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Adaptation of global hemostasis to therapeutic hypothermia in patients with out-of-hospital cardiac arrest: Thromboelastography study

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Abstract

Background: *The use of mild therapeutic hypothermia (MTH) in patients after out-of-hospital cardiac arrest (OHCA) who are undergoing primary percutaneous coronary intervention (pPCI) can protect patients from thromboembolic complications. The aim of the study was to evaluate the adaptive mechanisms of the coagulation system in MTH-treated comatose OHCA survivors.*

Methods: *Twenty one comatose OHCA survivors with acute coronary syndrome undergoing immediate pPCI were treated with MTH. Quantitative and qualitative analyses of physical clot properties were performed using thromboelastography (TEG). Two analysis time points were proposed: 1) during MTH with in vitro rewarming conditions (37°C) and 2) after restoration of normothermia (NT) under normal (37°C) and in vitro cooling conditions (32°C).*

Results: *During MTH compared to NT, reaction time (R) was lengthened, clot kinetic parameter (α) was significantly reduced, but no effect on clot strength (MA) was observed. Finally, the coagulation index (CI) was significantly reduced with clot fibrinolysis attenuated during MTH. The clot lysis time (CLT) was shortened, and clot stability (LY^{60}) was lower compared with those values during NT. In vitro cooling generally influenced clot kinetics and reduced clot stability after treatment.*

Conclusions: *Thromboelastography is a useful method for evaluation of coagulation system dysfunction in OHCA survivors undergoing MTH. Coagulation impairment in hypothermia was associated with a reduced rate of clot formation, increased weakness of clot strength, and disturbances of fibrinolysis. Blood sample analyses performed at 32°C during MTH, instead of the standard 37°C, seems to enhance the accuracy of the evaluation of coagulation impairment in hypothermia.* (Cardiol J 2019; 26, 1: 77–86)

Key words: coagulation, hypothermia, cardiac arrest, thromboelastography

Introduction

Despite the fact that 60% of out-of-hospital cardiac arrests (OHCA) survivors are treated by emergency medical service personnel, their prognosis is still very poor with survival rates reaching only 9.5% (8.8–10.2%) [1–5]. Two independent, randomized control trials for myocardial infarction patients who had OHCA confirmed an improvement in survival and neurological outcomes resulting from the application of mild therapeutic hypothermia (MTH) [6, 7]. The postulated mechanism of MTH action occurs via a decrease in cerebral oxygen demand, which reduces excitatory amino acid

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release and improves brain glucose utilization [8, 9]. There are limited data in the literature regarding hypothermia effects on coagulation. Proper evaluation of the coagulation system during hypothermia in patients with acute coronary syndrome (ACS) complicated by cardiac arrest will avoid bleeding and thrombotic complications. However, it is known that MTH interacts with the coagulation system mainly through change of platelets shape, impairment of its function and shortening of its life span, emphasizing its influence on the ability of platelets to respond to activating stimuli, as well the role of shear-induced platelet aggregation by increasing blood viscosity. Expression of von Willebrand factor (vWF) in endothelial cells is higher, and retention on the cell surface is prolonged, as well recognition of vWF with factor VIII is reduced at low temperatures [10].

As a result of cardiac arrest (CA), complex processes such as systemic ischemia/reperfusion responses, brain injury, and acidosis come into play [11]. MTH by lowering the cardiac output that modulates the level of plasma cytokines and increasing the level of endotoxins involved in systemic inflammation has an indirect effect on coagulation abnormalities [12–14].

Thromboelastography (TEG), is routinely performed at 37°C, however some authors have suggested that this approach may not reflect the real hemostatic function in hypothermic patients [15].

Some studies suggest that hypothermia may modify the coagulation cascade due to regulation of coagulation factor activities; most of which are thermo-sensitive [16, 17]. In practice, this anticoagulation effect may have an additional cardioprotective action, especially for CA patients who usually have an imbalanced coagulation cascade toward thrombosis [1, 2, 18]. In these patients a strict control of the coagulation system is crucial for proper treatment and prevention of undesirable effects. It should be noted that the previously published results were obtained by both TEG and throboelastometry methods at 37°C *in vitro*; no clinical data regarding MTH patients performed under cooling *in vitro* conditions are available [19]. There are a few studies investigating hypothermic effects on coagulation in OHCA patients, some of these have indicated a prolonged clot initiation during hypothermia and others contradicted this finding [20, 21]. Moreover, there are a lack of studies conducted under conditions that reflect the *in vivo* hypothermia environment, and those that are available were performed on healthy volunteers in model conditions [16, 17]. In the current study, the impact was evaluated of hypothermia on the coagulation system's adaptive mechanisms under normo- and hypothermic conditions *in vivo* and *in vitro* of comatose OHCA survivors.

