The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 4, e1660–e1672
doi:10.1210/clinem/dgaa936
Clinical Research Article

Clinical Research Article

Procoagulant Imbalance in Klinefelter Syndrome Assessed by Thrombin Generation Assay and Whole-Blood Thromboelastometry

Rita Indirli,[1,2*] Emanuele Ferrante,[1*] Erica Scalambrino,[3] Eriselda Profka,[2] Marigrazia Clerici,[3] Tommaso Lettera,[4] Andreea Liliana Serban,[1,5] Walter Vena,[2,6] Alessandro Pizzocaro,[6] Marco Bonomi,[7,8] Biagio Cangiano,[7,8] Giulia Carosi,[1,5] Gherardo Mazziotti,[6,9] Luca Persani,[7,8] Andrea Lania,[6,9] Maura Arosio,[1,2] Flora Peyvandi,[3] Giovanna Mantovani,[1,2] and Armando Tripodi[3]

[1]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, 20122 Milan, Italy; [2]Department of Clinical Sciences and Community Health, University of Milan, 20122 Milan, Italy; [3]Fondazione IRCCS Ca' Granda Ospedale Maggiore, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, 20122 Milan, Italy; [4]Laboratorio analisi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; [5]Department of Experimental Medicine, Sapienza University of Rome, 00161 Rome, Italy; [6]Endocrinology, Diabetology and Andrology Unit, Humanitas Clinical and Research Center IRCCS, Rozzano, 20089 Milan, Italy; [7]IRCCS Istituto Auxologico Italiano, Department of Endocrine and Metabolic Diseases and Lab of Endocrine and Metabolic Diseases, 20149 Milan, Italy; [8]Department of Medical Biotechnology and Translational Medicine, University of Milan, 20129 Milan, Italy; and [9]Humanitas University, Department of Biomedical Sciences, Pieve Emanuele, 20090 Milan, Italy

ORCiD numbers: 0000-0001-5642-0563 (R. Indirli); 0000-0002-0556-7650 (E. Ferrante); 0000-0001-5454-6074 (M. Bonomi); 0000-0002-2658-744X (B. Cangiano); 0000-0003-2068-9581 (L. Persani); 0000-0003-3988-3616 (M. Arosio); 0000-0002-9065-3886 (G. Mantovani).

*R.I. and E.F. contributed equally to this work.

Abbreviations: APTT, activated partial thromboplastin time; BMI, body mass index; ETP, endogenous thrombin potential; FVIII, factor VIII; HbA_{1c}, glycated hemoglobin A_{1c}; KS, Klinefelter syndrome; MCF, maximal clot firmness; MPO, myeloperoxidase; NET, neutrophil extracellular trap; PC, protein C; PPP, platelet-poor plasma; PRP, platelet-rich plasma; PT, prothrombin time; rTF, relipidated tissue factor; TGA, thrombin generation assay; TM, thrombomodulin.

Received: 18 September 2020; Editorial Decision: 10 December 2020; First Published Online: 31 December 2021; Corrected and Typeset: 1 February 2021.

Abstract

Context: Klinefelter syndrome (KS) is a condition at increased risk of thrombosis compared to 46,XY men.

Objective: This work aimed to investigate the coagulation balance of KS patients by thrombin generation assay (TGA) and thromboelastometry.

Methods: An observational, cross-sectional study was conducted at 3 tertiary endocrinological centers in Milan, Italy. Fifty-eight KS patients and 58 age-matched

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

healthy controls were included. Anticoagulant or antiplatelet therapy and known coagulation disorders were exclusion criteria. TGA was performed in platelet-poor plasma (PPP) and platelet-rich plasma (PRP). Whole-blood thromboelastometry and activities of coagulation factors were assessed. Endogenous thrombin potential (ETP), the area under the thrombin generation curve, assessed with and without thrombomodulin (ETP-TM$^+$ and ETP-TM$^-$), and their ratio (ETP ratio), were considered as indexes of procoagulant imbalance.

Results: Patients with KS displayed higher PPP-ETP-TM$^+$ (mean 1528 vs 0.1315 nM × min; $P < .001$), PPP-ETP ratio (0.78 vs 0.0.70; $P < .001$), factor (F)VIII (135% vs 0.107%; $P = .001$), fibrinogen (283 vs 0.241 mg/dL; $P < .001$), and FVIII/protein C ratio (1.21 vs 0.1.06; $P < .05$) compared to controls. Protein C was comparable in the 2 groups. Similar results were observed in PRP. The ETP ratio was positively associated with FVIII ($\rho = 0.538$, $P < .001$) in KS. Thromboelastometry parameters confirmed evidence of hypercoagulability in KS.

Conclusion: Patients with KS display a procoagulant imbalance expressed by increased thrombin generation both in PPP and PRP, which is at least in part explained by increased FVIII levels. The procoagulant imbalance, which was confirmed by thromboelastometry, may be responsible for the thrombotic events observed in these patients. Further investigation on the benefit/risk ratio of antithrombotic prophylaxis is warranted.

Key Words: Klinefelter syndrome, thrombin generation assay, thromboelastometry, thrombosis, hypogonadism, testosterone

Klinefelter syndrome (KS) is the most common chromosomal aneuploidy in men, with an estimated incidence of 1:650 newborn males (1, 2). Increased morbidity and mortality from all causes are described in KS (3), with a reduction in life expectancy of 2.1 years compared with the general population (4). Arterial and venous thromboses are from 3 to 20 times more frequent in KS patients of any age (5-9) and are among the leading causes of death in this condition (8, 10). Deep venous thrombosis, pulmonary embolism (collectively known as venous thromboembolism), mesenteric ischemia, venous insufficiency, leg ulcers (5, 6, 11), as well as cerebrovascular diseases (6, 12) are the main clinical thrombotic events observed in KS. However, the pathophysiological significances underlying this thrombotic predisposition are largely unknown and presumptive mechanisms are surmised mostly from case reports or small studies.

According to the Virchow triad, venous thromboembolism can result from hemodynamic changes such as reduction of blood flow or turbulence, endothelial injury or dysfunction, and blood hypercoagulability. A few studies have investigated endothelial parameters in KS, unveiling increased levels of apoptotic endothelial microparticles, enhanced expression of vitronectin receptor (13), and high concentrations of asymmetric dimethylarginine, a marker of endothelial dysfunction (14). Clues on hypercoagulability come from sparse studies, which suggest increased platelet reactivity (15) and impaired fibrinolysis due to increased levels of plasminogen activator inhibitor-1 (16). However, whether and to what extent other hemostasis abnormalities such as the balance between naturally occurring procoagulants and anticoagulants operating in plasma are influenced by metabolic derangements, hypogonadism, or testosterone treatment are unclear (8).

