The Effect of Shock Duration on Trauma-Induced Coagulopathy in a Murine Model

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Abstract

Background

Trauma-induced coagulopathy (TIC) is a life-threatening condition associated with high morbidity and mortality. TIC can present with different coagulation defects. In this study, the aim was to determine the effect of shock duration on TIC characteristics. We hypothesized that longer duration of shock leads to a more hypocoagulable rotational thromboelastometry (ROTEM) profile compared to a shorter duration of shock.

Methods|

Male B57BL/6J(c) mice (n=5 to 10 per group) were sedated and mechanically ventilated. Trauma was induced by bilateral lower limb fractures and crush injuries to the liver and small intestine. Shock was induced by blood withdrawals until a mean arterial pressure of 25-30 mmHg was achieved. Groups reflected trauma and shock for 30 min (TS30) and trauma and shock for 90 min (TS90). Control groups included ventilation only (V90) and trauma only (T90).

Results

Shock groups had increased base deficit compared to control groups (V90 and T90). Mortality was 10% in TS30 and 30% in TS90. ROTEM profile was more hypocoagulable, as shown by significantly lower maximum clot firmness (MCF) in the TS30 group (43.5 [37.5 – 46.8] mm) compared to the TS90 group (52.0 [47.0 – 53.0] mm, p=0.008). ROTEM clotting time and parameters of clot build-up did not significantly differ between groups.

Conclusions

TIC characteristics change with shock duration. Contrary to the hypothesis, a shorter duration of shock was associated with decreased maximum clotting amplitudes compared to longer duration of shock. The effect of shock duration on TIC should be further assessed in trauma patients.

Background

Haemorrhage after trauma is a leading cause of preventable mortality worldwide.[1] Haemorrhaging trauma patients frequently present with trauma-induced coagulopathy (TIC), which is associated with increased transfusion requirements and mortality.[2, 3] TIC can manifest with hypocoagulable, hypercoagulable or mixed characteristics.[4] A hypocoagulable state is often present early after trauma and is characterised by coagulation factor depletion, dysfunctional platelets and hyperfibrinolysis.[5–7] These components lead to an instable clot formation and strength, resulting in a disability to control the ongoing haemorrhage.[8] Hypocoagulable profiles can shift towards a more hypercoagulable state, characterised by increased thrombin generation, platelet activation and fibrinolytic shutdown.[9–11]

Hypercoagulability often develops later on after trauma, but can also be present as early as minutes to hours after trauma.[12–14] The mechanisms underlying TIC characteristics are largely unknown. Shock is thought to play a major role in hypocoagulation, and its presence is associated with adverse outcomes.[2] The duration of shock differs between trauma patients, however it is currently unknown how the duration of shock influences TIC.[15] Unravelling the modulatory effects of shock duration on TIC characteristics has implications for the timing of treatment strategies aimed at reducing TIC. In this study the aim was to compare the effects of shock duration on TIC. We hypothesised that longer duration of shock, is associated with a more hypocoagulable profile compared to short duration of shock.

Methods

Ethics

Experiments were performed with approval of the Institutional Animal Care and Use Committee of the Amsterdam UMC, location AMC. Procedures were performed in accordance with the European Parliament directive (2010/63/EU) and the Dutch national law the Experiments on Animals Act (Wod, 2014). Male B57BL/6J (c) mice were ordered from Charles River (USA) and housed in the on-site animal housing facility seven days before the experiment. Animals had excess to food (Teklad global 16% protein, Envigo, USA) and water ad libitum with regular 12-hour day-night cycle. All mice were 8 weeks old during the experiment with a weight of 20-30g.

Animal model

Mice were sedated with 3-4% isoflurane (Isoflutek, Karizoo, Spain) and injected intraperitoneally with 0.06mg/kg fentanyl (Hameln, Germany). During the tracheostomy procedure, mice received mask ventilation (2% isoflurane, 50% Fio2). After tracheostomy, mice were mechanically ventilated (VentElite, Harvard Apparatus, USA) with tidal volumes of 7 ml/kg, respiratory rate of 160 breaths per minute, inspiratory/expiratory ratio of 1:1.5, FiO_2 of 40% and 1-2% isoflurane. An inspiratory sigh of 20% was performed every 30 minutes as recruitment manoeuvre.