Methods

Study design

This prospective study was conducted as a single-center evaluation study. The protocol was approved by the Jagiellonian University Ethics Committee in Krakow. Patients were consecutively recruited between January 2014 and September 2015 to the cardiac intensive care unit of the Department of Interventional Cardiology at John Paul II Hospital in Krakow, Poland. Written informed consent was obtained from patients regaining consciousness after cardiac arrest. MTH was achieved with the use of an endovascular cooling device (Zoll Medical Corporation Chelmsford, MA) which was set to a target temperature of 32.0°C and was maintained for 24 h. Subsequently, rewarming was performed at a rate of 0.2°C/h. Inclusion criteria required ACS complicated by OHCA with return of spontaneous circulation. Patients had to be > 18 years of age and undergo primary percutaneous coronary intervention (pPCI) to a culprit artery with implantation of a coronary stent. Because patients with ST-segment elevation myocardial infarction (STEMI) were admitted directly to the catheterization laboratory without additional diagnostic tests, we compared a group with STEMI vs. a non-ST-segment elevation myocardial infarction (NSTEMI) group. Patients were excluded if they had recognized coagulopathy or they had been treated with MTH before hospital admission. Quantitative and qualitative measurements of clot physical properties were assessed using TEG (Haemoscope Corp., Niles, Illinois, USA) [22, 23]. At discharge, neurological outcomes were assessed according to the Pittsburgh Cerebral Performance Category (CPC) [24]. CPC scores of 1 and 2 were defined as good neurological outcomes, whereas CPC scores of 3, 4, and 5 showed an unfavorable neurological outcome.

Patient management

During the procedure, each patient received unfractionated, intravenous (i.v.) heparin, (according to their weight) and a dual antiplatelet drugs *via* a nasogastric tube. A loading dose of 180 mg ticagrelor and 300 mg acetylsalicylic acid (ASA) was administered upon admission, followed by ticagrelor 90 mg twice daily and 75 mg ASA once

Table 1. Summary of the drug use in study intervals.

Parameters	Basal (n = 21)	MTH (n = 20)	NT (n = 19)
UFH $_{(ptn)}$	4 (19%)	0 (0%)	0 (0%)
LMWH$_{(ptn)}$	1 (5%)	2 (10%)	1 (5%)
ASA$_{(ptn)}$	8 (38%)	20 (100%)*	17 (90%) ^
P2Y$_{12\ (ptn)}$	2 (10%)	20 (100%)*	19 (100%) ^
Clopidogrel $_{(ptn)}$	2 (10%)	0 (0%)	0 (0%)
Ticagrelor $_{(ptn)}$	0 (0%)	20 (100%)	19 (100%) ^

*$p < 0.05$ (MTH vs. Basal); ^ $p < 0.05$ (NT vs. Basal)

ASA — acetylsalicylic acid; LMVH — low molecular weight heparin; MTH — mild therapeutic hypothermia; NT — normothermia; P2Y$_{12}$ — inhibitor P2Y$_{12}$; UFH — unfractionated heparin; ptn — patients

(1) All patients in hypothermia received dual antiplatelet therapy (ASA$_{MTH}$ = 100% vs. ASA$_{Basal}$ = 38%; $p < 0.05$) and (P2Y$_{12MTH}$ = 100% vs. P2Y$_{12Basal}$ = 10%; $p < 0.05$).

(2) In 2 normothermic patients, ASA was discontinued due to bleeding from the respiratory tract and suspicion of central nervous system bleeding.

(3) All patients in normothermia received P2Y$_{12}$.

Table 2. Summary of basic parameters of thromboelastography (TEG).