Coagulation is a complex and integrated mechanism, and the investigation of its individual components (procoagulants and anticoagulants) or the traditional tests, such as the prothrombin time (PT) or activated partial thromboplastin time (APTT), do not reflect the situation occurring in vivo. The thrombin generation assay (TGA) is an in vitro procedure based on the continuous registration of thrombin generation (mediated by procoagulants) and decay (mediated by anticoagulants) and is considered the closest approximation to the process occurring in vivo (17). TGA can be performed with or without the addition of thrombomodulin (TM), the physiological activator of protein C (PC) located on endothelial cells and needed to explore the contribution of this anticoagulant to the overall coagulation balance (17-19). TGA can be performed in platelet-poor plasma (PPP), to assess coagulation factors only, but also in platelet-rich plasma (PRP), to also investigate the contribution of the platelet procoagulant function. Whole-blood thromboelastometry assesses the viscoelastic changes of clotting blood under experimental conditions that mimic the contribution of plasma, blood cells, and platelets in vivo.

The primary aim of this study was to assess the contribution of plasma to thrombin generation performed in PPP in KS patients and age-matched healthy controls. Secondary aims were 1) to evaluate the contribution of the platelet procoagulant activity to thrombin generation performed in PRP, and 2) to evaluate the coagulation potential of KS patients by means of whole-blood thromboelastometry.

Materials and Methods

Participants

We selected adult men with a cytogenetically confirmed diagnosis of KS among those followed up at 3 tertiary endocrinological centers in Milan (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; IRCCS Humanitas Clinical and Research Center; IRCCS Istituto Auxologico Italiano). We excluded patients with known hereditary coagulation disorders and individuals on anticoagulation (parenteral or oral) or antiplatelet treatment.

Clinical information regarding smoking habit, arterial hypertension, dyslipidemia, testosterone replacement therapy, and the most recent assessment (ie, within the previous 3 months) of body mass index (BMI), fasting plasma glucose, and glycated hemoglobin A_{1c} (HbA_{1c}) were extracted from hospital records. Total testosterone and gonadotropin concentrations were assessed on blood samples collected during study visits.

Dyslipidemia was defined as triglycerides greater than 150 mg/dL, and/or HDL cholesterol less than 40 mg/dL (20), and/or LDL cholesterol above the risk category-specific thresholds recommended by the 2019 European Society of Cardiology/European Atherosclerosis Society guidelines for the management of dyslipidemias (21), and/or prescription of lipid-lowering treatment. Arterial hypertension was defined as systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than 90 mm Hg (22), and/or prescription of antihypertensive treatment. Impaired fasting glucose and diabetes mellitus were defined according to American Diabetes Association criteria (23). Testosterone therapy was initiated in KS following current recommendations, that is, when symptoms and signs of testosterone deficiency became evident with endogenous testosterone levels below 12 nmol/L (24-26).

Healthy adults, recruited among male students and hospital staff in the 3 centers involved in the study, were included as controls. They were matched by age to the patient population and were free from current and past thrombotic events, anticoagulant drugs, or coagulation disorders known to affect TGA.

All study procedures were in accordance with the principles set out in the Declaration of Helsinki. The study was approved by the Milan Area 2 ethics committee (approval ID 1173). Written informed consent was obtained from all individuals included in the study.

Blood sampling and plasma preparation

Blood was collected from an antecubital vein into vacuum tubes containing 1/10 volume of trisodium citrate 109 mM (Becton Dickinson). For KS patients on testosterone replacement therapy, blood samples were taken 2 hours after application of testosterone transdermal gel, or at the end of the dosing interval in case of injectable long-acting testosterone undecanoate (24). One portion of citrated whole blood was used for thromboelastometry and the remaining was centrifuged (at controlled room temperature) with 2 different procedures. The first consisted of centrifugation for 20 minutes at 2880*g* and separation of PPP. The second consisted of centrifugation for 15 minutes at 150*g* to obtain PRP. PRP was then diluted into autologous PPP to obtain a standard platelet count of 150×10^9/L. The resultant PRP was eventually tested for thrombin generation immediately after the preparation. PPP was aliquoted in plastic-capped tubes, quickly frozen by immersion in liquid nitrogen, and stored at –70 °C until testing. To limit between-assay variability, an equal number of samples from patients and controls were prepared and tested in the same run. All the experimental procedures were conducted at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Ospedale Maggiore Policlinico, Milan.

Thrombin generation assay

TGA was assessed according to Hemker et al (27) as described (28). Testing was based on the activation of coagulation after addition to plasma of human recombinant relipidated tissue factor (rTF, 1 pM) (Recombiplastin 2G, Werfen) and synthetic phospholipids (PL, 1.0 μM) (Avanti Polar) as coagulation triggers in PPP, or rTF alone in PRP. Testing was performed in 2 plasma aliquots, with and without the addition of rabbit TM (Haematologic Technologies) (2 nM). Registration of thrombin generation was obtained with a fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem) (617 μM) by means of a fluorometer (Fluoroskan Ascent, Thermo Labsystems). The readings were recorded and analyzed with dedicated software (Thrombinoscope, Thrombinoscope BV), which displays the curve of thrombin concentration as a function of time and calculates the following parameters: the time (minutes) between the addition of the triggers and the initiation of thrombin generation (lag time); the thrombin peak (nM); the time (minutes) needed to reach the peak (TT peak); the area under the curve, defined as endogenous

thrombin potential (ETP) and expressed as nM × min; and the velocity index, defined as [peak/(TT peak – lag time)] and expressed as nM/min. Results were also expressed as ETP ratio, that is, the ratio of ETP measured in the presence of TM (ETP-TM$^+$) to the ETP measured in its absence (ETP-TM$^-$).

Measurement of individual coagulation parameters

Whole-blood cell counts were performed with Micros-60 (Horiba ABX). PT and APTT were measured with Recombiplastin 2G or ThrombosIL APTT (Werfen). Both tests were performed on an automated coagulometer (ACL Top, Werfen) and results were expressed as the ratio of the patient plasma relative to the pooled normal plasma. PC and antithrombin were measured as chromogenic activity by means of commercial kits (Hemosil antithrombin and Hemosil PC; Werfen). Factor (F)VIII, II, and fibrinogen were measured as described (29) and results reported as percentage activity relative to pooled normal plasma with an (arbitrary) activity of 100%.

Myeloperoxidase

Because a mediator of thrombosis has been identified in extracellular nuclear chromatin, released from dead cells or inflammatory cells, especially in the form of neutrophil extracellular traps (NETs) (30), myeloperoxidase (MPO) was assessed as one of the NET-related variables. Plasma MPO activity was measured by a chromogenic assay employing the enzyme-linked immunosorbent assay (ELISA) kit Myeloperoxidase (human) (Cayman).

