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ORIGINAL PAPER

Cardiovascular risk factors are important determinants of platelet-dependent thrombin generation in adult survivors of childhood cancer

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Abstract

Cardiovascular disease is the most frequent non-malignant cause of morbidity and mortality in adult survivors of childhood or adolescent cancer. Thrombin generation (TG) analysis gives insight in hypercoagulability as an important mechanism linked to cardiovascular risk factors (CVRFs). In 200 individuals, from the cardiac and vascular late sequelae in long-term survivors of childhood cancer study, TG in platelet-rich plasma (PRP) and platelet-free plasma (PFP) at 1pM tissue factor was investigated. Endogenous thrombin potential (ETP) and peak height were the analysed parameters of a TG curve. Sex-specific multivariable linear regression analysis adjusted for age and CVRFs was used to assess the clinical determinants of TG. Females presented with higher ETP and peak height compared to males, both in PRP and PFP. Hypertension (beta estimate, ß: 184.8 [90.7; 278.8]), obesity (ß: 161.9 [63.9; 259.5]), and HbA1c (ß: 715.6 [97.4; 1333.8]) were associated with higher ETP in PRP only. ETP in PRP was positively associated with obesity and HbA1c in both males and females and with dyslipidemia (ß: 253.07 [72.92; 433.22]) and systolic hypertension (ß: 436.7 [119.02; 754.39]) in females only. CVRFs showed no association with TG variables in PFP. In conclusion, this study presents an important relation between traditional CVRFs and TG in the presence of platelets only. Sex-specific differences in TG with females presenting with higher TG, particularly those with dyslipidemia and systolic hypertension, were demonstrated. These results highlight the potential of the platelet-coagulant function in identifying cancer survivors at higher risk for adverse cardiovascular events.

Keywords Cancer survivors · Hypertension · Thrombin generation · Platelets · Epidemiology · Females

Introduction

Adult survivors of childhood or adolescent cancer face an increased risk of cardiovascular disease, the most frequent non-malignant cause of morbidity and mortality in this population. Cardiotoxicity related to chemotherapy and radiotherapy accounts for part of this increased risk [1, 2]. However, the magnitude of risk and manifestations in individuals are influenced by numerous other factors including tumor- and host-related factors [3]. Signs of early onset of subclinical or intermediate atherosclerosis have been identified in this vulnerable group, particularly in survivors of leukemia [4]. Host-related factors include metabolic syndrome, frequently described in survivors of a range of pediatric malignancies [5–8]. Hypertension among adult survivors of a childhood cancer significantly increased the risk for cardiovascular disease (CVD), particularly coronary artery disease, heart failure, valvular disease, and arrhythmia and was further independently associated with risk of cardiac death [9]. Epidemiological studies further demonstrated sex differences in the health status of cancer survivors. Whereas females from the general population have a longer life expectancy than males, female cancer survivors present with poorer health and

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higher risk of adverse events than male cancer survivors [10, 11]. The mechanisms underlying this sex difference are largely unknown. Greater vulnerability to cancer treatment-related toxicity among females has been suggested [11]. Hormonal differences between sexes have obviously been suggested; however, up to time of puberty, these differences are minimal. Whether endocrine disturbances following cancer therapy and cancer itself in the adolescence and adulthood could be held responsible for female predominance of inferior long-term outcome is not yet fully elucidated.

Hypercoagulability is a known feature of active cancer patients leading to increased risk of morbidity and mortality [12]. However, hypercoagulability may also predispose to or aggravate atherosclerosis and atherothrombotic diseases. Beyond the well-known role of platelets in thrombosis and hemostasis, platelet–cancer crosstalk has been increasingly appreciated in promoting cancer progression and metastasis on one hand and enhancing thrombopoiesis in cancer patients on the other hand [13]. This is further supported by the increasing evidence for the role of antiplatelet agents in cancer prevention, particularly in the case of colorectal cancer [14]. In addition, platelet activation has been associated with atherosclerosis development and with the severity of the disease [15]. In part, this may be caused by pro-inflammatory actions of platelets, key mediators in the initiation, and maintenance of a chronic pro-inflammatory state by secretion of autocrine and paracrine effector molecules [16].