Reaction time	R	The latency period from the time that blood was placed in the TEG analyzer until initial fibrin formation. Represents enzymatic reaction
Clot kinetics	K	A measure of the speed to reach 20 mm amplitude. Represents clot kinetics
	α	A measure of fibrin build-up rapidity and cross-linking (clot-strengthening) by factor XIII; it also depended on platelet participation in clot formation and the concentration of fibrinogen and fibrin polymerization ability. Represents fibrinogen level
Clot strength	MA	A direct function of maximum dynamic properties of fibrin and platelet bonding via glycoprotein IIb/IIIa. Represents maximum platelet function.
Coagulation index	CI	A linear combination of R, K, alpha, MA
Clot stability	LY^{60}	A measure of the rate of amplitude reduction 60 min after MA. Estimates %lysis based on amplitude reduction after MA
Clot lysis time	CLT	The elapsed time between MA and 2 mm amplitude or less post MA

daily. During the procedure, glycoprotein (GP) IIb/IIIa receptor inhibitor was administered at the discretion of the interventional cardiologist. After the intervention, patients were hospitalized in the Cardiology Intensive Care Unit and received standard treatment. The pharmacological treatment summary during the study is presented in Table 1.

Blood sampling

Whole blood for hematology and coagulation analyses was collected at admission prior to performing pPCI (basal, an average body temperature of 35.3 ± 0.6°C), 22 ± 4 h after induction of hypothermia (MTH, at an average core temperature of 32.2 ± 0.1°C) and 21 ± 9 h after patients were rewarmed to normothermia (NT, at an average core temperature of 36.7 ± 0.2°C). The second set of samples was drawn for biochemical analysis. For TEG, blood was collected from the femoral vein into tubes containing 2.8 mL of citrate. Subsequently, 1 mL of venous blood was transferred into a vial containing kaolin and mixed by slow inversion. Then, 320 μL of blood was immediately transferred into two heparinized caps with 20 μL of 0.2M $CaCl_2$ and analyzed by TEG simultaneously at *in vitro* temperatures of 37°C and 32°C.

TEG analysis

Using the TEG analyzer, key parameters were determined under two temperature conditions (37°C and 32°C; Table 2). There were two key TEG parameters reflecting the function of plasma coagulation: R — time from the start of a sample run until the first significant level of detectable clot formation, which resulted from cleavage of fibrinogen by thrombin; α — rate of thrombin burst and fibrin formation and cross-linking. Platelets and fibrinogen activity are represented by MA, a measure of the dynamic properties of fibrin and platelets by binding to GP IIb/IIIa receptor. Together with

the K parameter, MA demonstrates the strength of fibrin clot. The K kinetics achieve a certain clot firmness. Fibrinolysis properties are documented by LY^{60}, and CLT. Coagulation index (CI) defines global clotting.

With regard to standard coagulation tests, the international normalized ratio, activated partial thromboplastin time, thrombin time, and fibrinogen concentrations were analyzed employing the BCS XP System (Siemens, Healthcare, Poland). The complete blood count test was done by the Sysmex XN1000 haematology analyzer (Sysmex Corporation, Japan).

Statistical analysis

Statistical analyses were performed using Statistica 10.0 package. Distribution of variables was tested by Kolmogorov-Smirnov normality test. Quantitative variables were characterized using descriptive statistics. Analyzing the relationships of qualitative data was done with Pearson's χ^2 test and Fisher's exact, two-sided test. Continuous variables were reported as mean ± standard deviation (SD) or medians and interquartile ranges as appropriate. Student t-test or Mann Whitney test was used to determine significance between the variables R, K, α, MA, TMA, LY^{60}, and CI levels in each group. A p-value ≤ 0.05 was considered statistically significant.

Results

Clinical characteristics

In a cohort of 21 consecutive OHCA patients (67 ± 11 years) 3 deaths occurred: 2 patients during assessment and 1 after collecting the final samples. Baseline demographic and resuscitation characteristics of those with and without STEMI undergoing coronary angiography are shown in Table 3.

Laboratory investigations

Standard laboratory values measured at 37°C during admission (basal), MTH and normothermia after rewarming (NT) are shown in Table 4.

Variables

Variable R. Under *in vivo* conditions, the value of R during MTH (32.2 ± 0.1°C) was 9.9 ± ± 2.0 min and did not differ significantly compared to the R during NT (36.7 ± 0.2°C), which was 9.1 ± 2.4 min. *In vitro*, there was no significant difference between the values of $R_{MTH37°C}$ vs. $R_{MTH32°C}$ and $R_{NT37°C}$ vs. $R_{NT32°C}$ (Table 5).