The right carotid artery (arterial blood pressure monitoring) and jugular vein were cannulated where after mice received intravenous fentanyl 0.12mg/kg (Pump 11 Pico Plus Elite, Harvard Apparatus, USA) and 20ml/kg maintenance fluids consisting of Ringer's lactate supplemented with 15.3mM glucose and 2mM sodium bicarbonate (BBraun Perfuser, Germany). Temperature was monitored continuously with a rectal thermometer and kept at 37°C using a heated table and heat lamp.

Mice were randomised to one of the following groups: 90 minutes ventilation (V90), trauma + 90min ventilation (T90), trauma + 30min shock (TS30), trauma + 90min shock (TS90). Trauma consisted of bilateral lower limb fractures using two haemostatic forceps. Median laparotomy was performed to induce crush injury by clamping the small intestine distally of Treitz ligament five times for two seconds. Liver injury was achieved by clamping 1cm of the right lobe for two seconds. Following trauma the abdomen was closed.

In group TS30 and TS90, preceding trauma, 200ul blood was drawn through the carotid artery. Following trauma, additional blood was drawn to achieve a target mean arterial pressure (MAP) of 25-30mmHg. Temperature was lowered to 35°C during shock. At the end of the experiment, blood was drawn though the carotid artery or via heart puncture. An overview of the experimental setup is shown in **supplemental Figure 1**.

Blood sampling

At the end of the experiment, the first 50ul blood/saline was discarded, after which 200ul blood was collected in a heparin coated syringe for arterial blood gas analysis. The next 50ul blood was discarded to prevent heparin contamination and the remaining blood was collected in 3.2% sodium citrate (1:9 ratio). A part of the collected citrated whole blood was used for rotational thromboelastometry (ROTEM). The remaining citrated blood was centrifuged twice at 2500G for 15min at 4°C (centrifuge 5430R; rotor FA-45-30-11, Eppendorf, Hamburg, Germany) and frozen in liquid nitrogen before storage at -80°C until further analysis.

Rotational thromboelastometry

The ex-tem assay measures the tissue factor pathway by addition of 7 µl ex-tem reagent (containing tissue factor) and 7 µl star-tem (containing phospholipids and calcium) to 105ul citrated whole blood sample. EXTEM was performed using ROTEM minicups (Werfen, Germany), according to manufacturer's guidelines. Clotting time (CT) measures the initiation of clot formation, the alpha angle represents the angle between the baseline and the tangent through the 2mm point. Maximum clot firmness (MCF) depicts the final clot strength and maximum lysis (ML) shows the maximum lysis in percentage detected during the 90 minutes run time.

Enzyme-linked immunosorbent assay (ELISA)

D-dimer levels were measured using ELISA according to manufacturer's instructions (Elabscience, USA).

Organ wet/dry ratios

The left lung, part of the liver, and left kidney were collected and wet weight was determined at after the experiment. After drying the organs at 37°C for seven days they were weighted again to determine wet/dry ratios.

Sample size analysis

Based on pilot experiments, we determined 8 mice were needed to detect a 10mm difference in ROTEM MCF between TS30 and TS90 with a common standard deviation (SD) of 5mm ($\alpha = 0.05$ and a power of 80%). To account for 20% mortality in our model, 10 mice were used in the TS30 and TS90 group.

Statistical analysis

Data were analysed using SPSS version 25.0 (IBM, New York, USA). Graphs were made using GraphPad Prism version 9.0 (San Diego, USA). The histograms of all parameters were visually inspected for

distribution. Parametric data were presented as mean with standard deviation (SD) and were analysed with Student T test. Non-parametric data were presented as median with interquartile range (IQR) and analysed with Mann Whitney test. Binominal data were analysed with the Fisher's exact test. A p-value of less than 0.05 was considered to be statistically significant.

Results

Trauma, shock and mortality

The amount of blood withdrawn to reach the predefined MAP target was similar between groups: 330ul (±40ul) in TS30 group and: 350ul (±60ul) in the TS90 group. Both TS30 and TS90 shock resulted in increased base deficit compared to the control groups (Figure 1). Mortality in the TS30 group was 10%, compared to 30% in the TS90 shock group (p=0.58). All mice in the control groups survived.