Potential modulation of platelet function could possibly modify the release and expression of platelet-derived inflammatory mediators and disrupt the formation of platelet–leukocyte hetero-aggregates involved in the development of the atherosclerotic lesion.

Circulating coagulation proteases have also been linked to atherosclerosis and cardiovascular disease development [17]. Various coagulation proteases like factors VIIa, Xa, and thrombin may influence atherogenesis via protease-activated receptor cell signalling, inducing different inflammatory and proangiogenic pathways. Moreover, complex molecular interactions may be effective, including factor XI-platelet-linked prothrombotic effects, driven by specific cardiovascular risk factors (CVRFs) like hypertension [18].

To explore the element of hypercoagulability as a factor in early onset CVD in adult survivors of childhood cancer, we utilized an integral assay, the thrombin generation potential, in the presence and absence of platelets in individuals enrolled in the "cardiac and vascular late sequelae in long-term survivors of childhood cancer (CVSS) study" in Mainz, Germany. Since common risk factors for CVD were carefully documented in this study, we could specifically address the contribution of such risk factors, as well as age and sex, to determine whether clinical determinants of thrombin generation differ between male and female survivors.

Methods

Study design and participants

The CVSS study is an epidemiological, observational study designed to investigate German childhood cancer survivors who had neoplasia according to the International Classification of Childhood Cancer (ICCC 3) [19] prior to the age of 15 years between 1980 and 1990 with respect to cardiovascular health. Survivors with the second malignant neoplasia were excluded. Adult cancer survivors, treated in Mainz and treatment centers within a radius of approximately 300 km, were invited. All participants pursued a 5 h examination at the study center following standard operating procedures by certified medical technical assistants. The subjects for the present substudy ($n = 200$) were randomly selected from the CVSS cohort in the period between February 2015 and January 2016. In parallel time, between February 2015 and October 2016, the control group ($n = 407$) was randomly selected from the population-based Gutenberg Health Study (GHS), as described elsewhere [20]. Excluding cancer history and intake of anticoagulant agents (cancer only = 49, cancer and anticoagulant use = 4, and anticoagulant use only = 19) resulted in 335 GHS subjects available for further analyses as a population-based control group. Assessment and definition of traditional CVRFs, CVD, and categorization of current medications are described in part A of the Supplemental Material. The study protocol and documents were approved by the local Ethics Committee and the study has been conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before entering the study.

Blood sampling and plasma preparation

Venous blood sampling was performed using tubes containing K3-ethylenediaminetetraacetic acid (EDTA) for routine biochemistry analysis including blood cell count or trisodium citrate (0.129 M, 1:9 vol:vol) for investigation of thrombin generation. Samples were collected under standardized conditions according to standardized operating procedures. All subjects were in fasting state for at least 11 h before the blood draw. Platelet-rich plasma was isolated by centrifugation of whole blood for 10 min at 200×*g* at room temperature. The rest of the blood sample was centrifuged at 2000×*g* for 15 min to obtain platelet poor plasma that was utilized to dilute platelet-rich plasma to obtain 150,000 platelets/µl. Platelet-free plasma was isolated by two centrifugations: first, the blood sample was centrifuged for 5 min at 2000×*g* at room temperature

and second, the obtained plasma was centrifuged again for 10 min at 10,000×*g*. The isolated platelet-free plasma was stored at − 80 °C until lab testing.