Variable α. Under *in vivo* conditions, during MTH, α was 54 ± 8°. This value indicated a significant impairment of fibrin build-up compared to NT after rewarming, in which α was 65 ± 7°; $p < 0.05$ (Table 6). The sample examination at different temperatures *in vitro* demonstrated significant differences in the values of $\alpha_{MTH37°C}$ vs. $\alpha_{MTH32°C}$ and $\alpha_{NT37°C}$ vs. $\alpha_{NT32°C}$ (Table 5).

Variable K. During MTH, a significant increase of clot kinetic properties (K) in comparison to NT conditions was observed: 2.8 ± 1.1 vs. 2.0 ± 0.7 min; $p < 0.05$ (Table 6). The sample examination at different temperatures *in vitro* demonstrated no differences in $K_{MTH37°C}$ vs. $K_{MTH32°C}$ and $K_{NT37°C}$ vs. $K_{NT32°C}$ (Table 5).

Variable MA. Under *in vivo* conditions, during MTH, there was no change in MA. In the NT, a significant increase in clot strength from MA_{MTH} 63 ± 7 to MA_{NT} 69 ± 6 mm, $p < 0.05$ was observed. Under *in vitro* conditions, no significant differences in $MA_{MTH37°C}$ vs. $MA_{MTH32°C}$ and $MA_{NT37°C}$ vs. $MA_{NT32°C}$ were observed (Table 5).

Variable CI. In the NT a significant increase in CI from CI_{MTH} –3.5 ± 2.4 to CI_{NT} –1.1 ± 2.8/s was observed. Under *in vitro* conditions, no significant differences in the parameter $CI_{MTH37°C}$ vs. $CI_{MTH32°C}$ and $CI_{NT37°C}$ vs. $CI_{NT32°C}$ were observed (Table 5).

Variable LY^{60}. *In vivo* conditions, during MTH, a significant reduction the clot stabilization from $LY^{60}{}_{MTH}$ 1.4 ± 1.4% to $LY^{60}{}_{NT}$ 3.7 ± 2.3% ($p < 0.05$) was observed. *In vitro*, a significant difference in the parameter LY^{60} between the intervals: $LY^{60}{}_{MTH37°C}$ vs. $LY^{60}{}_{MTH32°C}$; $LY^{60}{}_{NT37°C}$ vs. $LY^{60}{}_{NT32°C}$ was observed (Table 5).

The parameter CLT

There were no significant differences in CLT both under *in vivo* and *in vitro* conditions, during MTH and NT (Table 5).

Discussion

Major findings of the study were: coagulation impairment during hypothermia manifested by a reduced rate of clot formation, increased weakness of clot strength, and disturbances of fibrinolysis with reduced fibrinogen levels. Based on previous studies, the impairment of the coagulation system after application of MTH to OCHA patients should be considered as an effect of reduced temperature and ischemic-reperfusion damage resulting from CA. Changes include inhibition of enzyme function (zymogens) and abnormal platelet function as well as increased fibrinolysis or a combination of

Table 3. Baseline characteristics of patients after out-of-hospital cardiac arrest due to acute myocardial infarction undergoing mild therapeutic hypothermia.

