Thrombosis Research 174 (2019) 121–128

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Enhanced thrombin generation in patients with arterial hypertension

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ARTICLE INFO

Keywords:
Arterial hypertension
Thrombosis
Thrombin generation

ABSTRACT

Background: Arterial hypertension is associated with greater risk of cardiovascular diseases and thrombotic complications, suggesting that hypertension is a prothrombotic state.
Objectives: To investigate the relationship between arterial hypertension and thrombin generation, and between blood pressure level and thrombin generation in hypertensive patients.
Methods: A total of 165 hypertensive patients and 47 healthy adults controls were include in the study. Thrombin generation was assessed in both groups by the Calibrated Automated Thrombogram (CAT) method. Ambulatory blood pressure monitoring (ABPM) was also performed for all patients in the hypertensive group.
Results: Hypertensive patients had significantly higher levels of ETP and peak heights compared to healthy controls; means of ETP 1720.6 ± 267 and 1544.7 ± 302, respectively ($P < 0.001$) and means of peak height were 297.26 ± 48 and, 273 ± 53, respectively ($P < 0.001$). On multivariate linear regression analysis, hypertension remained independently associated with increased ETP ($\beta = 0.185$, $P = 0.047$). Analysis restricted to the hypertensive group with ABPM measurement showed statistically significant correlations between all measures of diastolic blood pressure (DBP) and ETP, and multivariate analysis showed that awake DBP was significantly associated with ETP ($\beta = 0.194$ for each 1-mm Hg increase in awake DBP, $P = 0.012$). Furthermore, hypertensive patients with cardiovascular complications had statistically elevated levels of peak height compared to hypertensive patients without cardiovascular complications.
Conclusions: Hypertensive patients possess enhanced thrombin generation compared healthy controls. Diastolic blood pressure level is independently correlated with increased thrombin generation in hypertensive patients. These findings suggest that arterial hypertension is a prothrombotic state.

1. Introduction

Arterial hypertension is highly prevalent in adult populations and affects almost one billion people worldwide [1]. According to the Global Burden of Disease Study, elevated arterial blood pressure is the leading risk factor for death and disability-adjusted life years lost [2]. Although blood vessels are exposed to high pressure in arterial hypertension, the main complications of arterial hypertension (i.e. myocardial infarction and stroke) are, paradoxically, thrombotic in nature rather than hemorrhagic [3]. While much attention has been focused on the renin-angiotensin system, catecholamines and other neurohormonal mechanisms involved in the pathogenesis of arterial hypertension, the study of the prothrombotic state in arterial hypertension has been relatively neglected. Nevertheless, a growing body of evidence suggests that arterial hypertension confers a hypercoagulable state [4].

The capacity of blood to form thrombin is generally considered the most important blood-borne determinant of hemostasis and thrombosis.

According to a previous study, patients with essential hypertension demonstrate elevated levels of prothrombin fragments (F1 + 2) compared to normotensive individuals. This elevation was predominant in patients with higher blood pressure and vascular complications [5].

In recent years the assessment of thrombin generation, an indicator of overall thrombotic activity, has become readily available with the calibrated automated thrombogram (CAT), yielding reasonably

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https://doi.org/10.1016/j.thromres.2018.11.027
Received 11 October 2018; Received in revised form 14 November 2018; Accepted 27 November 2018
Available online 13 December 2018

consistent results when the same individuals are tested over time [6–9].

A recent study has demonstrated association between CAT parameters and acute ischemic stroke; ETP and peak height were independent predictors of acute ischemic stroke. [10].

This study aim was to investigate whether plasma from patients with arterial hypertension has increased thrombin generation compared to healthy adults, and whether a positive correlation exists between thrombin generation and the level of arterial blood pressure (systolic or diastolic) in hypertensive cohort as measured by 24-h ABPM. Such correlation will further support the hypothesis that hypertension is a prothrombotic state.

2. Materials and methods

2.1. Study population

The study included consecutive ambulatory hypertensive patients referred for the performance of 24-h ABPM in the hypertension clinic at Emek Medical Center (EMC) in Afula, Israel between April 1 and August 31, 2015. Exclusion criteria were age under 18 years, pregnancy, inability to give informed consent, use of anticoagulants (vitamin K antagonists, factor Xa inhibitors, or direct thrombin inhibitors), diagnosis of chronic renal failure, and previous diagnosis of hypercoagulability. In addition we included a group of healthy adults as control group. The local ethics committee approved the study.

2.2. Study procedure

After giving informed consent, participants were interviewed personally, with a detailed questionnaire that included demographic information, previous diagnoses, smoking history, and medication. Blood samples for thrombin generation were collected at the time of enrollment in the control group, while in the study group they were collected after the ABPM devices had been returned. All in all, 165 hypertensive patients were enrolled in the study (hypertension group). Five patients were excluded from the analysis: three because of missing blood samples, and two because of technically unsatisfactory ABPM results. Forty-seven healthy participants from the healthy adult laboratory registry were included as a control group.

2.3. 24 h ABPM

24-h ABPM was performed with the use of an Oscar II device, (Suntech, California). All studies were performed during weekdays. Patients were asked to conduct a normal schedule, but refrain from exercise. The devices were programmed to measure the blood pressure every 20 min during awake hours and every 30 min during sleep hours. In each case, the awake and asleep time was based on patient predictions at the time of the ABPM programming. However, on retrieval of the devices the awake and asleep hours were adjusted according to the actual awake and asleep times the patients reported. Cutoffs for hypertension were 130/80 mm Hg for 24 h, 135/85 mm Hg for the awake period, and 120/70 mm Hg for the asleep period [11]. A technically satisfactory ABPM study had to include at least 20 measurement during the awake period and 7 measurements during the asleep period [11].

2.4. Thrombin generation assay

Six mL of blood were collected using a butterfly needle with tubing of 21 or 23 gauge on sodium citrate into two tubes. The samples were centrifuged twice, first for 15 min at 2500 RPM and then for 10 min at 2000 RPM, within one hour of blood collection to obtain platelet free plasma and stored the plasma at −70 °C for later analysis.

Calibrated automated thrombogram: Thrombin generation was measured on platelet-poor plasma with the CAT technique and the use of 5 pmol/L TF and 4 μmol/L phospholipids. The thrombogram generated 4 parameters by dedicated software (Thrombinoscope™ B.V., Maastricht, the Netherlands): (1) lag time, (2) endogenous thrombin potential (ETP), (3) peak height, and (4) time to peak. Short lag time/time to peak and high ETP/peak height point at hypercoagulable (prothrombotic) state and prolonged lag time/time to peak and decreased ETP/peak height indicate a hypocoagulable (prohemorrhagic) state [9].

2.5. Statistical analyses

Categorical variables were presented with the use of frequencies and percentages and compared with a Chi-squared test; continuous variables were presented via mean standard deviation scores. For each patient, the mean 24-h ABPM was calculated. Thrombin generation parameters were compared between the study group (hypertensive patients) and control group (healthy participants) with the unpaired Student's *t*-test.

One-way ANOVA was used to compare more than two groups. Post-hoc analyses by the Scheffe method was used to assess the differences between the groups.