Thrombin generation measurement

Measurement of thrombin generation was performed using the calibrated automated thrombogram (CAT) assay (Thrombinoscope BV, Maastricht, The Netherlands) according to previously described standardized protocols in the platelet epidemiology laboratory of the Center for Thrombosis and Hemostasis, University Medical Center in Mainz [21, 22]. Thrombin generation in platelet-rich plasma was assessed in fresh material (available in 200 CVSS and 335 GHS individuals), whereas in platelet-free plasma in stored material (available in 188 CVSS and 335 GHS individuals). For thrombin generation measurements in platelet-rich plasma, coagulation was triggered by adding 20 µl exogenous 1pM tissue factor to 80 µl plasma (with 150,000 platelets/µl). For measurements in platelet-free plasma, 20 µl exogenous 1pM tissue factor together with 4 µM phospholipids was added to 80 µl plasma. After 10 min prewarming at 37 °C in the fluorimeter, the reaction was started by adding 20 µl of a low-affinity fluorogenic substrate for thrombin (Z-Gly-Gly-Arg-AMC) and calcium chloride mixture (FluCa). To correct for inner filter effects and substrate consumption, thrombin generation measurements were calibrated against a signal from the calibration well obtained in a sample from the same plasma (80 µL platelet-rich or platelet-free plasma, respectively) supplemented with a fixed amount of thrombin–α2-macroglobulin complex (20 µL of thrombin calibrator) and 20 µL of FluCa by means of Thrombinoscope software (Thrombinoscope BV). All CAT reagents were obtained from Stago Deutschland GmbH (Düsseldorf, Germany).

Flow cytometric analysis of platelet surface P-selectin

As reported before [23], citrated whole blood samples were diluted 1:20 with PBS and incubated for 20 min at room temperature in the dark after double staining with the following antibodies: PerCP labelled anti-CD42a as platelet identifying monoclonal antibody and FITC labelled anti-CD62p. After incubation, the samples were analysed using the BD Accuri C6 cytofluorimeter (BD Biosciences, San Jose, CA, USA).

Statistical analysis

All clinical and laboratory data of the present investigation underwent quality control by a central data management unit. Data were reviewed for completeness by predefined algorithms and plausibility criteria. Normally distributed continuous variables were described using mean ± standard deviation (SD) and tested with *T* test, whereas categorical variables are expressed as absolute numbers and percentages and tested with Chi-squared test. Relations between continuous variables were explored by Spearman rank correlation coefficients. Multivariable linear regression analysis was used to identify determinants of thrombin generation parameters with endogenous thrombin potential and peak height as dependent variables and clinical parameters as independent variables. Because of only two subjects with diabetes and due to highly skewed distribution of Haemoglobin A1c (HbA1c), the natural log of HbA1c was used in the regressions. Because of the explorative character of the analysis, *p* values should be interpreted as a continuous measure of statistical evidence and no significance threshold was defined. Statistical analysis was performed with software program R, version 3.4.3 (http://www.R-project.org).

Results

Clinical characteristics of cancer survivors

Cancer survivors' characteristics for the presence of traditional CVRFs, current drug treatment, and childhood cancer type are depicted sex specifically in Table 1. Obesity was more frequent in females, whereas arterial hypertension and dyslipidemia were more frequent in male cancer survivors. There were only two female subjects with diabetes. In total, 47 survivors (23% of the total sample) had arterial hypertension, but only 17 (37% of those with hypertension: ten females and seven males) were taking antihypertensive medication. Out of 59 cancer survivors with dyslipidemia (30%), only one male individual was taking lipid-modifying agents. The largest group of survivors comprised of hematologic malignancies survivors with 54% of the individuals, followed by sarcomas (16.5%) and brain cancer survivors (15.5%). The group of "other" cancers (14%) included germ-cell cancers, liver cancer, and nasal cavity cancer. Compared to population-based control group, childhood cancer survivors were considerably younger (mean age = 35 ± 5.2 vs 57.9 ± 10.4 years). The percentage of females was 6% lower in the survivor group (43.5%) compared to the population-based control group (49.3%). The proportion of obesity in female cancer survivors was higher as compared to female controls (27.1% vs 23%), whereas it was lower in male cancer survivors as compared to male control subjects (16.8% vs 27.6%). The rates of smoking were also higher in cancer survivors compared to the control group. The other CVRFs such as diabetes, hypertension, and dyslipidemia were more prevalent in the GHS group compared to cancer survivors.