	STEMI (n = 11)	NSTEMI (n = 10)	P
Demographic data			
Males	9 (82%)	8 (80%)	0.9
Age	65 ± 9	68 ± 13	0.5
History of myocardial infarction	0	1 (10%)	0.3
History of ischemic heart disease	1 (9%)	2 (20%)	0.5
Arterial hypertension	7 (64%)	7 (70%)	0.8
Diabetes mellitus type 2	2 (18%)	4 (40%)	0.3
Dyslipidemia	4 (36%)	5 (50%)	0.5
Obesity	4 (36%)	4 (40%)	0.9
Tobacco smoking	4 (36%)	2 (20%)	0.4
Revascularization	0	2 (20%)	0.1
Chronic kidney disease stage 5	0	1 (10%)	0.5
Atrial fibrillation	0	2 (20%)	0.1
Arrest data			
Initial cardiac arrest rhythm:			
VF/VT	10 (91%)	7 (70%)	0.2
Asystole	0	2 (20%)	0.1
Pulseless electrical activity	1 (9%)	1 (10%)	0.9
Time to ROSC [min]	27 ± 15	19 ± 16	0.2
Witness (yes)	11 (100%)	10 (100%)	1
Bystander CPR (yes)	7 (64%)	7 (70%)	0.8
Hospital patients on admison data			
GCS score (3–4)	7 (64%)	6 (60%)	0.9
GCS score (5–6)	4 (36%)	4 (40%)	0.9
Medium HR [bpm]	102 ± 26	98 ± 13	0.7
Medium MAP [mmHg]	94 ± 26	96 ± 23	0.9
Cardiogenic shock (yes)	3 (27%)	1 (10%)	0.3
Time to MTH [min]	117 ± 37	154 ± 66	0.2
STEMI location			
Anterior	5 (45%)	NA	
Inferior	2 (18%)	NA	
Lateral	1 (9%)	NA	
Anterior-lateral	2 (18%)	NA	
Inferior-lateral	1 (9%)	NA	
LBBB	0	2 (20%)	0.1
RBBB	2 (18%)	2 (20%)	0.9
Coronary angiographic			
Culprit leasion:			
Left main artery	0 (0%)	0 (0%)	–
Left anterior descending artery	6 (55%)	2 (20%)	0.1
Diagonal artery	0 (0%)	1 (10%)	0.3
Intermediate artery	1 (9%)	0 (0%)	0.3
Left circumfex artery	1 (9%)	2 (20%)	0.5
Marginal artery	2 (18%)	2 (20%)	0.9
Right coronary artery	1 (9%)	2 (20%)	0.5
MVD (more than 1-VD)	0 (%)	1 (10%)	0.3
Culprit occlusions	8 (73%)	4 (40%)	0.1

→

Table 3 (cont). Baseline characteristics of patients after out-of-hospital cardiac arrest due to acute myocardial infarction undergoing mild therapeutic hypothermia.

	STEMI (n = 11)	NSTEMI (n = 10)	P
Intervention findings			
PCI	10 (91%)	10 (100%)	0.3
PCI type:			
Bare metal stent	1 (9%)	0 (0%)	0.3
Drug eluting stent	9 (82%)	10 (100%)	0.2
Used of GP IIb/IIIa inhibitors	1 (9%)	0 (0%)	0.3
Time from cardiac arrest to door to balloon [min]	107 ± 33	155 ± 68	0.06
Time PCI [min]	29 ± 18	18 ± 9	0.1
Hospital patients at discharge			
Echocardiogram:			
Normal (EF > 50%)	6 (55%)	4 (40%)	0.5
Mild-moderate (EF 30–49%)	3 (27%)	6 (60%)	0.1
Severe (EF < 30%)	2 (18%)	0 (0%)	0.2
Medication			
ASA	10 (91%)	8 (80%)	0.5
Clopidogrel	0 (0%)	1 (10%)	0.3
Ticagrelor	11 (100%)	9 (90%)	0.3
LMWH	3 (27%)	6 (60%)	0.1
Statin	4 (36%)	7 (70%)	0.1
ACEI	6 (54%)	5 (50%)	0.8
Digoxin	0 (0%)	1 (10%)	0.3
Beta-blocker	6 (54%)	4 (40%)	0.5
Diuretic	8 (72%)	5 (50%)	0.3
Cordarone	1 (9%)	1 (10%)	0.9
Clinical characteristics			
Cerebral edema	1 (9%)	3 (30%)	0.2
Stroke	0 (0%)	2 (20%)	0.1
Stent thrombosis	0 (0%)	0 (0%)	–
Pneumonia	6 (55%)	6 (60%)	0.8
Bleeding	2 (18%)	5 (50%)	0.1
Cardiogenic shock (yes)	5 (45%)	4 (40%)	0.8
Re-cardiac arrest	3 (27%)	2 (20%)	0.7
Neurological outcome			
Good neurological outcome	7 (64%)	5 (50%)	0.5
Death	2 (18%)	1 (10%)	0.6

ACEI — angiotensin-converting-enzyme inhibitor; ASA — acetylsalicylic acid; CPR — cardiopulmonary resuscitation; EF — ejection fraction; GCS — Glasgow Coma Scale; GP — glycoprotein; HR — heart rate; LBBB — left bundle branch block; LMWH — low molecular weight heparin; MAP — mean arterial pressure; MTH — mild therapeutic hypothermia; MVD — multivessel disease; NSTEMI — non-ST-segment elevation myocardial infarction; PCI — percutaneous coronary intervention; RBBB — right bundle branch block; ROSC — return of spontaneous circulation; STEMI — ST-segment elevation myocardial infarction; VT/VF — ventricular tachycardia/ventricular fibrillation