Coagulation

Ex-tem clotting time and alpha angle were not significantly different between groups (Figure 2). However, maximum clotting amplitude was significantly decreased in the TS30 compared to TS90, p=0.008 (Figure 2). Median max lysis (ML) and D-dimer levels did not differ significantly between groups (Figure 2 and Table 2).

Table 1
The effect of shock on arterial blood gas analysis

	V90	T90	TS30	TS90
pH	7.31 (7.20 - 7.34)	7.27 (7.15 - 7.31)	7.23 (7.21 - 7.28)	7.08 (7.01 - 7.31)
pCO_2 (mmHg)	34.0 (32.7 - 49.1)	40.6 (31.2 - 48.4)	36.2 (25.7 - 39.4)	38.5 (30.1 - 51.7)
pO_2 (mmHg)	158.7 (106.5 - 194.6)	168.7 (138.0 - 194.0)	195.9 (179.4 - 214.4)	182.9 (140.7 - 207.8)
HCO_3 (mM)	17.4 (16.0 - 19.2)	16.9 (14.5 - 18.1)	15.2 (10.7 - 17.0)	13.7 (10.3 - 16.6)
sO2 (%)	98.0 (96.1 - 98.3)	97.7 (96.3 - 98.4)	98.0 (97.3 - 98.5)	96.8 (95.5 - 98.0)
Na^+ (mM)	146.7 (143.1 – 147.4)	142.4 (141.8 – 144.8)	143.2 (140.1 – 144.0)	143.0 (140.8 – 143.6)
K^+ (mM)	5.8 (5.5 – 6.4)	6.5 (6.4 – 7.0)	6.5 (6.2 – 6.9)	7.0 (5.8 – 8.1)
Ca^{2+} (mM)	0.96 (0.88 – 1.03)	1.01 (0.93 – 1.03)	1.12 (1.07 – 1.15)	1.10 (1.07 – 1.22)
Glucose (mM)	6.9 (6.1 - 9.4)	8.0 (6.3 - 10.8)	9.3 (8.0 - 11.2)	7.7 (5.4 - 10.6)
Lactate (mM)	3.54 (2.73 - 4.03)	3.46 (3.05 - 3.98)	4.48 (3.26 - 7.25)	6.14 (4.15 - 9.12)

Data are presented as median with interquartile range. V90= 90min ventilation, T90= trauma + 90min ventilation, TS30=trauma + 30min shock, TS30=trauma + 90min shock.

Table 2
Blood counts, coagulation and organ oedema

	V90	T90	TS30	TS90
Haemoglobin (mM)	8.5 (8.1 – 8.8)	8.4 (8.0 – 9.6)	7.1 (6.7 – 7.8)	7.1 (6.7 – 7.5)
Haematocrit (%)	40 (39 – 42)	40 (38 – 46)	34 (32 – 37)	34 (32 – 36)
Leukocytes (x 10^9/L)	1.35 (0.93 – 1.63)	2.30 (1.95 – 3.55)	1.90 (1.50 – 3.20)	2.05 (1.23 – 2.83)
Platelet count (x 10^9/L)	791 (711 – 942)	855 (804 – 875)	655 (299 – 799)	693 (533 – 784)
D-dimer (ng/ml)	1535 (1071 – 1595)	1060 (902 – 1362)	1067 (848 – 1278)	920 (865 – 938)
Organ wet/dry ratios				
Lung	3.9 (3.6 – 4.3)	3.9 (3.3 – 4.7)	4.0 (3.7 – 4.5)	4.1 (3.4 – 5.0)
Kidney	3.5 (3.4 – 3.8)	3.3 (3.2 – 3.6)	3.6 (3.4 – 3.8)	3.6 (3.5 – 3.7)
Liver	3.3 (3.2 – 3.4)	3.3 (3.1 – 3.4)	3.2 (3.1 – 3.3)	3.4 (3.1 – 3.5)

Data are presented as median with interquartile range. V90= 90min ventilation, T90= trauma + 90min ventilation, TS30=trauma + 30min shock, TS30=trauma + 90min shock.

Organ oedema

Trauma and shock did not result in significant differences in lung, kidney and liver wet/dry ratios, compared to ventilation and trauma controls (Table 2). Shock duration did not significantly influence organ oedema.