Table 1 Clinical characteristics of the cancer survivors and control sample

	Cancer survivors (CVSS)		Control group (GHS)	
	Males	Females	Males	Females
Number	113	87	170	165
Age (years)	35.5 (5.3)	34.6 (5.1)	57.6 (10.1)	58.2 (10.6)
Cardiovascular risk factors				
Obesity	16.8% (19)	27.1% (23)	27.6% (47)	23.0% (38)
Smoking	21.4% (24)	20.2% (17)	17.0% (48)	18.8% (31)
Diabetes	0% (0)	2.4% (2)	8.1% (23)	7.3% (12)
Hypertension	26.5% (30)	20.2% (17)	42.0% (119)	48.5% (80)
Dyslipidemia	37.2% (42)	20.0% (17)	45.9% (130)	35.8% (59)
FH MI/stroke	14.2% (16)	14.1% (12)	15.9% (45)	23.6% (39)
Cardiovascular drug therapy				
Antiplatelet agents	0% (0)	1.2% (1)	13.5% (23)	7.9% (13)
Antilipemic drugs	0.9% (1)	0% (0)	16.5% (28)	10.9% (18)
Antihypertensive drugs	0% (0)	1.2% (1)	2.4% (4)	1.2% (2)
ACE inhibitors	5.2% (6)	5.9% (5)	34.7% (59)	27.9% (46)
ARB	1.7% (2)	0% (0)	7.1% (12)	6.7% (11)
Calcium channel blockers	0% (0)	1.2% (1)	10.0% (17)	11.5% (19)
Beta blocker	4.3% (5)	7.1% (6)	16.5% (28)	15.2% (25)
Diuretics	0.9% (1)	2.4% (2)	3.5% (6)	6.1% (10)
History of cancer according to affected organ				
Hematologic	52.7% (57)	47.2% (51)	–	–
Sarcoma	75.7% (25)	24.2% (8)	–	–
Brain	70.9% (22)	29% (9)	–	–
Other*	39.3 (11)	60.7% (17)	–	–

Presented are clinical characteristics of CVSS and GHS individuals, sex specifically. Data are expressed as percentage (absolute number)

FH family history, *MI* myocardial infarction, *ACE* angiotensin converting enzyme, *ARB* angiotensin receptor blocker, *Other** germ-cell cancer, liver cancer, and nasal cavity cancer

Thrombin generation profile in cancer survivors compared to population-based control group

As presented in Supplemental Table S1, childhood cancer survivors presented with higher peak height in platelet-rich plasma only. No differences were observed for TG parameters measured in the absence of platelets. Sex-specific analysis (Table 2) between the two groups showed in platelet-rich plasma higher endogenous thrombin potential and peak height in female cancer survivors compared to female control subjects. Differently, male cancer survivors showed lower endogenous thrombin potential compared to male controls. Similarly, the results in platelet-free plasma showed higher TG parameters in female cancer survivors compared

Table 2 Thrombin generation in cancer survivors compared to population-based controls

	Males			Females		
	GHS	CVSS	*p* value	GHS	CVSS	*p* value
Number	170	113	–	165	87	–
Age (years)	57.6 (10.1)	35.5 (5.3)	< 0.0001	58.2 (10.6)	34.6 (5.1)	< 0.0001
PRP_ETP (nM*min)	1507 (254)	1410 (228)	0.0011	1511 (298)	1607 (358)	0.036
PRP_Peak height (nM)	98.4 (25.6)	93.5 (22.9)	0.095	96.2 (27.9)	115.8 (32.2)	< 0.0001
PFP_ETP (nM*min)	822 (184)	703 (236)	< 0.0001	810 (277)	1044 (383)	< 0.0001
PFP_Peak height (nM)	94.5 (27.7)	76.5 (35.8)	< 0.0001	96.6 (54.0)	123.8 (69.2)	0.0024

The table presents the thrombin generation results compared between CVSS and GHS participants, sex specifically. Presented data are mean (standard deviation)