Due to STEMI, patients were admitted directly to a catheterization laboratory (without additional diagnostic tests), and STEMI vs. NSTEMI groups were compared. No significant differences between groups were observed: (1) the main mechanism of cardiac arrest was ventricular fibrillation; (2) Arterial hypertension was the most commonly occurring risk factor for atherosclerosis in both groups; (3) Time frame to balloon was longer in NSTEMI patients (delay of revascularization resulted from the exclusion of non-cardiogenic reasons of cardiac arrest; p = 0.06); (4) In the STEMI group, > 50% left anterior descending artery was due to infarct-related artery; (5) Cardiogenic shock occurred at the same frequency in both groups (45% vs. 40%; p = 0.8); (6) The bleeding rates were greater in NSTEMI (STEMI vs. NSTEMI, 18% vs. 50%; p = 0.1); and (7) The number of favourable neurological outcomes (as identified by the Pittsburgh Cerebral Performance Category scores 1 and 2) were similar in patients in both groups (64% vs. 50%; p = 0.5).

Table 4. Summary of standard laboratory values from whole blood without using heparin.

Parameter	Basal	MTH	NT
AT III [%]	81 (10)	68 (8)*	76 (9)§
Fibrinogen [g/L]	3.0 (0.8)	3.1 (0.9)	5.4 (1.2)‡§
PLT [$10^{-3}/\mu$L]	192 (56)	151 (38)*	138 (50)‡
APTT [s]	28 (13)	34 (9)*	31 (6)‡
INR	1.2 (0.3)	1.2 (0.2)	1.1 (0.2)
DD [μg/L]	18316 (13865)	3253 (3538)*	1567 (1417)‡§
ACT [ms]	121 (21)	116 (14)	119 (14)

The data shown are the mean and standard deviation (SD).*p < 0.05 (MTH vs. Basal); ‡p < 0.05 (NT vs. Basal); §p < 0.05 (NT vs. MTH)

ACT — activated clotting time; APTT — activated partial thromboplastin time; AT III — antithrombin III; DD — D-dimer; INR — international normalized ratio; MTH — mild therapeutic hypothermia; NT — normothermia; PLT — platelets

Significant differences between time points MTH vs. Basal: (1) the mean of AT III; (2) the mean of PLT counts; (3) the mean of APTT; (4) the mean of DD.

Significant differences between time points NT vs. Basal: (1) the mean of fibrinogen; (2) the mean of PLT counts; (3) the mean of APTT; (4) the mean of DD.

Significant differences between time points NT vs. MTH: (1) the mean of AT III; (2) the mean of fibrinogen; (3) the mean of DD.

Table 5. A summary of the thromboelastography (TEG) parameters for patients with out-of-hospital cardiac arrest undergoing mild therapeutic hypothermia (MTH) during and after treatment (normothermia [NT]). TEG was performed at 32°C and 37°C *in vitro* conditions.

Parametr	MTH	P	NT	P
$R_{37°C\ (min)}$	9.3 ± 1.9	> 0.05	9.1 ± 2.4	> 0.05
$R_{32°C\ (min)}$	9.9 ± 2.0		9.1 ± 2.2	
$K_{37°C\ (min)}$	2.5 ± 1.3	> 0.05	2.0 ± 0.7	> 0.05
$K_{32°C\ (min)}$	2.8 ± 1.1		2.1 ± 0.6	
$\alpha_{37°C\ (deg)}$	58 ± 10	< 0.05*	65 ± 7	< 0.05*
$\alpha_{32°C\ (deg)}$	54 ± 8		62 ± 6	
$MA_{37°C\ (mm)}$	62 ± 7	> 0.05	69 ± 6	> 0.05
$MA_{32°C\ (mm)}$	63 ± 7		70 ± 5	
$LY^{60}{}_{37°C\ (\%)}$	2.7 ± 2.2	< 0.05*	3.7 ± 2.3	< 0.05*
$LY^{60}{}_{32°C\ (\%)}$	1.4 ± 1.4		2.3 ± 1.9	
$CI_{37°C\ (/s)}$	–2.8 ± 2.8	> 0.05	–1.1 ± 2.8	> 0.05
$CI_{32°C\ (/s)}$	–3.5 ± 2.4		–1.2 ± 2.3	
$CLT_{37°C\ (min)}$	491 ± 430	> 0.05	630 ± 502	> 0.05
$CLT_{32°C\ (min)}$	479 ± 446		628 ± 549	

The data shown are the mean and standard deviation of four separate assays.