Thromboelastometry

Whole-blood thromboelastometry is an in vitro global coagulation procedure that detects and quantifies the viscoelastic properties of whole blood during clotting and fibrinolysis (31). Thromboelastometry was performed by means of the 4-channel ROTEM Gamma equipment according to instructions and reagents provided by the manufacturer (TEM). Partial thromboplastin from rabbit origin, ellagic acid, and calcium chloride (INTEM) were employed to activate the intrinsic pathway of coagulation; in another aliquot, rTF was added (EXTEM) to assess the extrinsic coagulation pathway. Another aliquot was tested with the addition of cytochalasin D (FIBTEM) to inhibit platelet activity. The main parameters stemming from the procedure are (i) the clotting time, which is the time (seconds) needed for starting coagulation; (ii) the clot formation time, which is the time (seconds) needed for the clot to reach a predefined amplitude; (iii) the α angle (degrees [°]), representing the velocity of clot growth; and (iv) the maximal clot firmness (MCF), which is the maximal amplitude of the clot (in millimeters [mm]) and represents the strength of clot.

Hormonal assays

Circulating total testosterone concentrations were assessed by an Elecsys Testosterone II (Calibrator reference: 05200067190) test marketed by Roche Diagnostics. This method is standardized via isotope dilution–gas chromatography/mass spectrometry. The assay has a lower limit of detection of 0.087 nmol/L, a functional sensitivity of 0.4 nmol/L, and interassay or intra-assay coefficients of variation of less than 5%.

Luteinizing hormone and follicle-stimulating hormone concentrations were measured by the electrochemiluminescence immunoassay "ECLIA" from Roche Diagnostics (Roche Diagnostics GmbH). Luteinizing hormone and follicle-stimulating hormone assays have a lower limit of detection of 0.1 mIU/mL and a functional sensitivity of 0.2 mIU/mL. The interassay or intra-assay coefficients of variation were less than 5% in all assays.

Sample size calculation

We chose the ETP ratio as the outcome variable for sample size calculation. Because no previous data were available for ETP ratio in KS, we elected to use the mean ETP ratio (SD) value obtained in a group of healthy participants corresponding to 0.67 (0.11) (32). We then postulated a 10% increase of the ETP ratio in the KS population relative to controls. These assumptions led to a sample size of 58 patients and 58 controls when setting a power of 90% and a 5% probability of type I error.

Statistical analysis

Distribution of quantitative variables was assessed by Shapiro-Wilk test. Normally distributed quantitative variables were expressed as mean and SD, whereas variables with a skewed distribution were reported as median and range; qualitative variables were represented as absolute and percentage frequencies. Paired or unpaired *t* test was performed to compare means of normally distributed variables. Alternatively, the nonparametric Mann-Whitney test was used. The Fisher exact test was employed to compare frequencies of qualitative variables between 2 groups. Univariate association analysis was performed by linear regression, Pearson correlation

test or Spearman rank correlation test as appropriate. Multivariable analysis was conducted by multiple linear regression. A 2-sided *P* value was considered statistically significant when less than .05. Analysis was performed with IBM SPSS Statistics (version 26) or GraphPad Prism (version 9).

Results

From April 2018 to December 2019, 58 patients affected by KS (median age, 39.7 years [range, 18-78 years]) and 58 age-matched controls (median age, 37.5 years [range, 22-77 years], *P* = .93) were enrolled in the study. Table 1 summarizes the characteristics of the study population. Controls had a lower frequency of dyslipidemia, a nonsignificantly lower median BMI, and lower median gonadotropin levels as expected, compared to KS patients, while other explored variables were comparable between the 2 groups.

One KS patient reported one past episode of pulmonary embolism, another had experienced a transient ischemic attack several years earlier when he was on testosterone treatment, and 3 patients suffered from chronic venous insufficiency in the lower limbs.

Thirty-five patients were receiving testosterone replacement therapy at study entry; 12 hypogonadal patients were tested before starting testosterone treatment, while the remaining were testosterone-naive patients with still normal endogenous testosterone levels. Patients treated with testosterone were (nonsignificantly) older than patients not on testosterone replacement therapy, had a higher frequency of impaired fasting glucose (n = 7), and higher mean hematocrit levels (see Table 1); however, hematocrit was within normal range in all patients and controls. No one presented with overt diabetes.

Thrombin generation in platelet-poor plasma

Mean ETP-TM$^+$ (SD) as measured in PPP was significantly higher in KS patients than controls (1527 [358] vs 1315 [351] nM × min; *P* < .001). No significant difference was observed in TGA performed in the absence of TM (Fig. 1). The median (minimum-maximum) ETP ratio (with/without TM) was significantly increased in KS patients compared to controls (0.78 [0.43-0.99] vs 0.70 [0.19-0.88]; *P* < .001) (see Fig. 1 and Table 2). KS displayed also higher thrombin peak (*P* = .001) and velocity index than controls (*P* = .01) (Fig. 2), with no significant difference in lag time and TT peak (see Fig. 2).

Higher ETP-TM$^+$ (median 1567, minimum-maximum 939-2176 nM × min), ETP ratio (median 0.81, minimum-maximum 0.43-0.99), and nonsignificantly increased ETP-TM$^-$ (mean 2028, SD 282 nM × min) as compared to controls (*P* = .0002, *P* < .001, and *P* = .06, respectively) were still observed in the KS group after exclusion of patients with mosaic 46,XY/47,XXY and 48,XXXY karyotypes. In the latter 2 categories, ETP-TM$^+$, ETP-TM$^-$, and ETP ratio were within the nonmosaic 47,XXY range.

Thrombin generation in platelet-rich plasma

PRP was available in 52 KS patients and 43 controls. In PRP assays, KS again displayed higher median ETP-TM$^+$ compared to controls (1543 [1046-2379] vs 1399 [781-1742] nM × min, *P* = .004). ETP-TM$^-$ was comparable in the 2 groups. Median ETP ratio was higher in KS patients than controls (see Table 2). The relative difference of the median ETP ratio values between KS patients and controls did not increase in the PRP assay compared to PPP (see Table 2).

Other coagulation parameters

PT, APTT, and platelet count did not differ in the 2 groups. Higher FVIII (135% [range, 78%-430%] vs 107% [range, 47%-380%]; *P* = .001), fibrinogen (283 mg/dL [range, 179-424 mg/dL] vs 241 mg/dL [range, 170-359 mg/dL] *P* < .001), and FVIII/PC ratio (1.21 [range, 0.65-3.85] vs 1.06 [range, 0.48-3.28]; *P* < .05] were observed in KS patients compared to controls. The levels of the remaining procoagulant and anticoagulant factors were similar in the 2 groups.