The correlation between the thrombin generation measurements and the mean 24-h ABPM measurements was estimated by means of the Pearson or Spearman correlation. The associations between categorical or continuous variables and each of the thrombogram parameters (lag time, ETP, peak height, and time to peak) were examined with multivariate general linear model (GLM). The multivariate analysis was performed with the stepwise algorithm (the final model included only variables with $P < 0.05$).

The patients were classified into two groups according to ETP levels. The classification was made according to the sample median ETP. In order to demonstrate the 24-h ABPM trends in both ETP groups, non-parametric local regression (LOESS) was implemented. The graphical presentation included the LOESS trend along with the 95% confidence interval (CI). The statistical analyses were performed with SAS 9.3.

3. Results

3.1. Descriptive statistics

A total of 165 hypertensive patients were enrolled in the study group and 47 healthy participants in the control group.

The baseline characteristics of the study population and the control group are summarized in (Table 1). The mean age of the hypertensive group was 60 ± 13.4 years versus 40 ± 7.5 in the control group, with a significant difference of 19.1 years between the two groups ($P = 0.0001$). The mean BMI was 28.9 ± 5.5 kg/m^2 in the hypertensive group versus 25.66 ± 3.6 in the control group ($P = 0.0002$).

Seventy-five (45.5%) patients were male in the hypertensive group versus 22 (48%) in the control group ($P = 0.9$), 45 (27.3%) were diabetic, 18 (10.9%) suffered from ischemic heart disease, 16 (9.7%) had suffered an ischemic stroke in the past, and 8 (4.9%) suffered from peripheral arterial disease, 22 (13.6%) had at least from one cardiovascular disease (stroke, ischemic heart disease or peripheral arterial disease). Of all patients, 129 (78.2%) had a previous diagnosis of arterial hypertension. Of hypertensive patients, 77.6% had received medication for the reduction of arterial blood pressure. The average number of hypertension drugs per patient was 2.3 ± 1.1 (maximum 5). Forty six percent of hypertensive patients were on one or two hypertensive drugs; 31.5% were on three or more drugs. The most prevalent class of hypertension medication were calcium channel blockers (CCB), taken by 39.4% of hypertensive patients. Forty-six patients (27.9%) received an angiotensin converting enzyme inhibitor (ACEI) and 63 (32.1%) patients received an angiotensin receptor blocker. Other blood pressure lowering drugs included beta-blockers (38.2%), thiazide diuretics (17.6%), alpha-blockers (10.9%), spironolactone (6%), and furosemide (3.6%). Sixty-six patients (44%) were treated

Table 1
Baseline demographic and clinical characteristics of hypertensive patients and controls, and ABPM measurement results in hypertensive patients.

	Hypertension (N = 165)	Control (N = 47)	*P* Values	CI (95%)
Demographic characteristics				
Age (years)	60 ± 13.4	40.87 ± 7.5	$P < 0.0001$	−22.1 to −16.1
Gender (male)	75 (45.5%)	22 (46%)	$P = 0.91$	−16% to 17%
BMI (kg/m^2)	28.9 ± 5.5	25.66 ± 3.74	$P = 0.0002$	−1.51 to −4.87
History of hypertension	129 (78.8%)	0 (0%)	–	–
Current smoker	30 (18.8%)	23 (48%)	$P < 0.0001$	12.9% to 45.2%
Ischemic heart disease	18 (10.9%)	0 (0%)	–	–
Peripheral arterial disease	8 (4.85%)	0 (0%)	–	–
History of ischemic stroke	16 (9.7%)	0 (0%)	–	–
Diabetic	45 (27.3%)	0 (0%)	–	–
Cardiovascular disease	22 (13.6%)	0 (0%)		
Medications				
ACE-I	46 (27.9%)	0 (0%)		
ARB	53 (32.1%)	0 (0%)		
Beta blockers	63 (38.2%)	0 (0%)		
CCB	65 (39.3%)	0 (0%)		
Spirolactone	10 (6.1%)	0 (0%)		
Thiazide	29 (17.6%)	0 (0%)		
Furosemide	6 (3.6%)	0 (0%)		
Alpha blockers	18 (10.9%)	0 (0%)		
Anti-platelet	66 (45%)	0 (0%)		
Statins	75 (45%)	0 (0%)		
Mean 24-h blood pressure measurements				
Mean awake SBP (mm Hg)	139.5 ± 18.5 [138.5, 92–210]	N/A	–	–
Mean awake DBP (mm Hg)	78.7 ± 11.7 [78, 53–119]	N/A	–	–
Mean asleep SBP (mm Hg)	130.1 ± 20.6	N/A	–	–
Mean asleep DBP (mm Hg)	70 ± 11.3	N/A	–	–
Mean overall SBP (mm Hg)	136.6 ± 18.3	N/A	–	–
Mean overall DBP (mm Hg)	76 ± 11.1	N/A	–	–

BMI (body mass index); SBP (systolic blood pressure); DBP (diastolic blood pressure); ETP (endogenous thrombin potential). ACE-I (angiotensin converting enzyme inhibitors); ARB (angiotensin receptor blocker); CCB (calcium channel blockers); Cardiovascular disease (Peripheral artery disease, Ischemic heart disease or Stroke), anti-plaetelets (aspirin or clopidogrel). Categorical variables are presented as frequencies; continuous variables are presented as mean ± standard deviation, CI (confidence interval of the difference).

with either aspirin or clopidogrel. Seventy-five patients (45.5%) received a statin. The average 24-h systolic blood pressure (SBP) was 136.6 ± 18.3 mm Hg. The average 24-h diastolic blood pressure (DBP) was 76 ± 11.1 mm Hg. The average awake SBP was 139.5 ± 18.5 mm Hg. The average awake DBP was 78.7 ± 11.7 mm Hg. The average asleep SBP was 130.1 ± 20.6 mm Hg. The average asleep DBP was 70 ± 11.3 mm Hg. The average 24-h pulse rate was 68.8 ± 9.4 beats per minute (BPM). 37.7% of patients were defined as dippers, meaning that there was a decrease of > 10% in SBP during sleep.

3.2. Association of hypertension (binary variable) and thrombogram parameters

Table 2 compares thrombogram parameters performed by calibrated automated thrombogram between hypertensive study cohort and control group: respectively a) hypertensive patients versus b) healthy participants.

The mean lag time (in minutes) was 3.6 ± 0.58 versus 3.59 ± 0.72 ($P = 0.06$). The mean ETP (nmol/L min) was 1720.6 ± 267.1 versus 1588.7 ± 314 ($P < 0.005$). The mean peak height (nmol/L) was 297.26 ± 48.25 versus 281.1 ± 55.1 ($P = 0.02$). The time to peak (in minutes) was 6.44 ± 0.89 versus 6.6072 ± 1 ($P = 0.81$). Thrombin generation (ETP and peak height) was significantly enhanced in hypertensive patients compared to healthy participants.

