue M, Jackson C. Early protein C activation is reflective of burn injury severity and plays a critical role in inflammatory burden and patient outcomes. Burns. 2022 Feb;48(1):91-103.
49. Guilabert P, Martin N, Usua G, Vendrell M, Colomina MJ, Barret JP. Coagulation Alterations in Major Burn Patients: a narrative review. J Burn Care Res. 2022 Nov 29:irac177.
50. Renné T, Schmaier AH, Nickel KF, Blombäck M, Maas C. In vivo roles of factor XII. Blood. 2012 Nov 22;120(22):4296-303. doi: 10.1182/blood-2012-07-292094. Epub 2012 Sep 19.
51. Schaden E, Hoerburger D, Hacker S, Kraincuk P, Baron DM, Kozek-Langenecker S. Fibrinogen function after severe burn injury. Burns. 2012 Feb;38(1):77-82.
52. Veigas PV, Callum J, Rizoli S, Nascimento B, da Luz LT. A systematic review on the rotational thrombelastometry (ROTEM®) values for the diagnosis of coagulopathy, prediction and guidance of blood transfusion and prediction of mortality in trauma patients. Scand J Trauma Resusc Emerg Med. 2016 Oct 3;24(1):114. doi: 10.1186/s13049-016-0308-2. PMID: 27716278; PMCID: PMC5048662.
53. Schaden E, Kimberger O, Kraincuk P, et al. Perioperative treatment algorithm for bleeding burn patients reduces allogeneic blood product requirements. Br J Anaesth. 2012;109:376-381.