In KS patients with mosaic 46,XY/47,XXY or 48,XXXY karyotypes, FVIII and fibrinogen levels and FVIII/PC ratio were within the minimum to maximum range of nonmosaic 47,XXY patients.

Myeloperoxidase

MPO activity was not significantly different between KS patients (27 ng/mL [range, 9-66 ng/mL]) and controls (23 ng/mL [range, 9-82 ng/mL]; *P* = .39). The mean percentage (SD) of neutrophils was similar in the 2 groups, as well (KS 61.1 [7.4]; controls 59.4 [7.2]; *P* = .33).

Thromboelastometry

Thromboelastometry performed in 52 KS patients and 43 controls showed a procoagulant imbalance in the former. A greater α angle and MCF with only the former reaching a statistical significance (*P* = .01) were observed when testing for EXTEM in KS patients compared to controls (Fig. 3).

Table 1. Clinical and biochemical characteristics of Klinefelter syndrome patients and controls

	Controls	KS, all	*P* controls vs KS, all	T-treated KS	Non–T-treated KS	*P* T-treated vs non–T-treated KS
No.	58	58	–	35	23	–
Age, y, median (range)	37.5 (22-77)	39.7 (18-78)	.93	41 (20-58)	27 (18-78)	.08
Nonmosaic 47,XXY	–		–		21	
n (%)	–	53 (91.4)	–	32		
Mosaic 46,XY/47,XXY					2	
n (%)		4 (6.9)		2		.66
48,XXXY					0	
n (%)	–	1 (1.7)	–	1		
BMI, kg/m^2, median (range)	24.6 (18.5-34.1)	26.1 (16.1-36.4)	.06	26.5 (18.3-36.4)	25.3 (16.1-32.0)	.47
Fasting plasma glucose, mg/dL, mean (SD)	NA	93.5 (6.5)	–	94.3 (7.3)	92.3 (4.9)	.43
Glycated hemoglobin mmol/mol, mean (SD)	NA	34 (3.96)	–	36 (3.7)	35 (4.4)	.59
Impaired fasting glucose, n (%)	3 (5%)	7 (12)	.73	7 (20)	0 (0)	.03
Dyslipidemia, n (%)	5 (9%)	26 (45)	.001	18 (51)	9 (39)	.43
Arterial hypertension, n (%)	5 (9%)	3 (5.2)	.26	3 ()	0 (0)	.27
Smoking habit, n (%)	9 (16%)	15 (26)	.81	10 (29%)	5 (22)	.76
T levels nmol/L, median (range)	16.7 (12.1-28.4)	15.8 (0.9-35.7)	.59	20.2 (3.7-35.7)	11.8 (0.9-33.6)	.001
LH mIU/mL, median (range)	5.8 (2.3-9.2)	16.0 (0-52.2)	.01	3.8 (0-43.1)	25.4 (13.1-52.2)	< .001
FSH mIU/mL, median (range)	3.8 (0.8-10.6)	18.9 (0-75.0)	< .001	6.6 (0-22.9)	38.9 (15.1-75.0)	< .001
T replacement therapy, n (%)	0	35 (60)	< .001	35	0	–
T formulation						
Long-acting injectable T undecanoate, n (%)	–	24 (69)	–	24	–	–
Daily 2% transdermal T gel, n (%)	–	11 (31)	–	11	–	–
Hematocrit, %, mean (SD)	39.6 (3.0)	40.9 (4.1)	.10	41.8 (3.6)	39.2 (4.6)	.04
Arterial thrombotic events, n (%)	0	1 (1.7)	> .99	1	0	> .99
Venous thrombotic events, n (%)	0	1 (1.7)	> .99	0	1	.40
Chronic venous insufficiency, n (%)	0	3 (5.2)	.24	2	1	> .99

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; KS, Klinefelter syndrome; LH, luteinizing hormone; NA, not available; T, testosterone.

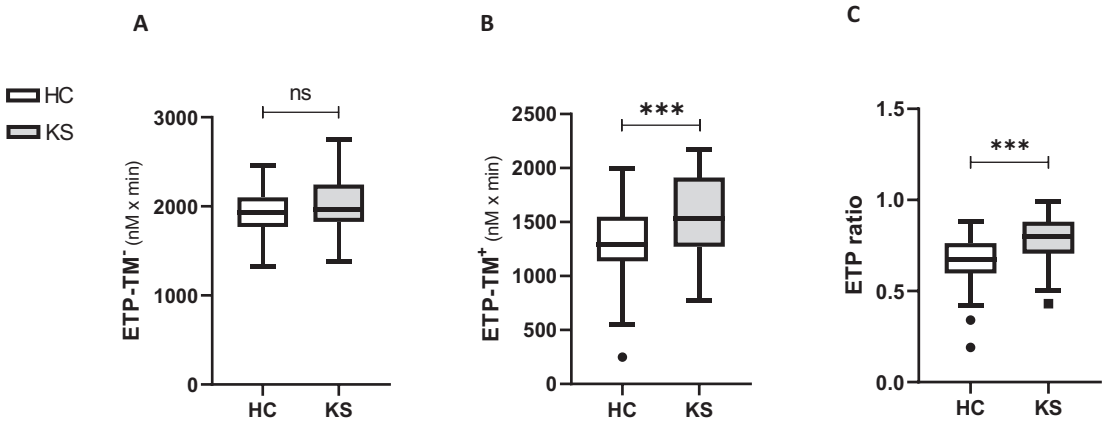

Figure 1. Comparison of endogenous thrombin potential (ETP) between controls (HC, white boxes) and patients with Klinefelter syndrome (KS, gray boxes). A, ETP assessed without addition of thrombomodulin (TM^-). B, ETP assessed with addition ofTM (TM^+). C, ETP ratio calculated as [$ETP\text{-}TM^+$/$ETP\text{-}TM^-$]. *P less than .05; **P less than .01; ***P less than .001.