*Statistically significantly different from that at 32°C at the p < 0.05 level.

Significant differences at different time points for MTH and NT between parameters at 37°C vs. 32°C: 1) the mean of α; 2) the mean of LY^{60}.

Table 6. A summary of the thromboelastography parameters for patients with out-of-hospital cardiac arrest undergoing mild therapeutic hypothermia (MTH) during and after treatment (normothermia [NT])

Parameter	MTH	NT
R [min]	9.9 ± 2.0	9.1 ± 2.4
α [deg]	54 ± 8	65 ± 7*
K [min]	2.8 ± 1.1	2.0 ± 0.7*
MA [mm]	63 ± 7	69 ± 6*
CI [s^{-1}]	–3.5 ± 2.4	–1.1 ± 2.8*
LY^{60} [%]	1.4 ± 1.4	3.7 ± 2.3*
CLT [min]	479 ± 446	630 ± 502

The data shown are the mean and standard deviation; *p < 0.05 (NT vs. MTH)

α — angle to define clot kinetics; CI — coagulation index; CLT — clot lysis time; LY^{60} — rate of clot stability after 60 min; K — time to reach 20 mm amplitude; MA — maximum amplitude; R — time to define clot kinetics. All abbreviations are defined in Table 2.

any of those factors [11, 25, 26]. TEG is a bedside test which allows assessment of the interaction between coagulation factors, platelets, fibrin and fibrinolysis. Routinely, TEG is performed at 37°C, nevertheless some studies were dedicated to test the influences of temperature on TEG measurements [27, 28]. The results of the current TEG analysis under *in vivo* conditions demonstrated that application of MTH impaired both propagation of coagulation (α-angle), as well as K and MA, yet did not affect the R time. In contrast, a delay in clot lysis (reflected by low LY^{60} and CI) was detected in the present study. Some observations are consistent with previous studies in which hemostasis in OHCA survivors treated with MTH, using both TEG® as well as rotational thromboelastogram (ROTEM®) analysis was investigated and the im-