Discussion

In this murine model of trauma and shock, we showed that short duration of shock is associated with hypocoagulability compared to longer duration of shock.

This is in line with previous research showing that hypocoagulability is present as early as minutes after traumatic injury.[2, 16] Both the severity of tissue injury as well as the presence of shock worsens TIC.[2] Acidosis impairs fibrin polymerisation and clot strength in vitro and in vivo.[17, 18] Furthermore, shock and hypoperfusion are major contributors for the release of tissue plasminogen activator (tPA), converting plasminogen into plasmin, resulting in hyperfibrinolysis after trauma.[19, 20] In our model, mean values of maximum lysis and D-dimer levels did not significantly differ between groups. This could be explained by the different fibrinolytic system in mice compared to humans (i.e. shorter tPA half-life, clots are more resistant to endogenous breakdown).[21]

Our main finding, that persistence of shock reduces hypocoagulablity, was contrary to our hypothesis and may seem counterintuitive, since hypoperfusion and acidosis, the driving forces behind the hypocoagulable state, are still present. However, studies also show that minimal amounts of coagulation factors are required for relatively normal thrombin generation.[22] In fact, it is not uncommon that thrombin generation is increased after trauma.[10] In addition, endothelial dysfunction, increasing presence of circulating procoagulant platelets and exhaustion of anti-coagulant pathways may explain the shift we observe in TIC characteristics.[11, 23, 24] The observed effect could also be inflammation-induced, as pro-inflammatory pathways are tightly linked with hypercoagulability and thrombosis.[25, 26]

Our results add to the existing literature by showing that a reduction in hypocoagulability can occur early after trauma and is influenced by shock duration. Of note, the increased clotting amplitude after 90 minutes of shock was driven by endogenous responses, as animals did not receive treatment.

Our findings of the effect of shock duration on TIC characteristics may have several implications. Our results underline the rationale of early aggressive treatment of TIC, as shown in trials investing early transfusion of blood components, as well as tranexamic acid.[27, 28] With persistence of shock, targeting dysfunctional platelets and immunomodulation may convey benefits for the severely injured trauma patient. However, these aspects of trauma-induced shock and coagulopathy need further explorations.

There are limitations to this study. Our model of traumatic shock consists of traumatic injury in combination with controlled blood withdraw. Although our abdominal trauma results in bleeding, it is unlikely that mice continue to bleed excessively during the shock period. This means that after the blood withdraws a relatively stable state ensues, which differs somewhat form the trauma patient with uncontrolled bleeding. Also, since more mice died after 90min shock compared to 30min shock, survival bias might explain part of the observed effect. Lastly, we have not dissected the precise mechanistic coagulation pathways explaining the difference in maximum clot firmness in the ROTEM. We can therefore only speculate about the mechanisms.

In conclusion, hypocoagulability is part of early endogenous TIC and alters with prolonged shock duration. More research is needed to unravel the mechanisms behind this compensation in order to develop more targeted treatments for trauma-induced shock.

Abbreviations

CT Clotting time

ELISA Enzyme linked immune sorbent assay

FiO_2 Fractional inspired oxygen

MAP Mean arterial pressure

MCF	Maximum clot firmness
ML	Maximum lysis
ROTEM	Rotational thromboelastometry
TIC	Trauma-induced coagulopathy

Declarations

Ethics approval and consent to participate

Experiments were performed with approval of the Institutional Animal Care and Use Committee of the Amsterdam UMC, location AMC

Consent for publication

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

MWH is executive section editor pharmacology with Anesthesia & Analgesia, Section Editor Anesthesiology with Journal of Clinical Medicine and associate editor with Frontiers.

NPJ is editor in Chief with Intensive Care Medicine Experimental.

All other authors declare no conflict of interest.

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The experiments performed in this study were funded from institutional resources.

Authors' contributions

PHS collected the data, performed data analysis and drafted the manuscript. MAWM performed the surgical procedures for the experiment. MWH, NPJ and DJBK supervised the project and revised the manuscript. All authors read and approved the final manuscript.