To overcome the age differences between the cohort and the control group, hypertensive patients were subdivided into two groups: age ≤ 45 ($n = 35$) and age > 45 ($n = 125$). Their age, ETP, and peak height were compared to those of the control group using One-way ANOVA. Fig. 1A compares the mean age between the control group and the two hypertensive groups. The mean ages were 40.87 ± 7.5 (control group) 40.36 ± 7.9 and 65 ± 8.5 (hypertensive patients). The first two groups were of a similar age, significantly lower age than that of the third group ($P < 0.001$). Fig. 1B compares the mean ETP between the three groups, with mean ETP 1588 ± 314,1765 ± 279, and 1708 ± 263 respectively. Both hypertensive groups had statically significant elevation in mean ETP, compared to healthy adults (($P < 0.05$). Fig. 1C compares the peak height between the groups. The mean peak height was 281 ± 52, 286 ± 52, 304 ± 46 respectively. Hypertensive patients older than 45 demonstrated a significant elevation of peak height compared to the control group ($P < 0.001$); the rest

Table 2
Comparison of the thrombogram parameters between patients with hypertension and healthy controls.

Variables		Hypertension ($N = 162$)	Control ($N = 46$)	*P*-Value	CI (95%)
Thrombin generation	ETP (nM∗min)	1720.6 ± 267.1	1588.7 ± 314	$P < 0.005$	−223.5 to −40.4
	Peak height	297.26 ± 48.25	281.1 ± 55.1	$P = 0.02$	−35.4 to −2.9
	Lag time	3.61 ± 0.58	3.59 ± 0.72	$P = 0.06$	−0.17 to 0.23
	Time to peak	6.44 ± 0.89	6.6072 ± 1	$P = 0.81$	−0.34 to 0.26

ETP (endogenous thrombin potential). Continuous variables are presented as mean ± standard deviation. CI: Confidence interval of the difference.

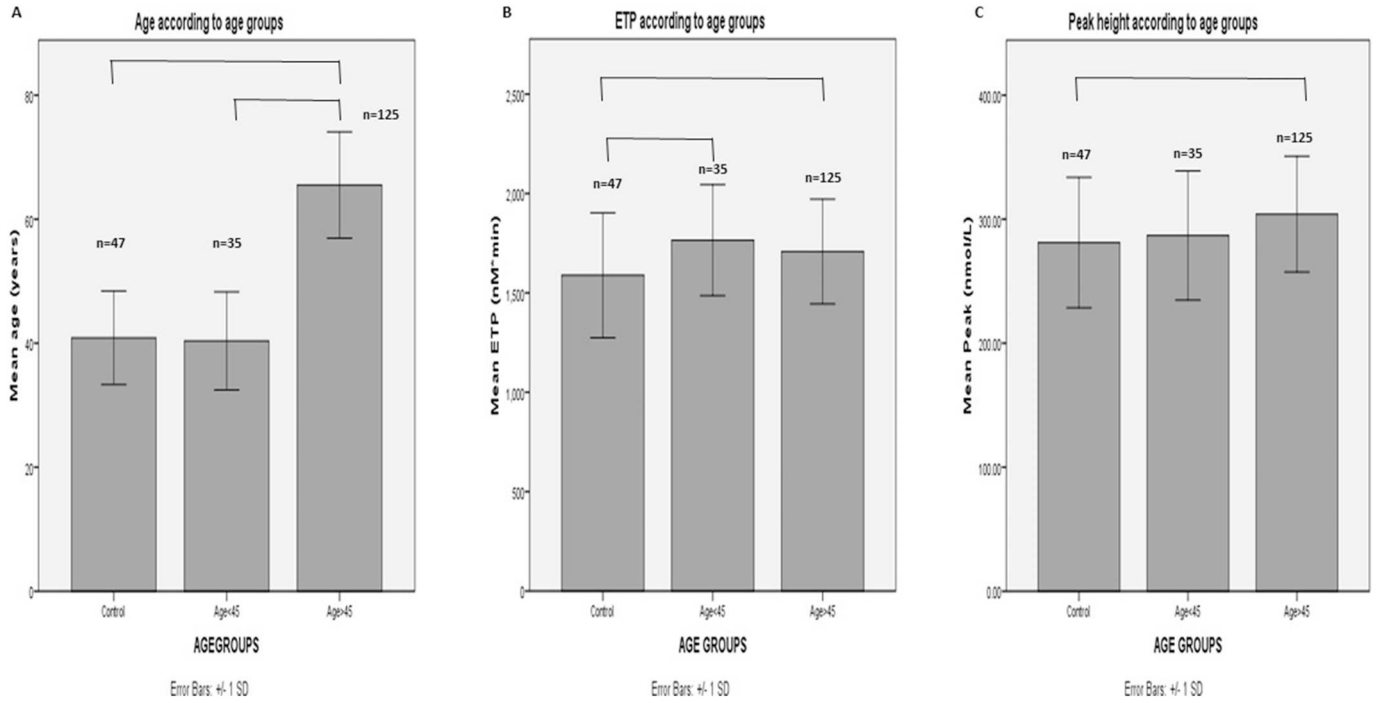

Fig. 1. Comparison of age, ETP and peak height according to age groups, statistically significant differences between groups are connected by a line ($P < 0.05$).

of the groups had similar peak heights.

Similarly, hypertensive patients were subdivided into two groups: BMI ≤ 30 ($n = 91$) and age > 45 ($n = 69$). Their BMI, ETP, and peak height were compared to those of the control group. Fig. 2A compares the mean BMI between control group, hypertensive groups with BMI ≤ 30 and BMI > 30 respectively. The mean BMI was 25.66 ± 3.7, 25.2 ± 2.4 and 33.7 ± 4.6 respectively. The BMI of the first two groups was similar, but significantly lower than that of the third group ($P < 0.0001$). Fig. 2B compares the mean ETP between the groups with mean ETP 1588 ± 314, 1671 ± 256, 1786 ± 268 respectively. Hypertensive patients with BMI above 30 had statically significant elevation in mean ETP compared to hypertensive patients with BMI ≤ 30 ($P < 0.05$) and healthy adults ($P < 0.0001$). Fig. 1C compares the peak height between the same groups. The mean peak height was 281 ± 52, 288 ± 47, 316 ± 45 respectively. Hypertensive patients with BMI above 30 had statically significant elevation in the mean peak height compared to hypertensive patients with BMI ≤ 30 ($P < 0.0001$) and healthy adults ($P < 0.0001$).

As shown in Table 3, hypertension remained independently associated with increased ETP (β = 0.185, $P = 0.047$) on multivariable linear regression analysis that accounted for relevant potential confounders.