pairment of hemostasis during MTH application was proved. Variables resulted during both analyses were comparable [21, 25, 29]. The R variable, corresponding to clotting time in ROTEM®, is the value most influenced by activity of coagulation factors. In this study, R was not extended during MTH, compared to NT, under both *in vivo* and *in vitro* conditions. This agreed with previous observations but was inconsistent with others [10, 21, 30–32]. The first inconsistency could be explained by negligible temperature dependence of coagulation factors that form part of the extrinsic coagulation cascade. This step consists of two reactions: the coupling of factor VII/VIIa with tissue factor and the subsequent activation of factor X. Active cooling to 32°C did not influence activity and plasma levels of factor VII, which is the coagulation factor with the shortest half-life [32]. The second inconsistency, a significant R prolongation after induction of MTH, was observed in studies mainly involving surgical patients [32, 33] and could be a consequence of inhaled anaesthetics and propofol effects, which are believed to have an effect on hemostatic function [34]. Variables corresponding to α, K, and MA in TEG are defined as α, clot formation time, and maximum clot firmness in ROTEM, respectively. Decreases in their values may reflect the impairment of the platelet count and activity as well as significantly lower fibrinogen levels during MTH compared to basal NT and NT after rewarming [30]. In this study, fibrinogen levels were significantly higher at NT compared to those under MTH and basal conditions. In the present observation, as fibrinogen levels have an impact on blood clot strength, stability, and velocity of formation, its levels corresponded with values of α-angle and MA, respectively. Such relationships were observed *in vivo* (α-angle, MA) as well as *in vitro* (α-angle), which is consistent with previous observations [21, 30]. The most likely explanation for this finding is that elevated levels of fibrinogen in NT were related to acute phase reaction to ischemic-reperfusion injury caused by earlier cardiac arrest in OHCA survivors [11, 35]. MTH had an impact on decreased platelet blood count, compared to basal NT which has been referred to in previous studies [21, 36]. In this study, the decrease remained within the normal range during MTH had no clinical impact on coagulation impairment. A tendency toward platelet count decrease during MTH may be explained as platelet margination and formation of platelet and platelet/leukocyte aggregates (which may depend on the level and duration of hypothermia) or by hepatic and splenic sequestration [37–39]. Another explanation of this phenomenon may be a cold-induced decrease in bone marrow function [40]. The persistent decrease in a platelet count, which was observed in the observed group after rewarming, may also be explained by perioperative blood loss (during pPCI), which is consistent with previous studies involving invasively-treated patients [29]. TEG analysis of fibrinolysis dynamics was brought to a conclusion that one of the possible mechanisms leading to reduced clot solubility during therapeutic hypothermia is a higher secretion of tissue plasminogen activator by endothelial cells and a slower conversion of plasminogen to plasmin. Similar findings have been previously observed in other studies [41, 42]. The dysfunction in coagulation and fibrinolysis systems observed *in vitro* may be clinically relevant as a tendency toward bleeding complications via impaired clot formation. However, the present study group consisted of a relatively small number of patients to thoroughly study this relationship; the statistical power of this group was insufficient to bring us to such clinical conclusions. As a standard feature, in most TEG studies blood tests were performed at 37°C, regardless of patient body temperature. This study examined whether the sample temperature had an impact on coagulation impairment during the TEG evaluation, similar studies has been performed for different patient groups with samples collected during MTH [27, 31, 35]. The MTH effects on coagulation tests from both *in vivo* (patient treatment) and in *in vitro* conditions (TEG) were analyzed. The *in vitro* results were different with respect to *in vivo* data; the delayed (prolonged) initiation of clot formation (lower α) and impaired tendency to clot lysis (lower LY^{60}) were observed when TEG was performed at 37°C, while there were no effects on the MA, K and CI. These last findings were consistent with those previously obtained in other *in vitro* hypothermic studies [20, 28, 36, 43]. The present approach to analyze blood samples collected from patients during MTH at 32°C and 37°C allowed a more accurate assessment of cooling-induced coagulation impairment. The finding that TEG results may be affected by the *in vitro* temperatures have already been confirmed in some studies [15, 28, 31, 43], while in other studies it was considered insignificant [19, 44]. However, in the largest randomized clinical trial conducted to date by Nielsen et al. [45], it was shown that there were no differences in risk of death and neurological outcome between group with 33°C (MTH) vs. 36°C of targeted temperature management (TTM)

This takes into consideration new ILCOR guidelines which recommend both therapies — MTH or targeted temperature management are nowadays the standard procedures in OHCA patients. In this prospective study, previous findings were confirmed that TEG is an effective tool for the effect of MTH on coagulation disorders both *in vivo* and *in vitro* [26]. Blood samples collected at regular intervals after OHCA have allowed a reliable comparison of blood coagulation parameters during MTH and then in the rewarming phase. In conclusion, based on TEG evaluation, hypothermia appears to impair coagulation and platelet function.

Limitations of the study

The limitation of the TEG method is low reproducibility, when performed by unqualified personnel. In this context, routine use of TEG could help prevent the occurrence of severe bleeding or thromboembolic complications, thus increasing the survival chances of patients with OHCA undergoing pPCI. Some limitations have to be considered. The first limitation is the low number of patients admitted to this study (21), which did not allow correlation of TEG results with clinical events. A second limitation was the lack of a control group; the current guidelines recommend the use of MTH in all patients with OHCA, and ethical standards will most likely prevent further randomized trials that withhold hypothermia in order to establish a control group. Although validation of results using a randomized controlled approach seems preferable, according to the design of this study, patients actually served as their own controls. Finally, TEG variables were not measured before hypothermia induction.

Conclusions

Thromboelastography appears to be a useful method for evaluation of coagulation system dysfunction in OHCA survivors undergoing therapeutic hypothermia. Coagulation impairment in hypothermia was associated with a reduced rate of clot formation, increased weakness of clot strength, and disturbances of fibrinolysis and most likely results from reduced fibrinogen levels and platelet activity. Blood sample analyses performed at 32°C during MTH, instead of the standard 37°C, seems to enhance the accuracy in evaluation of coagulation impairment in hypothermia.

Conflict of interest: None declared

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