PRP platelet-rich plasma, *ETP* endogenous thrombin potential, *PFP* platelet-free plasma

Table 3 Clinical determinants of thrombin generation according to the presence of platelets

	Platelet-rich plasma				Platelet-free plasma			
	ETP β (2.5%; 97.5%)	*p* value	Peak height β (2.5%; 97.5%)	*p* value	ETP β (2.5%; 97.5%)	*p* value	Peak height β (2.5%; 97.5%)	*p* value
Age	−1.71 (−9.19; 5.77)	0.65	0.05 (−0.72; 0.83)	0.89	−0.63 (−9.44; 8.19)	0.89	−0.58 (−2.11; 0.95)	0.46
Sex (female)	217.12 (138.6; 295.6)	**< 0.001**	22.24 (14.12; 30.36)	**< 0.001**	347.1 (255.2; 438.99)	**< 0.001**	49.62 (33.65; 65.59)	**< 0.001**
Obesity	161.9 (63.9; 259.9)	**0.0014**	5.12 (−5.02; 15.25)	0.32	97.94 (−15.72; 211.6)	0.093	5.42 (−14.3; 25.17)	0.59
Smoking	−41.8 (−134.3; 50.6)	0.38	2.09 (−7.47; 11.65)	0.67	−56.49 (−163.87; 50.89)	0.30	−12.63 (−31.29; 6.03)	0.19
ln HbA1c*	715.6 (97.4; 1333.8)	**0.024**	65.51 (1.56; 129.45)	**0.046**	−94.41 (−802.87; 614.06)	0.79	−73.5 (−196.64; 49.6)	0.24
Hypertension	184.8 (90.7; 278.8)	**< 0.001**	5.82 (−3.91; 15.55)	0.24	121.4 (14.89; 227.97)	**0.027**	17.63 (−0.89; 36.14)	0.064
Dyslipidemia	36.2 (−51.6; 123.99)	0.42	−1.1 (−10.2; 7.98)	0.81	63.95 (−38.41; 166.31)	0.22	10.75 (−7.04; 28.54)	0.24
FH MI/stroke	23.3 (−84.34; 130.96)	0.67	2.0 (−9.13; 13.14)	0.73	25.6 (−99.64; 150.84)	0.69	12.75 (−9.01; 34.52)	0.25

Multivariable linear regression analysis of endogenous thrombin potential (ETP) and peak height as dependent variables adjusted for age, sex, and cardiovascular risk factors (presented in the table) in platelet-rich and platelet-free plasma

FH family history, *MI* myocardial infarction

*The model includes the natural log of HbA1c as a continuous variable for diabetes risk, as only two subjects with diabetes were in the sample

to females control subjects, whereas in males, TG parameters were higher in male control subjects when compared to male cancer survivors.

Clinical determinants of thrombin generation in cancer survivors

Higher endogenous thrombin potential and peak height were identified in females compared to males, in both platelet-rich and platelet-free plasma (Supplemental Table S2). The univariate analysis, as presented in Supplemental Table S3, showed an increased endogenous thrombin potential measured in platelet-rich plasma of subjects with hypertension, obesity, and diabetes. In addition, obese individuals presented with higher peak height in platelet-rich plasma and higher endogenous thrombin potential and peak height measured in platelet-free plasma. No differences in parameters of thrombin generation were observed for subjects with dyslipidemia, smoking, or those with history of myocardial infarction and/or stroke, both in platelet-rich and platelet-free plasma.