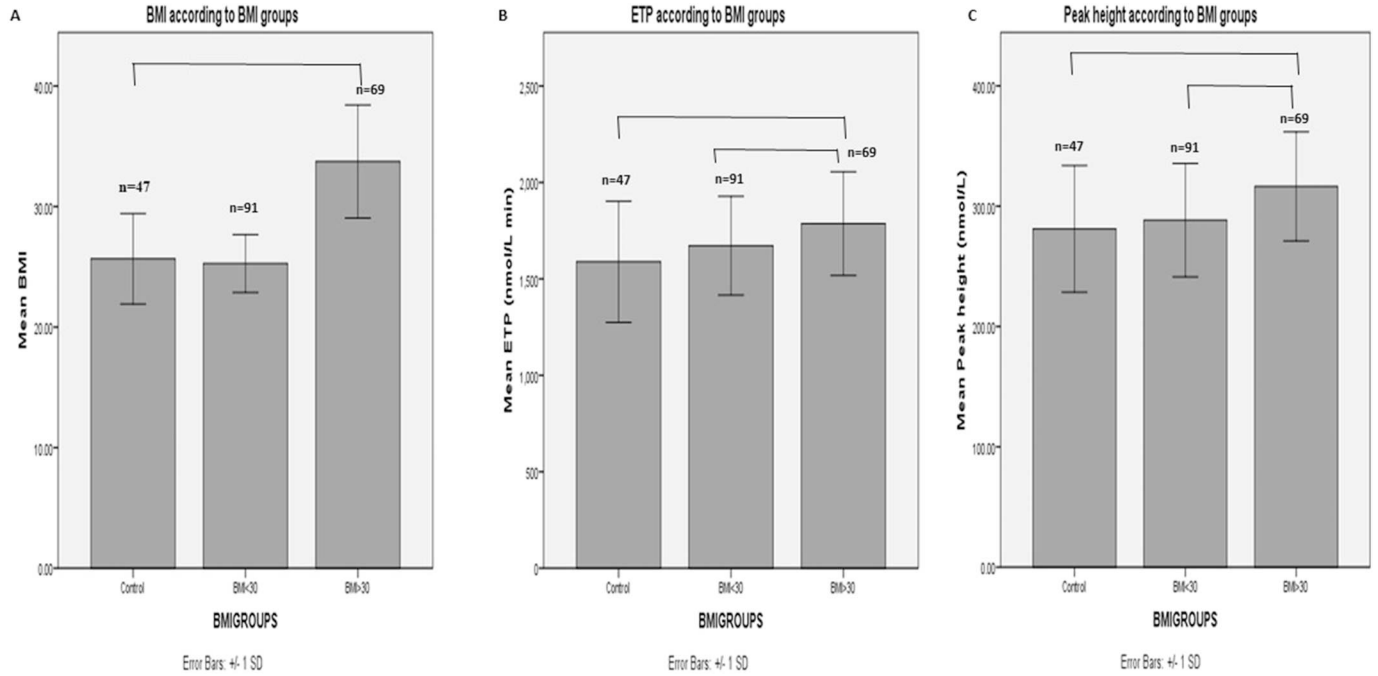

Fig. 2. Comparison of BMI, ETP and peak height according to BMI groups, statistically significant differences between groups are connected by a line ($P < 0.05$).

Table 3
The association of hypertension (binary variable) and thrombogram measurements ($N = 212$).

Model adjustments	ETP		Peak height		Lag time		Time to peak	
	β	*P*	β	*P*	β	*P*	β	*P*
Crude	0.194	0.05	0.16	0.021	0.022	0.758	−0.016	0.819
Age and sex	0.27	0.001	0.084	0.307	0.061	0.455	0.11	0.173
Age, sex, BMI, smoking	0.188	0.032	0.032	0.712	0.035	0.688	0.084	0.346
*Multivariable model	0.185	0.047	0.001	0.993	0.063	0.508	0.121	0.21

β; the standardized regression coefficients are shown, BMI (body mass index), ETP (endogenous thrombin potential), * Adjusted for age, sex, BMI, smoking status, diabetes mellitus, ischemic heart disease, stroke, peripheral artery disease, ACE-I, ARB, CCB, spirolactone, alpha blockers, thiazides anti-platelet and statins.

Table 4
Pearson correlation coefficient for the relationship between the thrombogram parameters and blood pressure (24-ABPM) values among patients with hypertension ($N = 160$).

	Lag-time	ETP	Peak	Time to peak
Age	−0.079	−0.110	0.159*	−0.231**
BMI	0.214	0.220**	0.273**	0.079
No. of drugs	0.035	−0.017	0.204**	−0.092
PP	0.087	−0.037	0.102	−0.024
SBP-AW	0.103	0.103	0.147	0.031
DBP-AW	0.056	0.206**	0.118	0.076
SBP 24H	0.113	0.109	0.170*	0.025
DBP 24H	0.070	0.235**	0.147	0.075
SBP-ASL	0.131	0.081	0.194*	0.010
DBP-ASL	0.103	0.219**	0.175*	0.062
P 24H	0.117	0.174*	0.203*	0.033

ETP (endogenous thrombin potential); PP (pulse pressure); SBP-AW (awake systolic blood pressure); DBP-AW (awake diastolic blood pressure); SBP 24H (overall SBP); DBP 24H (overall DBP); SBP-ASL (asleep SBP); DBP-ASL (asleep DBP); *P* 24H (overall pulse).

* $P < 0.05$.

** $P < 0.01$.

3.3. Association of blood pressure level and thrombogram measurements among hypertensive patients

The correlation between the thrombogram parameters, age, BMI and blood pressure are depicted in Table 4. BMI had a statistically significant, positive but weak, correlation with ETP and peak height; the correlation coefficients were 0.22 and 0.273 respectively. Age also had a significant, positive but weak, correlation with peak height and time to peak, the correlation coefficients were 0.159 and 0.231 respectively.

Diastolic blood pressure and 24-h pulse had a significant, positive but weak, correlation with ETP. The correlation coefficients of mean awake DBP, mean asleep DBP, mean 24-h DBP and ETP were 0.21, 0.22, and 0.24, respectively. The correlation coefficient of mean 24-h pulse and ETP was 0.174 (Table 4).

In addition, systolic blood pressure and 24-pulse pulse had a significant, positive albeit very weak, correlation with peak height. The correlation coefficients of mean SBP, mean asleep SBP, mean 24-h DBP and peak height were 0.17, 0.194, and 0.203, respectively. The correlation coefficient of mean 24-h pulse and ETP was 0.174. The number of hypertension drugs had a significant, positive but weak, correlation with peak height; the correlation coefficient was 0.204. No significant correlation was detected between pulse pressure and the thrombogram parameters (Table 4).

Multiple linear regression analysis was conducted to assess the independent relationship between the levels the different blood pressure measurements and ETP. After adjustment for age, sex, BMI, smoking status, comorbid condition and medications only awake-diastolic blood pressure positively and significantly correlated with ETP with β = 0.194 and *P*-value = 0.012. However, awake-diastolic blood pressure was not independently associated with other thrombogram measurements (Table 5).

The equation that describes the model was ETP = 1084 + (9.921 ∗ BMI) + (4.472 ∗ mean DBP-AW) For each increase of one unit in BMI the ETP increased by 9.921 units on average. For each increase of one mm Hg in awake DBP, the ETP rose by 4.472 points on average.

In order to demonstrate the DBP trends over time, local regression (LOESS) curves were performed for patients with high and low ETP (classified by using the sample median ETP cutoff-point), showing higher DBP levels among patients with higher ETP (Fig. 3).