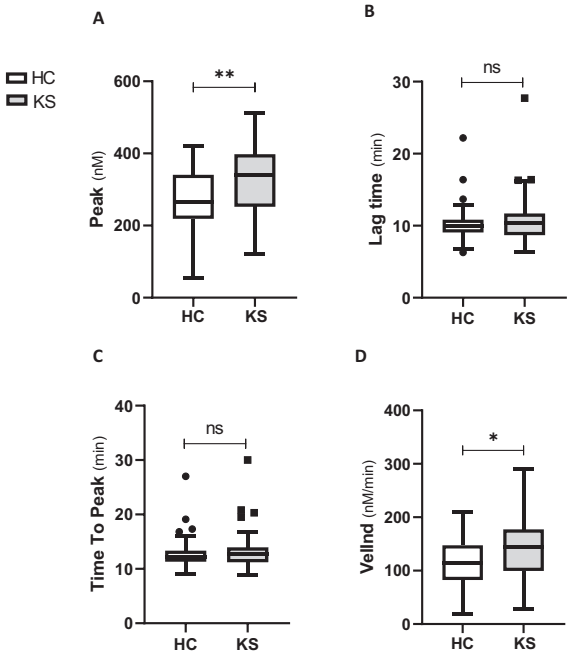

Figure 2. Comparison of other thrombin generation parameters between healthy controls (HC, white boxes) and patients with Klinefelter syndrome (KS, gray boxes). A, Peak-thrombin. B: Lag time, the time (minutes) from the addition of the trigger to the initiation of thrombin generation. C: Time-to-peak (minutes), the time needed to reach the peak-thrombin. D: Velocity index (VelInd), which depends on thrombin peak, the lag time, and the time-to-peak. *P less than .05; **P less than .01; ***P less than .001.

When cytochalasin D was added to inhibit platelet activity, a significant trend toward hypercoagulability was again evident in the KS group compared to controls in terms of greater MCF ($P < .001$) and α angle ($P < .001$) (see Fig. 3).

Association between procoagulant imbalance and clinical and biochemical characteristics

In the univariate analysis conducted in the KS patient group, a positive linear relationship was observed between $ETP\text{-}TM^+$ and FVIII ($\rho = 0.356$, $P = .006$), ETP ratio and FVIII ($\rho = 0.538$, $P < .001$), and ETP ratio and FVIII/PC ratio ($\rho = 0.448$, $P < .001$). Table 3 summarizes the correlation coefficients and statistical significance for the examined variables. Overall, no relationship was found for testosterone replacement therapy or serum testosterone levels with TGA parameters and coagulation factor concentrations. Conversely, age showed a positive association with $ETP\text{-}TM^+$, ETP ratio, and fibrinogen (Fig. 4). When patients were divided according to testosterone treatment (yes or not) and age range (≤ 29 years, 30-49 years, ≥ 50 years), younger patients on testosterone treatment showed lower thrombin generation and fibrinogen concentrations than untreated ones, but these differences did not reach statistical significance (see Fig. 4). Moreover, ETP (assessed with or without TM), ETP ratio, and fibrinogen were variably associated with dyslipidemia, BMI, and HbA_{1c} (see Table 3), whereas no such association was observed for FVIII or FVIII/PC ratio. Smoking habit, hematocrit, and arterial hypertension were not associated with the coagulation profile.

In a multivariable analysis in KS patients that included as independent variables age, testosterone concentrations, testosterone replacement therapy (yes/no), BMI, HbA_{1c}, dyslipidemia, and hematocrit, only age was confirmed as a significant predictor of $ETP\text{-}TM^+$ ($P = .009$, $\beta = 21.72$), whereas no parameter could predict ETP ratio or fibrinogen concentrations.

Table 2. Comparison of median (minimum-maximum) endogenous thrombin potential ratio between Klinefelter syndrome patients and healthy controls in assays performed on platelet-poor plasma and platelet-rich plasma

	ETP ratio			
	Controls	Klinefelter	Δ%	*P*
PPP	0.70 (0.19-0.88)	0.78 (0.43-0.99)	11	< .001
PRP	0.72 (0.45-0.93)	0.79 (0.51-0.99)	10	.009

Abbreviations: Δ%, percentage relative difference of median ETP ratio between KS and controls, calculated as {[(median ETP ratio in KS) – (median ETP ratio in controls)]/(median ETP ratio in controls)}; ETP, endogenous thrombin potential; ETP ratio: ratio of ETP measured in presence of thrombomodulin to ETP measured in its absence; KS, Klinefelter syndrome; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

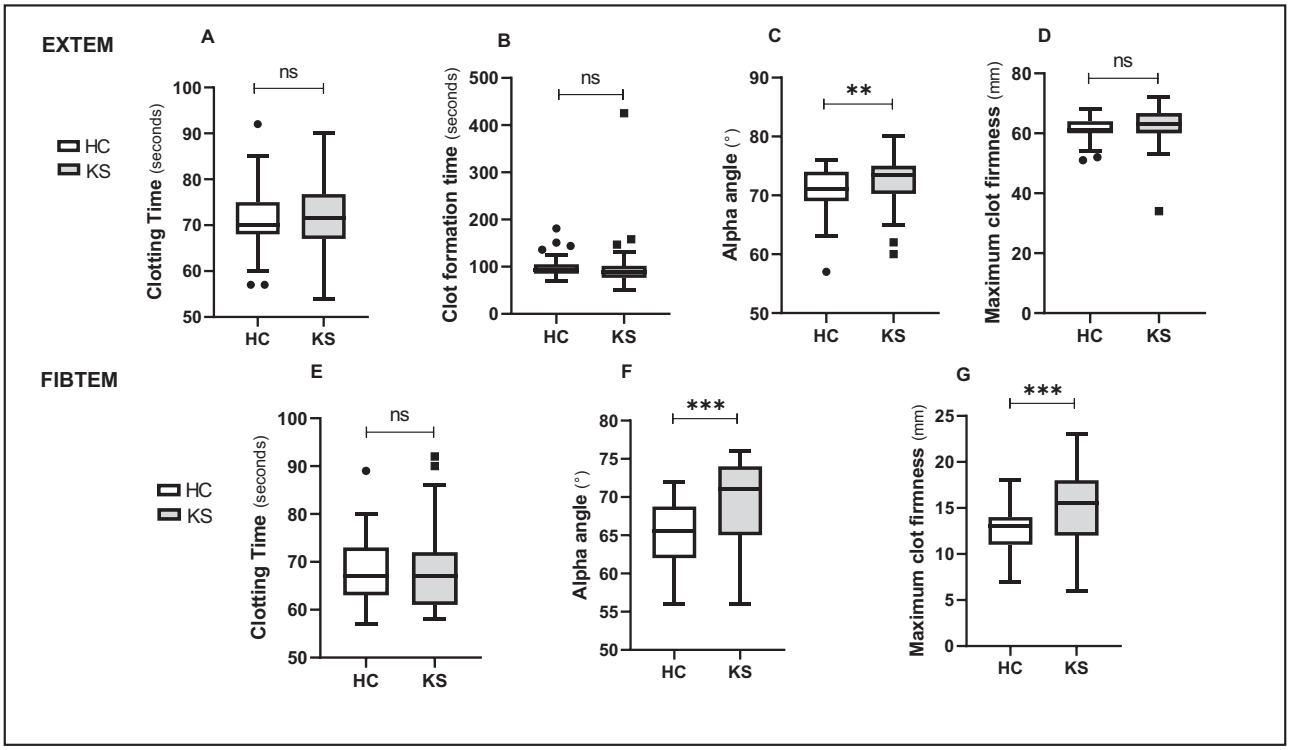

Figure 3. Thromboelastometry results in healthy controls (HC, white boxes) and patients with Klinefelter syndrome (KS, gray boxes). A to C, clotting time, clot formation time, α angle, and maximal clot firmness, respectively when measuring by the extrinsic pathway (EXTEM). E to G: clotting time, α angle, and maximal clot firmness, respectively, when assessed on platelets activity inhibition (FIBTEM). **P* less than .05; ***P* less than .01; ****P* less than .001.