Linear regression analysis for parameters of thrombin generation with adjustment for age, sex, and traditional CVRFs (natural log of HbA1c was used as a continuous variable indicating the risk for diabetes) found female sex (beta estimate, β: 217.12 [138.6; 295.6], $p < 0.001$), obesity (β: 161.9 [63.9; 259.9], $p = 0.0014$), arterial hypertension (β: 184.8 [90.7; 278.8], $p < 0.001$), and the natural log of HbA1c (β: 715.6 [97.4; 1333.8], $p = 0.024$) as relevantly associated to higher endogenous thrombin potential in platelet-rich plasma (Table 3). Female sex was further associated with higher peak height in platelet-rich plasma and with both higher endogenous thrombin potential and peak height in platelet-free plasma. Traditional CVRFs showed no important association with thrombin generation in platelet-free plasma in the adjusted model. To investigate whether hormonal factors such as oral contraceptives had impact on thrombin generation, we compared thrombin generation potential in females with and without intake of these drugs. Females on oral contraceptives compared to females not taking oral contraceptives showed higher endogenous thrombin potentials and peak height in both platelet-rich and platelet-free plasma (Supplemental Table S4).

Cardiovascular risk factors and endogenous thrombin potential in a sex-specific analysis

Table 4 presents a sex-stratified multivariable analysis for endogenous thrombin potential in platelet-rich plasma as dependent variable, adjusted for age, traditional CVRFs including high-normal and elevated systolic blood pressure, and the natural log of HbA1c as continuous variable indicating the risk for diabetes (there were only two subjects

Table 4 Sex-specific determinants of the endogenous thrombin potential

	Females		Males	
	β (2.5%; 97.5%)	p value	β (2.5%; 97.5%)	p value
Age (years)	6.96 (−7.38; 21.29)	0.34	−2.82 (−10.88; 5.24)	0.49
Obesity	200.18 (36.48; 363.88)	**0.019**	177.62 (56.88; 298.35)	**0.0048**
Smoking	−180.17 (−353.33; −7.01)	0.045	54.54 (−47.48; 156.57)	0.30
ln HbA1c*	910.78 (−111.4; 1932.99)	0.085	1059.0 (292.99; 1825.01)	**0.0079**
Dyslipidemia	253.07 (72.92; 433.22)	**0.0075**	−1.75 (−95.76; 92.26)	0.97
FH MI/stroke	−14.56 (−218.66; 189.54)	0.89	45.07 (−75.67; 165.81)	0.47
High-normal BP	−152.9 (−318.95; 13.098)	0.075	−7.26 (−100.79; 86.28)	0.88
Elevated BP	436.7 (119.02; 754.39)	**0.0088**	1.80 (−134.36; 137.97)	0.98

Multivariable linear regression analysis of the endogenous thrombin potential assessed in platelet-rich plasma as dependent variable stratified for males and females and adjusted for age, sex, cardiovascular risk factors (presented in the table), and systolic blood pressure categories (high normal = 120–139 mmHg; elevated = ≥ 140 mmHg)

BP blood pressure, *FH* family history, *MI* myocardial infarction

*Due to only two subjects with diabetes, the model includes the natural log of HbA1c as a continuous variable.

Table 5 Interaction of female sex and hypertension regarding the endogenous thrombin potential

	ETP β (2.5%; 97.5%)	p value
Age (10 years)	7.324 (−67.512; 82.159)	0.85
Sex (female)	170.671 (83.804; 257.538)	< **0.001**
Hypertension	133.717 (15.925; 251.509)	**0.027**
Female sex * hypertension	251.869 (64.281; 439.457)	**0.0092**

Multivariable linear regression analysis for endogenous thrombin potential (ETP) in platelet-rich plasma as dependent variable adjusted for age, sex, and female sex–hypertension interaction term

with diabetes in the sample). Obesity was linked with higher endogenous thrombin potential in both males and females. The results further showed a positive association with natural log of HbA1c, in both sexes and highly relevant in males. Dyslipidemia and elevated blood pressure (systolic blood pressure ≥ 140 mmHg) were associated with higher endogenous thrombin potential in females only, whereas no associations were observed in males. The results between endogenous thrombin potential and high-normal and elevated diastolic blood pressure revealed weaker association for elevated diastolic pressure in females only (Supplemental Table S5). Given the independent association of hypertension and female sex with endogenous thrombin potential, we next examined whether there is an interaction between these two risk factors. The results (Table 5) showed positive interaction of female sex and hypertension on endogenous thrombin potential as dependent variable in a model adjusted for age [interaction term, $\beta = 251.87$ (64.28; 439.46), $p = 0.0092$]. To investigate whether female factors such as menstrual bleeding and intake of oral contraceptives affect the relation between higher endogenous thrombin potential and CVRFs in females, an additional multivariable model further adjusted for these factors, showed an independent positive association between oral contraceptives and endogenous thrombin potential (Table 6), with slight reduction in the observed association for hypertension.