Thrombogram parameters were compared according to the presence or absence of cardiovascular diseases. Only peak height demonstrated a significant difference between the two groups, the mean peak height was 333.4 (nM) in cardiovascular patients versus 295.75 (nM) in later group ($P = 0.001$). In addition, patients with cardiovascular diseases were significantly older ($P = 0.001$).

The remaining thrombogram parameters showed no significant difference between the two groups; ETP ($P = 0.292$), lag time ($P = 0.086$), time to peak ($P = 0.719$). (Table 6).

A multiple linear regression analysis was conducted to assess the relationship between the thrombogram parameters and the presence of cardiovascular disease. After adjustment for age, sex, diabetes mellitus, smoking status, patients with cardiovascular disease had significantly higher peak height and prolonged lag time than patients without cardiovascular disease (Table 7, model 1). Similar coefficients were

Table 5
The association between awake-diastolic blood pressure values and thrombogram measurements.

Model adjustments	ETP		Peak height		Lag time		Time to peak	
	β	*P*	β	*P*	β	*P*	β	*P*
Crude	0.206	0.009	0.128	0.137	0.056	0.482	0.076	0.342
Age and sex	0.2	0.018	0.194	0.021	0.013	0.879	−0.008	0.925
Age, sex, BMI, smoking	0.185	0.027	0.174	0.033	−0.004	0.962	−0.014	0.864
*Multivariable model	0.194	0.012	0.133	0.077	0.044	0.576	0.011	0.992

B; standardized regression coefficients for 1-point increase in diastolic blood pressure. * Adjusted for age, sex, BMI, smoking status, diabetes mellitus, ischemic heart disease, stroke, peripheral artery disease, use of ACE-I, ARB, CCB, spirolactone, alpha blockers, thiazides, anti-platelet and statins. BMI (body mass index), ETP (endogenous thrombin potential).

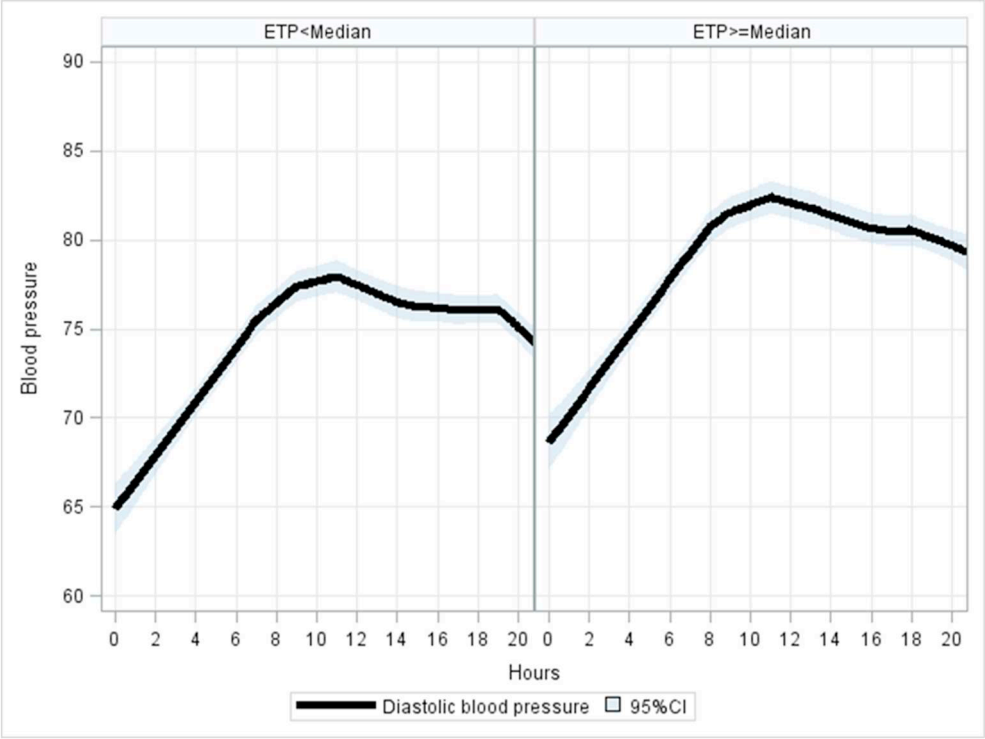

Fig. 3. Local regression of the DBP trends over 20 h, stratified by endogenous thrombin potential (ETP) levels in nM ∗ min, based on the sample ETP median (1716 nM ∗ min).

Table 6
Comparison of the thrombogram parameters in hypertensive patients according to the presence of cardiovascular disease.

Thrombogram parameters	Cardiovascular disease (n = 21) Mean ± SD	Non-cardiovascular disease (*n* = 139) Mean ± SD	*P* value	CI (95%)
Age	68.90 ± 3.51	58.66 ± 10.54	0.001	4.195 to 16.291
BMI	28.8176 ± 3.51	28.9804 ± 5.78	0.900	−2.729 to 2.4
ETP (nM ∗ min)	1780.02 ± 305.39	1713.90 ± 261.3	0.292	−57.47 to 189.720
Peak height (nM)	333.40 ± 49.68	295.75 ± 46.11	0.001	16.11 to 59.19
Lag time (min)	3.833 ± 0.62	3.595 ± 0.58	0.086	−0.034 to 0.51
Time to peak (min)	6.502 ± 0.897	6.579 ± 0.906	0.719	−0.494 to 0.342

BMI (body mass index); ETP (endogenous thrombin potential). Continuous variables are presented as mean ± standard deviation, CI (Confidence Interval) of the difference.

Table 7
Multiple linear regression analyses for the presence of cardiovascular disease.

Model	Model adjustments	ETP		Peak height		Lag time		Time to peak	
		β	*P*	β	*P*	β	*P*	β	*P*
0	Crude	0.086	0.276	0.67	0.001	0.134	0.089	−0.03	0.668
1	Age, sex, BMI, smoking, DM	0.131	0.1	0.254	0.001	0.176	0.03	0.031	0.69
2	Model 1 + DBP	0.132	0.095	0.255	0.001	0.172	0.03	0.034	0.67
3	Model 1 + SBP	0.123	0.124	0.247	0.002	0.173	0.033	0.033	0.688
4	*Multivariable model	0.112	0.146	0.248	0.001	0.125	0.111	0.036	0.655

β; the standardized regression coefficients are shown, BMI (body mass index), DM (diabetes melitus) ETP (endogenous thrombin potential), SBP (systolic blood pressure), DBP (diastolic blood pressure). * Adjusted for age, sex, BMI, smoking status, diabetes mellitus, use of ACE-I, ARB, CCB, spirolactone, alpha blockers, thiazides anti-platelet and statins.

demonstrated after adjusting for diastolic or systolic blood pressure and in the multivariable model (Table 7, model 2–4).

4. Discussion

Arterial hypertension is one of leading risk factors for thrombotic complications such as myocardial infarction and stroke [3]. Our study demonstrated that hypertensive patients have elevated levels of thrombin generation (ETP and peak height) compared to healthy young individuals. This is further evidence that arterial hypertension is a prothrombotic state.