Finally, in a multivariable analysis involving both KS patients and controls that included KS diagnosis (yes/no), total testosterone concentrations, testosterone replacement therapy (yes/no), BMI, and dyslipidemia as independent variables, the KS diagnosis was the only variable significantly associated with ETP-TM$^+$ ($P = .02$, $\beta = 317.4$) and ETP ratio ($P = .005$, $\beta = .1421$).

Discussion

KS patients are at increased risk of arterial/venous thrombosis (5-8) and death from thrombosis (8, 10) compared to the general population. A number of risk factors have been described encompassing autoimmune disorders like antiphospholipid syndrome (33), genetic predisposition (34-39), or increased levels of procoagulant and antifibrinolytic factors (36, 40). However, evidence relies on small series or anecdotal reports evaluating single components of the coagulation cascade (17). The only study available on TGA in KS patients has been reported by Chang et al (41), who used PPP. The authors did not find a significant difference in TGA parameters between controls and KS patients, irrespective of testosterone replacement therapy, and concluded that platelet activation, rather than plasmatic factors, are affected in KS individuals. However, in that study TGA was performed in the absence of TM. We performed TGA in the

Table 3. Association of thrombin generation test parameters and clinical and biochemical characteristics of Klinefelter syndrome patients in univariate analysis

	Age, y	BMI	HbA_{1c}	Dyslipidemia	Hematocrit	T treatment yes/no	TT levels
ETP-TM$^-$	0.232	**0.464**^{b}	**0.474**^{a}	0.214	0.176	–0.039	0.077
ETP-TM$^+$	**0.331**^{a}	0.226	**0.457**^{a}	0.263	–0.045	–0.049	0.217
ETP ratio	**0.285**^{a}	–0.047	0.309	**0.322**^{a}	–0.086	–0.040	0.161
Fibrinogen	**0.438**^{b}	**0.474**^{b}	**0.385**^{a}	**0.375**^{a}	–0.003	–0.103	–0.029
FVIII	0.144	0.194	0.201	0.242	–0.135	0.110	–0.055
FVIII/PC	0.094	0.103	–0.228	0.078	–0.210	0.015	0.004

Correlation coefficients are reported.
Abbreviations: BMI, body mass index; ETP, endogenous thrombin potential; ETP ratio, [(ETP-TM$^+$):(ETP-TM$^-$)] ratio; ETP-TM$^-$, endogenous thrombin potential without thrombomodulin; ETP-TM$^+$, endogenous thrombin potential with thrombomodulin; FVIII, factor VIII; FVIII/PC, factor VIII:protein C ratio; HbA_{1c}, glycated hemoglobin A_{1c}; T, testosterone; TT, serum total testosterone.
^{a}*P* less than .05; ^{b}*P* less than .01.

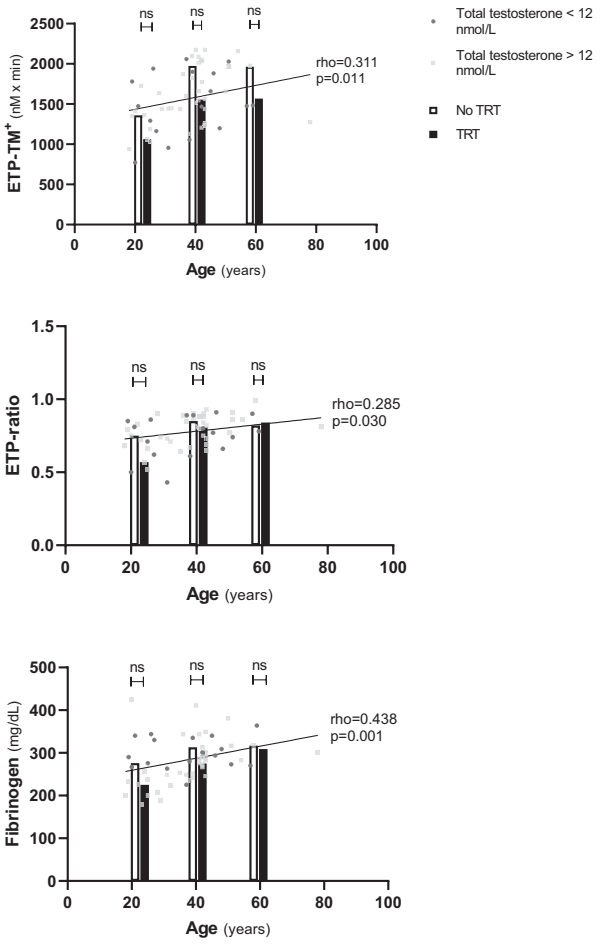

Figure 4. Association between age (years) and coagulation parameters in Klinefelter syndrome (KS) patients. Dark gray dots represent KS patients with total testosterone concentrations less than 12 nmol/L; light gray dots indicate KS patients with total testosterone concentrations greater than 12 nmol/L (25). Bars differentiate KS patients not taking any testosterone therapy at study entry (white bars, median) and patients on testosterone treatment (black bars, median) for each age range: 29 years or younger, 30 to 49 years, and 50 years or older. Correlation coefficient and *P* value are reported. ETP-TM$^+$, endogenous thrombin potential as assessed in platelet-poor plasma after addition of thrombomodulin; ETP ratio, ratio of ETP assessed with thrombomodulin to ETP assessed without thrombomodulin; TRT, testosterone replacement treatment.

presence of exogenous TM, which was aimed to mimic the relevant contribution of endothelial cells in the activation of PC as it occurs in vivo. Notably, these experimental conditions are sensitive to the FVIII-PC balance (17), hence it is not surprising that increased FVIII (the procoagulant) or decreased PC (the anticoagulant), make the ETP ratio with/without TM much more reliable than the TGA performed without TM to truly represent the conditions operating in vivo (17). This is further substantiated if one considers that activated PC is the physiological inhibitor to FVIII (17, 42). In the present study, we found that KS patients display increased ETP measured in the presence of TM, as well as

higher ETP ratio compared to controls, whereas ETP did not differ significantly between the 2 groups when assessed in the absence of TM. The latter findings are in line with data by Chang and colleagues (41). Overall, our results indicate that KS patients possess a plasma procoagulant imbalance, likely due to increased levels of FVIII (ie, one of the most potent triggers of thrombin generation) and to the increased FVIII/PC ratio. Interestingly, FVIII and FVIII/PC ratio are also directly correlated with ETP-TM$^+$ and ETP ratio. Increased FVIII in KS had already been described in previous reports (34, 36, 43). However, unlike other series investigating these changes in patients experiencing thrombotic events, we documented that they could occur in the whole KS population as a hallmark of a generalized prothrombotic state.