Correlation analysis between endogenous thrombin potential and platelet activation marker

The sex-stratified correlation analysis between endogenous thrombin potential, measured in platelet-rich plasma, and percentage of platelets expressing P-selectin showed a Spearman rank correlation for female cancer survivors of $r_s = 0.17$ and for male cancer survivors of $r_s = 0.11$. The correlation between these variables according to the presence of traditional CVRFs was observed for obesity ($r_s = 0.18$) and hypertension ($r_s = 0.12$) only.

Thrombin generation according to cancer type and CVRFs

The results based on cancer type showed no differences in thrombin generation parameters between survivors of different cancer types (Supplemental Table S6). Stratifying the individuals based on cancer type and arterial hypertension (Supplemental Table S7) resulted in increased endogenous thrombin potential in hypertensive individuals of hematologic ($p = 0.0042$), sarcoma ($p = 0.0097$), and brain cancer survivors ($p = 0.096$). Further stratification by sex, cancer type, and CVRFs was not feasible due to resulting small subgroups to compare.

Table 6 Female-specific determinants of the endogenous thrombin potential

	Model 1		Model 2	
	β (2.5%; 97.5%)	p value	β (2.5%; 97.5%)	p value
Age (years)	6.47 (−7.61; 20.55)	0.37	8.24 (−5.6; 22.09)	0.25
Obesity	129.98 (−28.93; 288.89)	0.11	148.09 (−4.92; 301.1)	0.062
Smoking	−167.19 (−336.36; 1.97)	0.057	−148.71 (−311.73; 14.32)	0.078
ln HbA1c*	807.67 (−192.0; 1807.36)	0.12	848.66 (−115.3; 1812.62)	0.089
Hypertension	318.16 (143.96; 492.36)	**<0.001**	263.28 (91.13; 435.42)	**0.0038**
Dyslipidemia	155.02 (−19.87; 329.9)	0.087	167.79 (−1.19; 336.78)	0.056
FH MI/stroke	34.77 (−163.92; 233.46)	0.73	31.52 (−159.17; 222.21)	0.75
Oral contraceptives	–	–	222.62 (66.15; 379.08)	**0.0068**
Menstrual cycle	–	–	−8.99 (−209.61; 191.64)	0.93

Multivariable linear regression analysis of endogenous thrombin potential (ETP) in platelet-rich plasma as dependent variable in females. Model 1 is adjusted for age and cardiovascular risk factors (variables in the table); Model 2 = Model 1 + additionally adjusted for female factors (i.e., intake of oral contraceptives and menstrual bleeding)

*Due to only two subjects with diabetes, the model includes the natural log of HbA1c as a continuous variable

Discussion

Thrombin generation analysis gives insight in hypercoagulability as an important mechanism linked to CVRFs. We have recently reported on cardiovascular risk in CVSS ($n = 951$ individuals) compared to age-matched control group (GHS). The findings showed an increased risk and premature occurrence of arterial hypertension [relative risk (RR) 1.38, 95% confidence interval (95% CI 1.21–1.57)], dyslipidaemia [RR 1.26 (95% CI 1.12–1.42)], and CVD [RR 1.89 (95% CI 1.34–2.66)] in childhood cancer survivors [24]. To our knowledge, this is the first study to investigate sex specifically the thrombin generation potential of randomly selected subjects from a large cohort of childhood cancer survivors. First, this study demonstrates sex-specific differences in the thrombin generation profile with female survivors presenting with higher thrombin generation compared to female population-based control subjects and compared to male survivors in both the presence and absence of platelets. Second, it identifies obesity, hypertension, and higher HbA1c as determinants of a higher thrombin generation potential in the presence of platelets only and, third, reveals an important interaction for hypertension-related hypercoagulability in the presence of platelets in female survivors only.