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None to report

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Figures

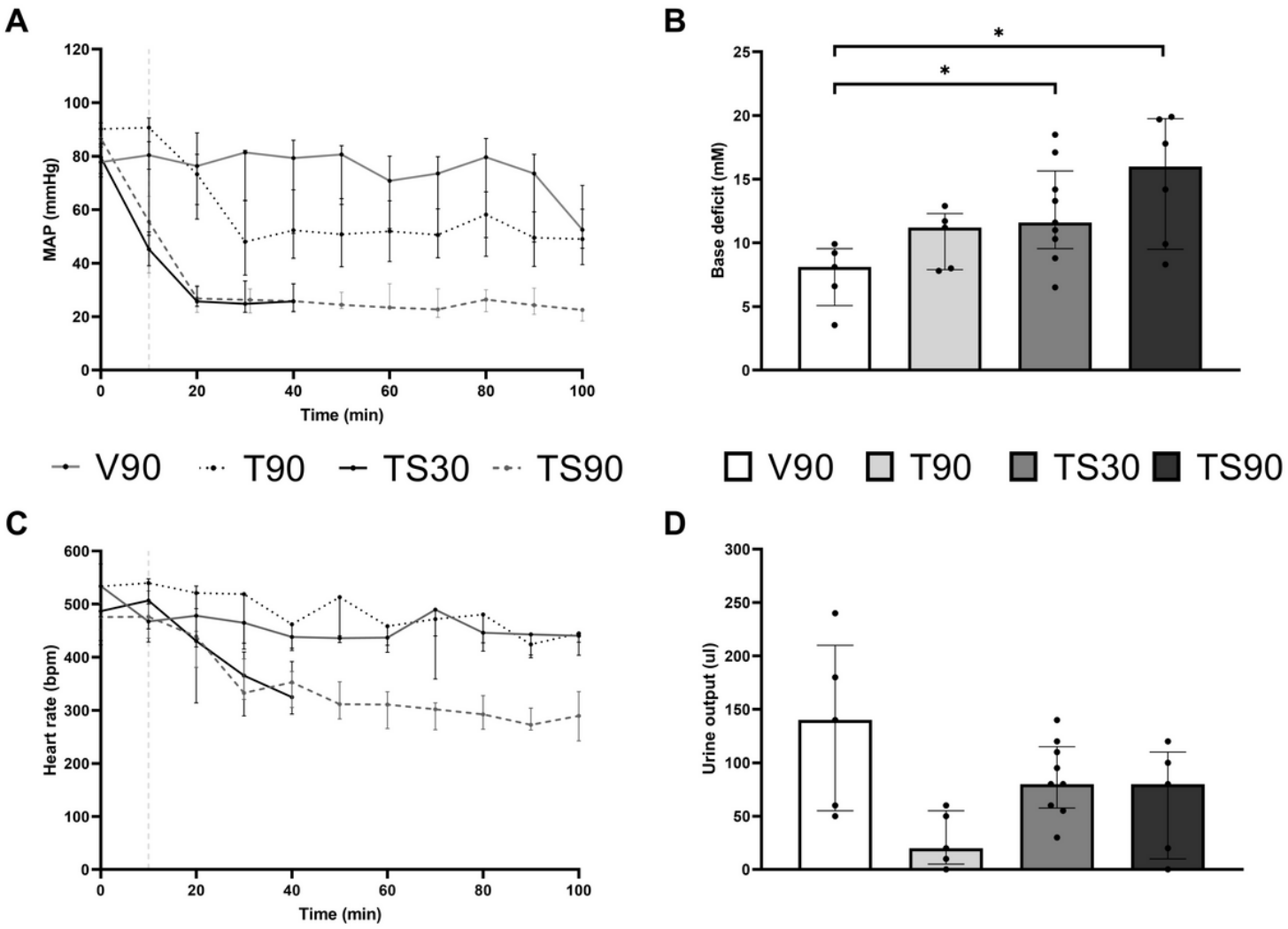

Figure 1

Hemodynamic parameters during different shock durations Data are presented as median with interquartile range. (A) Mean arterial pressure, (B) base deficit, (C) Heart rate, (D) Urine output. V90= 90min ventilation, T90= trauma + 90min ventilation, TS30=trauma + 30min shock, TS30=trauma + 90min shock. * $p<0.05$ between groups.