Furthermore, we studied whether a correlation exists between 24-h

ABPM and CAT parameters and found that awake DBP and BMI were independently associated with ETP. This positive correlation between the level diastolic blood pressure and ETP suggests that elevated diastolic blood pressure is associated with a prothrombotic state. Which, to our knowledge, is the first time such findings have been presented. Surprisingly, such correlation did not exist with systolic blood pressure.

Previous research has shown that among hypertensive patients, rheological and hemostatic factors — such as plasma viscosity, hematocrit, red cell rigidity, red blood cell aggregation, fibrinogen, von Willibrand factor (vWF), plasminogen activator inhibitor type 1 (PAI-1) level and tissue plasminogen activator (tPA) activity — are increased [12]. In addition, raised fibrinogen and vWF levels in hypertensive patients are associated with respectively left ventricular hypertrophy and microalbuminuria, which are established markers of target organ damage and predictors of increased cardiovascular risk [13,14]. Several epidemiological studies have related hemostatic and rheological factors to the incidence of ischemic heart disease and stroke in patients with hypertension [15–18].

Endothelial dysfunction may also play a role in the pathogenesis of thrombosis in arterial hypertension. A defective endothelial L-arginine/nitric oxide (NO) pathway characterizes arterial hypertension. This leads to the decreased bioavailability of NO in the vasculature of patients with arterial hypertension. Reduced NO bioavailability leads to the expression of proinflammatory, proatherosclerotic, and prothrombotic state in the vasculature [19]. These consequences are directly connected with complications of arterial hypertension, such as increased platelet activation and increased thrombotic risk [20].

The renin angiotensin aldosterone (RAAS) system may also play a role in inducing a prothrombotic state in hypertension. Numerous deleterious effects of angiotensin II — including vasoconstriction, sympathetic nervous activation, smooth muscle cell growth and proliferation, vascular inflammation, generation of reactive oxygen species and endothelial dysfunction — are mediated through the angiotensin II type 1 (AT1) receptor. Angiotensin II opposes the effect of NO, stimulates the production of adhesion factors and plasminogen activator inhibitor-1 (PAI-1), thus promoting the risk of thrombosis. It has been suggested that treating hypertensive patients with ACEI may reverse the prothrombotic state [21]. In our study, 99 patients (60%) were treated with either an ACEI or an angiotensin receptor blocker (ARB). However, we did not find a correlation between use of either ACEI or ARB and ETP. Experimental data also demonstrated that statins may exert a direct antithrombotic effect in models of arterial and venous thrombosis via a mechanism unrelated to cholesterol-lowering activity. This suggests that statins could inhibit several pathways of hemostasis, including platelet activation and coagulation cascade [22]. Tripodi et al. showed that treatment with statins over a period of two months resulted in a significant reduction of median ETP [23]. In our study, 75 patients (45.5%) were on statin therapy. However, we did not find a correlation between statin treatment and ETP.

This study demonstrated significant independent correlation between obesity and increased ETP and peak height. Beijers et al. showed that body fat mass, particularly a central pattern of fat distribution, was associated with higher levels of TG in elderly women, but not in men [24]. Ay et al. demonstrated a significant decrease in TG parameters in morbidly obese patients after bariatric surgery [25]. Finally, Sonnevi et al. showed that obesity correlated with increased TG in women with venous thromboembolism and that the main determinants of this hypercoagulable state were increased levels of fibrinogen and prothrombin [26].

In our study diabetes mellitus had no association with ETP, Our findings regarding diabetic patients are incongruous with previous studies by Tripodi et al. and Beijers et al., which demonstrated increased TG in patients with DM [27,28]. Furthermore, Kim et al. demonstrated that patients with DM have increased TG, accompanied by high levels of coagulation factors and a low level of protein C [29]. Nevertheless, our findings regarding diabetic patients should be interpreted with caution, because our study was not designed to assess diabetic patients.

The multivariate analysis we performed demonstrated that DBP and increased BMI are independently associated with ETP. Our results demonstrate a significant, positive, correlation between cardiovascular disease and peak height (Table 7), with a significant increase in mean peak height in hypertensive patients with cardiovascular complications, compared to hypertensive patients without cardiovascular complications (Table 5). According to Carcaillon et al. thrombin generation and peak height were associated with acute ischemic stroke [10]. Future studies should investigate whether peak height can be used as a biomarker to predict future cardiovascular events in patients with hypertension.

Unfortunately, our study suffered from several limitations. One of the study limitations was age and BMI, comorbidities and medications differences between hypertensive patients and the healthy control group. Age has been shown to correlate positively with CAT parameters, age-related thrombin-generation differences has predominantly been demonstrated between adult and children populations [30,31]. However, we demonstrated in Fig. 1B that hypertensive patients with similar mean age had statistically significant elevation in ETP compared to the control group. In addition, BMI was significantly higher in the hypertensive group, as shown in Table 1. hypertensive patients with low BMI had 83.2 mean difference from the healthy adults, however without reaching statistical significance ($P = 0.266$) probably due to low statistical power ($\beta = 0.48$). Therefore, our results regarding ETP differences between hypertensive patients and healthy individuals should be interpreted with caution.

However, the association between hypertension and ETP remained positive and statistically significant after adjusting for all potential and available confounders such as; age, BMI, gender, smoking status, comorbid conditions and medications (Table 3).

Another limitation of our study is lack of information regarding; waist circumference, lipid profile, insulin levels, markers of inflammation, other markers of thrombosis, or markers of endothelial dysfunction. In addition, no markers of target organ damage, such as urine albumin/creatinine ratio or left ventricular hypertrophy. Most importantly, we did not measure the effect of reducing arterial blood pressure on ETP, so that we cannot prove that increased DBP is the cause of increased ETP. Furthermore, despite the correlation between DBP and ETP being statistically significant, the correlation coefficient was only 0.2, which is relatively weak.

In conclusion, patients with arterial hypertension have elevated thrombin-generation parameters compared to healthy adults. Diastolic blood pressure level positively correlated with ETP among hypertensive patients. In addition, hypertensive patients with cardiovascular complications demonstrated increased peak height, compared to hypertensive patients without cardiovascular complications. Our study suggests that arterial hypertension points to a prothrombotic state.

Ethics and patient consent

Ethical approval to report this case was obtained from the ethics committee of Haemek Medical Center, Afula (Approval identifier: EMC-98-14). Written informed consent was obtained from the patient for their anonymized information to be published in this article.

Previous presentation of the whole or part of the work presented in the article

None.

Sources of support

None.

Disclosure of funding

None.

Potential conflicts of interest

None.

References

[1] P.M. Kearney, M. Whelton, K. Reynolds, P. Muntner, P.K. Whelton, J. He, Global burden of hypertension: analysis of worldwide data, Lancet 365 (2005) 217–223, https://doi.org/10.1016/S0140-6736(05)17741-1.