The procoagulant imbalance TGA showed in KS was not mirrored by shortened PT or APTT, which were comparable to controls. This is not surprising because these tests are instrumental for the diagnosis of hemorrhagic diseases but much less suitable to investigate prothrombotic conditions subsequent to congenital deficiency of naturally occurring anticoagulants (17). The procoagulant imbalance in KS was further supported by the results of thromboelastometry, which points at more rapid clot formation and greater clot strength as shown by the increased α angle and MCF that were observed in KS compared to controls.

Metabolic syndrome is another circumstantial determinant of thrombosis. Indeed, the prevalence of metabolic syndrome is greatly increased in KS (44). Furthermore, diabetes, obesity, and dyslipidemia are recognized risk factors for cardiovascular outcomes. Interestingly, whereas advancing age and some metabolic features displayed an association with fibrinogen and thrombin generation in the univariate analysis in the KS group, only advancing age was confirmed as a significant predictor of ETP (but not of ETP ratio) in the multivariable analysis. Overall, these findings suggest that coagulation abnormalities, including increased FVIII and fibrinogen concentrations, should be regarded as intrinsic features of KS, with only a minor contribution, if any, of the metabolic background. We can speculate that these features may hypothetically mirror altered gene expression. Several genes, located both on autosomal and sexual chromosomes, are differentially expressed in KS patients compared to 46,XX women and 46,XY men (16), probably as a result of epigenetic changes (45, 46), and in association with syndromic features. Admittedly, coagulative changes may still be related to other metabolic features (eg, fat mass, inflammatory status) that were not specifically addressed in the present study.

We also documented a lack of association between testosterone treatment or circulating testosterone levels and thrombin generation or coagulation factor concentrations. Although the association between testosterone treatment and increased thrombotic risk in the general male hypogonadal population is controversial (47), recent studies focusing on KS patients do not support such an association. In the study by Chang et al (41), thrombin generation was lower in treated than untreated KS patients. The same authors in a recently published KS cohort reported that testosterone replacement was (nonsignificantly) associated with lower incidence rates of venous thrombosis and thrombotic deaths (8), thus indirectly confirming that replacement therapy does not negatively affect the procoagulant imbalance of KS individuals. Whether and in which way restoration of normal testosterone concentrations may exert a protective role in KS have not been thoroughly investigated so far. Male hypogonadism has been associated with unfavorable procoagulant and antifibrinolytic changes (48, 49), while testosterone may enhance anticoagulant activity (50) and modulate platelet activation (51). Although similar data in KS patients are lacking, in the present study coagulation parameters showed no correlation with the androgenic state except for a (nonstatistically significant) reduction of thrombin generation and fibrinogen concentrations among young testosterone-treated patients. However, in a study by Haymana et al, concentrations of asymmetric dimethylarginine (a marker of endothelial dysfunction) were negatively associated with total testosterone levels ($\beta = -.412$, $P = .001$) in KS patients (14). Therefore, it is possible that testosterone acts on targets (eg, endothelial function) other than coagulation factors in KS. Further investigations are needed to assess the effects of testosterone treatment on the thrombotic profile of KS individuals.

Besides coagulation factors, platelets may play a direct role in the thrombosis risk of KS. Platelets in fact possess a dual function in hemostasis. First, they adhere to the subendothelial matrix at the site of vessel wall injury (or perturbation) and then aggregate to each other to form a platelet plug; this is collectively known as the hemostatic function of platelets. Second, platelet membranes on activation express negatively charged phospholipids that help assemble the coagulation factors that are instrumental to generating thrombin; this is collectively known as the platelet procoagulant function. The platelet hemostatic function is explored by tests of adhesion/aggregation and/or by the measurement of biochemical markers secreted by activated platelets. The procoagulant function is investigated by TGA performed in PRP, in which the phospholipids needed for coagulation factor alignment are provided by the platelets themselves. In thromboelastometry, platelet contribution can be assessed by comparison of parameters obtained in EXTEM and in FIBTEM, but the hemostatic and the procoagulant functions cannot be differentiated.

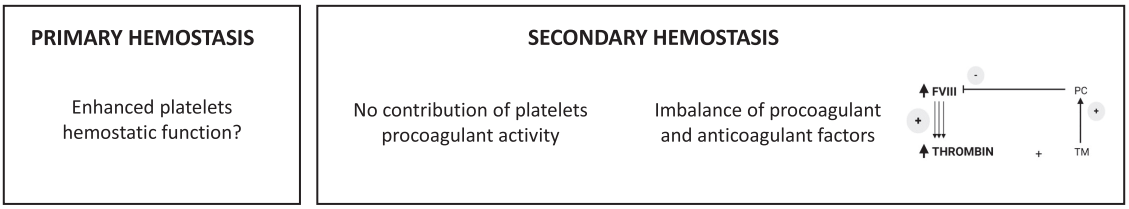

Figure 5. Hypothetical interactions of platelet function and components of the coagulation cascade in Klinefelter syndrome. FVIII, factor VIII; PC, protein C;TM, thrombomodulin.

Di Minno et al reported that platelets from KS patients express higher levels of the activation markers 8-iso-prostaglandin F2α and 11-dehydro-thromboxane-B_2, and require exposure to lower concentrations of arachidonic acid to reach adequate levels of aggregation (15). Norris and colleagues described the case of a 47,XXY patient with leg ulcers and evidence of enhanced platelet aggregation in response to a low concentration of adenosine diphosphate (52). These findings are consistent with a greater platelet hemostatic reactivity. However, in the KS cohort reported by Chang et al, platelet aggregation was not increased in testosterone-naive patients (53). The results on the hemostatic function of platelets when combined with the results of the present TGA study in PRP and PPP are consistent with the hypothesis depicted in Fig. 5, whereby the (enhanced) platelet hemostatic function (although still controversial) could play a role in the thrombotic risk observed in KS, but their contribution to secondary hemostasis (ie, thrombin generation) is unlikely because it is related much more to plasma than to platelets.