Female sex was strongly related to a higher endogenous thrombin potential, both in the presence and absence of platelets. The sex-related hypercoagulability could partly be explained by the intake of oral contraceptives, as shown in the present and previous studies [25]. Acquired resistance to protein C (APC) and impaired function of the tissue factor pathway inhibitor (TFPI) system including decreased TFPI levels have been recognized as important elements of the prothrombotic profile related to hormonal contraception [26, 27].

Obesity and hypertension were strongly related to higher endogenous thrombin potential in the presence of platelets. This finding is further supported by the correlation between endogenous thrombin potential and percentage of platelets expressing platelet surface activation marker P-selectin, particularly in subjects with obesity and hypertension. We have recently reported a significant relation between obesity and thrombin generation in the presence of platelets only in individuals from an adult general population-based study [20]. The present study additionally demonstrates an interaction between platelet-related hypercoagulability and arterial hypertension. Platelet-related hypercoagulability has been identified as an important mechanism in hypertension, both in animal models and humans, where platelet-localized factor XI is a driver of a vascular coagulation–inflammation network in arterial hypertension [18]. Female sex and hypertension presented an additive effect to a higher endogenous thrombin potential. The lack of an important association between hypertension and thrombin generation in the adult general population-based GHS study, as we previously reported, might be result of the intake of antihypertensive medications by these individuals [23]. Therefore, as hypertension is an important, modifiable risk factor, prompt, and effective management will certainly alleviate the high burden of cardiovascular morbidity and mortality in this vulnerable population [28–30].

Female cancer survivors, particularly those exposed to alkylating agents, have impaired ovarian reserve and often experience symptoms associated with hormone deprivation [31, 32]. Estrogen was shown to have an important role in

delaying the onset of atherothrombotic events in females by inducing the synthesis of prostacyclin, increasing nitric oxide bioavailability, and directly inhibiting platelet aggregation [33, 34]. Furthermore, the reduced platelet reactivity in pre-menopausal women has been related to the presence of estrogen receptors on the platelet surface [35]. Whether, estrogen deprivation due to the cancer-related therapy is the underlying cause of hypercoagulability in female cancer survivors, deserves further investigation.

This study showed no differences in the thrombin generation profile between survivors of different cancer groups, proposing that cancer type is not the major determinant of the increased hypercoagulable state in adult survivors of childhood cancer.

The strengths of this study are several. First, the thrombin generation was assessed in both platelet-rich and platelet-free plasma. We have recently shown that unlike thrombin generation in platelet-free plasma, thrombin generation in platelet-rich plasma is more substantially determined by cardiovascular risk factors and is the favorable assay to assess individual risk for CVD [20]. Second, a rigorous and detailed assessment of the individual's cardiovascular profile including standardized measurements of blood pressure, glucose, and lipid status was employed to ensure quality of collected data. Third, this is a first study demonstrating the clinical determinants of thrombin generation potential in the presence and absence of platelets from adult survivors of childhood cancer using a sex-specific approach. The results of this descriptive and hypothesis-generating study need to be confirmed in a hypothesis-driven study with a sizeable number of cases and age-matched population controls.

This study unravels a potentially important explanation for the observed difference in risk patterns between female and male cancer survivors. The increased thrombin generation was particularly determined by systolic hypertension, obesity, and dyslipidemia when assessed in the presence of platelets. This study proposes that thrombin generation is, in conjunction with platelet activation, an important mechanism in the pathogenesis of CV disease in cancer survivors, providing an explanation of their higher risk for adverse cardiovascular events. These results highlight the potential of the platelet-coagulant axis as important tool in identifying cancer survivors at higher risk for adverse cardiovascular events. Further investigation is needed to evaluate the benefits of existing prevention strategies to ameliorate the cardiovascular health status in this vulnerable population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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