[2] S.S. Lim, T. Vos, A.D. Flaxman, G. Danaei, K. Shibuya, H. Adair-Rohani, M.A. AlMazroa, M. Amann, H.R. Anderson, K.G. Andrews, M. Aryee, C. Atkinson, L.J. Bacchus, A.N. Bahalim, K. Balakrishnan, J. Balmes, S. Barker-Collo, A. Baxter, M.L. Bell, J.D. Blore, F. Blyth, C. Bonner, G. Borges, R. Bourne, M. Boussinesq, M. Brauer, P. Brooks, N.G. Bruce, B. Brunekreef, C. Bryan-Hancock, C. Bucello, R. Buchbinder, F. Bull, R.T. Burnett, T.E. Byers, B. Calabria, J. Carapetis, E. Carnahan, Z. Chafe, F. Charlson, H. Chen, J.S. Chen, A.T.-A. Cheng, J.C. Child, A. Cohen, K.E. Colson, B.C. Cowie, S. Darby, S. Darling, A. Davis, L. Degenhardt, F. Dentener, D.C. Des Jarlais, K. Devries, M. Dherani, E.L. Ding, E.R. Dorsey, T. Driscoll, K. Edmond, S.E. Ali, R.E. Engell, P.J. Erwin, S. Fahimi, G. Falder, F. Farzadfar, A. Ferrari, M.M. Finucane, S. Flaxman, F.G.R. Fowkes, G. Freedman, M.K. Freeman, E. Gakidou, S. Ghosh, E. Giovannucci, G. Gmel, K. Graham, R. Grainger, B. Grant, D. Gunnell, H.R. Gutierrez, W. Hall, H.W. Hoek, A. Hogan, H.D. Hosgood, D. Hoy, H. Hu, B.J. Hubbell, S.J. Hutchings, S.E. Ibeanusi, G.L. Jacklyn, R. Jasrasaria, J.B. Jonas, H. Kan, J.A. Kanis, N. Kassebaum, N. Kawakami, Y.-H. Khang, S. Khatibzadeh, J.-P. Khoo, C. Kok, F. Laden, R. Lalloo, Q. Lan, T. Lathlean, J.L. Leasher, J. Leigh, Y. Li, J.K. Lin, S.E. Lipshultz, S. London, R. Lozano, Y. Lu, J. Mak, R. Malekzadeh, L. Mallinger, W. Marcenes, L. March, R. Marks, R. Martin, P. McGale, J. McGrath, S. Mehta, Z.A. Memish, G.A. Mensah, T.R. Merriman, R. Micha, C. Michaud, V. Mishra, K.M. Hanafiah, A.A. Mokdad, L. Morawska, D. Mozaffarian, T. Murphy, M. Naghavi, B. Neal, P.K. Nelson, J.M. Nolla, R. Norman, C. Olives, S.B. Omer, J. Orchard, R. Osborne, B. Ostro, A. Page, K.D. Pandey, C.D. Parry, E. Passmore, J. Patra, N. Pearce, P.M. Pelizzari, M. Petzold, M.R. Phillips, D. Pope, C.A. Pope, J. Powles, M. Rao, H. Razavi, E.A. Rehfuess, J.T. Rehm, B. Ritz, F.P. Rivara, T. Roberts, C. Robinson, J.A. Rodriguez-Portales, I. Romieu, R. Room, L.C. Rosenfeld, A. Roy, L. Rushton, J.A. Salomon, U. Sampson, L. Sanchez-Riera, E. Sanman, A. Sapkota, S. Seedat, P. Shi, K. Shield, R. Shivakoti, G.M. Singh, D.A. Sleet, E. Smith, K.R. Smith, N.J. Stapelberg, K. Steenland, H. Stöckl, L.J. Stovner, K. Straif, L. Straney, G.D. Thurston, J.H. Tran, R. van Dingenen, A. van Donkelaar, J.L. Veerman, L. Vijayakumar, R. Weintraub, M.M. Weissman, R.A. White, H. Whiteford, S.T. Wiersma, J.D. Wilkinson, H.C. Williams, W. Williams, N. Wilson, A.D. Woolf, P. Yip, J.M. Zielinski, A.D. Lopez, C.J. Murray, M. Ezzati, A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010, Lancet 380 (2012) 2224–2260, https://doi.org/10.1016/S0140-6736(12)61766-8.

[3] G.Y.H. Lip, A.D. Blann, State? Endothelium and Fibrinolysis in Hypertension: Important Facets of a Prothrombotic, 52 (2008), pp. 218–219, https://doi.org/10.1161/HYPERTENSIONAHA.108.114876.

[4] G. Lip, Hypertension and the prothrombotic state, J. Hum. Hypertens. 14 (2000) 687–690, https://doi.org/10.1038/sj.jhh.1001051.

[5] A. Trifiletti, R. Scamardi, M.A. Pizzoleo, G. Sottilotta, N. Barbera, N. Frisina, Increased indexes of thrombin activation in advanced stages of hypertension, Pathophysiol. Haemost. Thromb. 31 (2001) 49–54, https://doi.org/10.1159/000048044.

[6] H.C. Hemker, P. Giesen, R. AlDieri, V. Regnault, E. de Smed, R. Wagenvoord, T. Lecompte, S. Béguin, The Calibrated Automated Thrombogram (CAT): a universal routine test for hyper- and hypocoagulability, Pathophysiol. Haemost. Thromb. 32 (2002) 249–253, https://doi.org/10.1159/000073575.

[7] R. Al Dieri, B. de Laat, H.C. Hemker, Thrombin generation: what have we learned? Blood Rev. 26 (2012) 197–203, https://doi.org/10.1016/j.blre.2012.06.001.

[8] G. Campo, R. Pavasini, A. Pollina, L. Fileti, J. Marchesini, M. Tebaldi, R. Ferrari, Thrombin generation assay: a new tool to predict and optimize clinical outcome in cardiovascular patients? Blood Coagul. Fibrinolysis 23 (2012) 680–687, https://doi.org/10.1097/MBC.0b013e328355111f.

[9] E. Castoldi, J. Rosing, Thrombin generation tests, Thromb. Res. 127 (Suppl. 3) (2011) S21–S25, https://doi.org/10.1016/S0049-3848(11)70007-X.

[10] L. Carcaillon, M. Alhenc-Gelas, Y. Bejot, C. Spaft, P. Ducimetiere, K. Ritchie, J.-F. Dartigues, P.-Y. Scarabin, Increased thrombin generation is associated with acute ischemic stroke but not with coronary heart disease in the elderly: the three-city cohort study, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 1445–1451, https://doi.org/10.1161/ATVBAHA.111.223453.

[11] E. O'Brien, G. Parati, G. Stergiou, R. Asmar, L. Beilin, G. Bilo, D. Clement, A. de la Sierra, P. de Leeuw, E. Dolan, R. Fagard, J. Graves, G.A. Head, Y. Imai, K. Kario, E. Lurbe, J.-M. Mallion, G. Mancia, T. Mengden, M. Myers, G. Ogedegbe, T. Ohkubo, S. Omboni, P. Palatini, J. Redon, L.M. Ruilope, A. Shennan, J.A. Staessen, G. van Montfrans, P. Verdecchia, B. Waeber, J. Wang, A. Zanchetti, Y. Zhang, European society of hypertension working group on blood pressure monitoring, European society of hypertension position paper on Ambulatory Blood Pressure Monitoring, J. Hypertens. 31 (2013) 1731–1768, https://doi.org/10.1097/HJH.0b013e328363e964.