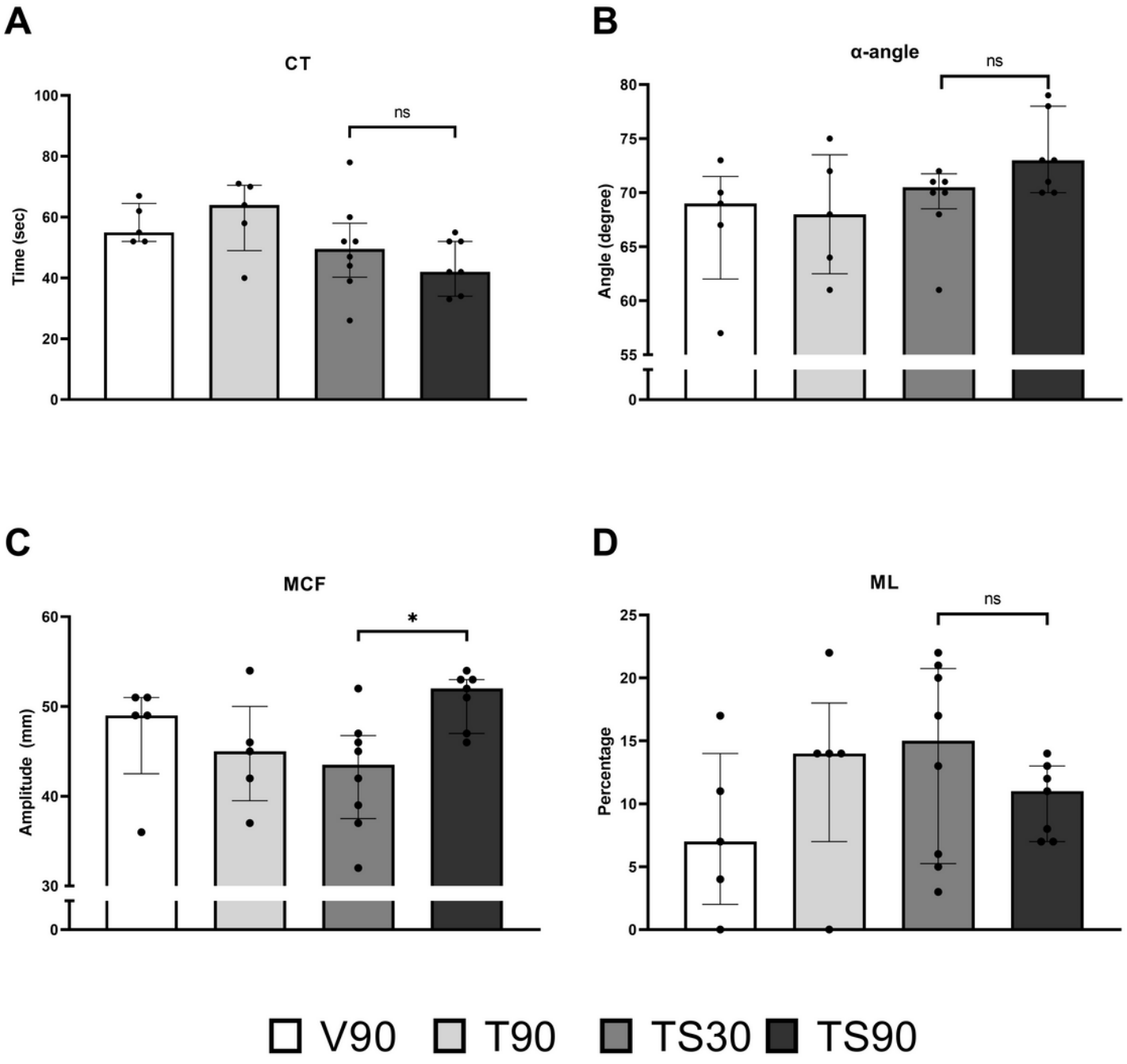

Figure 2

The effect of shock duration on ROTEM parameters Data are presented as median with interquartile range. (A) EXTEM clotting time, (B) EXTEM a-angle, (C) EXTEM maximum clot firmness, (D) EXTEM maximum lysis. V90= 90min ventilation, T90= trauma + 90min ventilation, TS30=trauma + 30min shock, TS30=trauma + 90min shock. * $p<0.05$ between groups, ns $p \geq 0.05$.

Supplementary Files

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- Sloosetal.ShockdurationandTICsupfig1.docx