[12] A. Lee, The role of rheological and haemostatic factors in hypertension, J. Hum. Hypertens. 11 (1997) 767–776, https://doi.org/10.1038/sj.jhh.1000556.

[13] G.Y. Lip, A.D. Blann, A.F. Jones, P.L. Lip, D.G. Beevers, Relation of endothelium, thrombogenesis, and hemorheology in systemic hypertension to ethnicity and left ventricular hypertrophy, Am. J. Cardiol. 80 (1997) 1566–1571 http://www.ncbi.nlm.nih.gov/pubmed/9416937 (accessed September 14, 2018).

[14] R. Pedrinelli, O. Giampietro, F. Carmassi, E. Melillo, G. Dell'Omo, G. Catapano, E. Matteucci, L. Talarico, M. Morale, F. De Negri, Microalbuminuria and endothelial dysfunction in essential hypertension, Lancet (London, England) 344 (1994) 14–18 http://www.ncbi.nlm.nih.gov/pubmed/7912295 (accessed September 14, 2018).

[15] J.W. Yarnell, I.A. Baker, P.M. Sweetnam, D. Bainton, J.R. O'Brien, P.J. Whitehead, P.C. Elwood, Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies, Circulation 83 (1991) 836–844 http://www.ncbi.nlm.nih.gov/pubmed/1999035 (accessed September 14, 2018).

[16] G.D. Lowe, A.J. Lee, A. Rumley, J.F. Price, F.G. Fowkes, Blood viscosity and risk of cardiovascular events: the Edinburgh artery study, Br. J. Haematol. 96 (1997) 168–173 http://www.ncbi.nlm.nih.gov/pubmed/9012704 (accessed September 14, 2018).

[17] M.C. Stone, J.M. Thorp, Plasma fibrinogen–a major coronary risk factor, J. R. Coll. Gen. Pract. 35 (1985) 565–569 http://www.ncbi.nlm.nih.gov/pubmed/4093900 (accessed September 14, 2018).

[18] S. Agewall, J. Wikstrand, B. Fagerberg, Prothrombin fragment 1 + 2 is a risk factor for myocardial infarction in treated hypertensive men, J. Hypertens. 16 (1998) 537–541 http://www.ncbi.nlm.nih.gov/pubmed/9797200 (accessed September 14, 2018).

[19] E. Gkaliagkousi, S. Douma, C. Zamboulis, A. Ferro, Nitric oxide dysfunction in vascular endothelium and platelets: role in essential hypertension, J. Hypertens. 27 (2009) 2310–2320, https://doi.org/10.1097/HJH.0b013e328330e89a.

[20] E. Gkaliagkousi, G. Passacquale, S. Douma, C. Zamboulis, A. Ferro, Platelet activation in essential hypertension: implications for antiplatelet treatment, Am. J. Hypertens. 23 (2010) 229–236, https://doi.org/10.1038/ajh.2009.247.

[21] A. Remková, H. Kratochvíl'ová, J. Ďurina, Impact of the therapy by renin–angiotensin system targeting antihypertensive agents perindopril versus telmisartan on prothrombotic state in essential hypertension, J. Hum. Hypertens. 22 (2008) 338–345, https://doi.org/10.1038/sj.jhh.1002328.

[22] F. Violi, C. Calvieri, D. Ferro, P. Pignatelli, Statins as antithrombotic drugs, Circulation 127 (2013) 251–257, https://doi.org/10.1161/CIRCULATIONAHA.112.145334.

[23] A. Tripodi, F. Pellegatta, V. Chantarangkul, L. Grigore, K. Garlaschelli, A. Baragetti, L. Lemma, A. Catapano, Statins decrease thrombin generation in patients with hypercholesterolemia, Eur. J. Intern. Med. 25 (2014) 449–451, https://doi.org/10.1016/j.ejim.2014.03.016.

[24] H.J.B.H. Beijers, I. Ferreira, H.M.H. Spronk, B. Bravenboer, J.M. Dekker, G. Nijpels, H. ten Cate, C.D.A. Stehouwer, Body composition as determinant of thrombin generation in plasma: the Hoorn study, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 2639–2647, https://doi.org/10.1161/ATVBAHA.110.211946.

[25] L. Ay, H.-P. Kopp, J.-M. Brix, C. Ay, P. Quehenberger, G.-H. Schernthaner, I. Pabinger, G. Schernthaner, Thrombin generation in morbid obesity: significant reduction after weight loss, J. Thromb. Haemost. 8 (2010) 759–765, https://doi.org/10.1111/j.1538-7836.2010.03766.x.

[26] K. Sonnevi, S.N. Tchaikovski, M. Holmström, J.P. Antovic, K. Bremme, J. Rosing, G. Lärfars, Obesity and thrombin-generation profiles in women with venous thromboembolism, Blood Coagul. Fibrinolysis 24 (2013) 547–553, https://doi.org/10.1097/MBC.0b013e32835f93d5.

[27] A. Tripodi, A. Branchi, V. Chantarangkul, M. Clerici, G. Merati, A. Artoni, P.M. Mannucci, Hypercoagulability in patients with type 2 diabetes mellitus detected by a thrombin generation assay, J. Thromb. Thrombolysis 31 (2011) 165–172, https://doi.org/10.1007/s11239-010-0506-0.

[28] H.J.B.H. Beijers, I. Ferreira, H.M.H. Spronk, B. Bravenboer, J.M. Dekker, G. Nijpels, H. ten Cate, C.D.A. Stehouwer, Impaired glucose metabolism and type 2 diabetes are associated with hypercoagulability: potential role of central adiposity and low-grade inflammation – the Hoorn study, Thromb. Res. 129 (2012) 557–562, https://doi.org/10.1016/j.thromres.2011.07.033.

[29] H.K. Kim, J.E. Kim, S.H. Park, Y. Il Kim, I.S. Nam-Goong, E.S. Kim, High coagulation factor levels and low protein C levels contribute to enhanced thrombin generation in patients with diabetes who do not have macrovascular complications, J. Diabetes Complicat. 28 (2014) 365–369, https://doi.org/10.1016/j.jdiacomp.2014.01.006.

[30] A.W.J.H. Dielis, E. Castoldi, H.M.H. Spronk, R. Van Oerle, K. Hamulyák, H. Ten Cate, J. Rosing, Coagulation factors and the protein C system as determinants of thrombin generation in a normal population, J. Thromb. Haemost. 6 (2007) 125–131, https://doi.org/10.1111/j.1538-7836.2007.02824.x.

[31] H. Haidl, C. Cimenti, B. Leschnik, D. Zach, W. Muntean, Age-dependency of thrombin generation measured by means of calibrated automated thrombography (CAT), Thromb. Haemost. 95 (2006) 772–775 http://www.ncbi.nlm.nih.gov/pubmed/16676066 (accessed September 15, 2018).