Our study has some limitations. First, owing to the relatively small sample size and consequently small number of thrombotic events, we could not assess whether there are TGA difference in KS patients with or without thrombosis. Second, we did not systematically investigate our cohort for the presence of prothrombotic mutations and polymorphisms, which may contribute to thrombosis. Metabolic parameters were not recorded at the time of study blood sampling but up to 3 months earlier, and this may have introduced potential bias to the association analysis. Third, we did not investigate extensively for the possible contribution to the heightened thrombin generation brought about by the molecular species released in plasma following neutrophil activation (ie, NETs). It is unlikely however that NETs are increased in our cohort because the levels of MPO (a surrogate marker of NETs) were similar in patients and controls. Similarly, we did not investigate for the possible contribution of microparticles derived from platelets, monocytes, and endothelial cells, which disseminate in the circulation the procoagulant asset derived from the parent cells (54, 55). Elevated levels of circulating microparticles are observed in a variety of diseases (54), and it has been shown that increased levels of microparticles are likely to contribute to thrombosis (56).

In conclusion, this study shows a plasma procoagulant imbalance in patients with KS as indicated by increased thrombin generation in the presence of TM, which is driven (at least in part) by increased levels of FVIII. The procoagulant imbalance is also substantiated by alterations of the parameters of thromboelastometry. These biochemical findings are in line with the relatively high thrombotic risk observed in these patients. Whether antithrombotic prophylaxis should be considered in these patients should be investigated by appropriate clinical trials.

Acknowledgments

Financial Support: This work was supported by Ricerca Corrente Funds from the Italian Ministry of Health to Fondazione IRCCS Ca' Granda Policlinico Ospedale Maggiore, and IRCCS Istituto Auxologico Italiano (Ricerca Corrente funds: O5C622_2016).

Additional Information

Correspondence: Giovanna Mantovani, MD, PhD, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Department: Endocrinology Unit, Via Francesco Sforza, 35, Milan, 20122, Italy. Email: giovanna.mantovani@unimi.it.

Disclosures: The authors have nothing to disclose.

Data Availability: Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Kanakis GA, Nieschlag E. Klinefelter syndrome: more than hypogonadism. *Metabolism*. 2018;**86**:135-144.
2. Bonomi M, Rochira V, Pasquali D, Balercia G, Jannini EA, Ferlin A; Klinefelter ItaliaN Group (KING). Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. *J Endocrinol Invest*. 2017;**40**(2):123-134.
3. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebæk A. Klinefelter syndrome: integrating genetics, neuropsychology, and endocrinology. *Endocr Rev*. 2018;**39**(4):389-423.

4. Bojesen A, Juul S, Birkebaek N, Gravholt CH. Increased mortality in Klinefelter syndrome. *J Clin Endocrinol Metab*. 2004;**89**(8):3830-3834.
5. Campbell WA, Price WH. Venous thromboembolic disease in Klinefelter's syndrome. *Clin Genet*. 1981;**19**(4):275-280.
6. Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. *J Clin Endocrinol Metab*. 2006;**91**(4):1254-1260.
7. Zöller B, Ji J, Sundquist J, Sundquist K. High risk of venous thromboembolism in Klinefelter syndrome. *J Am Heart Assoc*. 2016;**5**(5):e003567.
8. Chang S, Christiansen CF, Bojesen A, Juul S, Münster AB, Gravholt CH. Klinefelter syndrome and testosterone treatment: a national cohort study on thrombosis risk. *Endocr Connect*. 2020;**9**(1):34-43.
9. Calogero AE, Giagulli VA, Mongioì LM, et al; Klinefelter ItaliaN Group (KING). Klinefelter syndrome: cardiovascular abnormalities and metabolic disorders. *J Endocrinol Invest*. 2017;**40**(7):705-712.
10. Swerdlow AJ, Higgins CD, Schoemaker MJ, Wright AF, Jacobs PA; United Kingdom Clinical Cytogenetics Group. Mortality in patients with Klinefelter syndrome in Britain: a cohort study. *J Clin Endocrinol Metab*. 2005;**90**(12):6516-6522.
11. Becker KL. Clinical and therapeutic experiences with Klinefelter's syndrome. *Fertil Steril*. 1972;**23**(8):568-578.
12. Price WH, Clayton JF, Wilson J, Collyer S, De Mey R. Causes of death in X chromatin positive males (Klinefelter's syndrome). *J Epidemiol Community Health*. 1985;**39**(4):330-336.
13. Condorelli RA, Calogero AE, La Vignera S. Different profile of endothelial cell apoptosis in patients with Klinefelter's syndrome. *J Endocrinol Invest*. 2013;**36**(2):84-91.
14. Haymana C, Aydogdu A, Demirci I, et al. Increased endothelial dysfunction and insulin resistance in patients with Klinefelter syndrome. *Endocr Metab Immune Disord Drug Targets*. 2018;**18**(4):401-406.
15. Di Minno MN, Esposito D, Di Minno A, et al. Increased platelet reactivity in Klinefelter men: something new to consider. *Andrology*. 2015;**3**(5):876-881.
16. Zitzmann M, Bongers R, Werler S, et al. Gene expression patterns in relation to the clinical phenotype in Klinefelter syndrome. *J Clin Endocrinol Metab*. 2015;**100**(3):E518-E523.
17. Tripodi A. Detection of procoagulant imbalance: modified endogenous thrombin potential with results expressed as ratio of values with-to-without thrombomodulin. *Thromb Haemost*. 2017;**117**(5):830-836.
18. Tripodi A. Thrombin generation assay and its application in the clinical laboratory. *Clin Chem*. 2016;**62**(5):699-707.
19. Tripodi A, Legnani C, Chantarangkul V, Cosmi B, Palareti G, Mannucci PM. High thrombin generation measured in the presence of thrombomodulin is associated with an increased risk of recurrent venous thromboembolism. *J Thromb Haemost*. 2008;**6**(8):1327-1333.
20. Grundy SM, Cleeman JI, Daniels SR, et al; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation*. 2005;**112**(17):2735-2752.
21. Mach F, Baigent C, Catapano AL, et al; ESC Committee for Practice Guidelines (CPG); ESC National Cardiac Societies. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Atherosclerosis*. 2019;**290**:P140-P205.
22. Williams B, Mancia G, Spiering W, et al; Task Force for the Management of Arterial Hypertension of the European Society of Cardiology and the European Society of Hypertension. 2018 ESC/ESH guidelines for the management of arterial hypertension. *J Hypertens*. 2018;**36**(10):1953-2041.
23. American Diabetes Association. 2. Classification and diagnosis of diabetes: *Standards of Medical Care in Diabetes—2020*. *Diabetes Care*. 2020;**43**(Suppl 1):S14-S31.
24. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: an endocrine society. *J Clin Endocrinol Metab*. 2018;**103**(5):1715-1744.
25. Khera M, Adaikan G, Buvat J, et al. Diagnosis and treatment of testosterone deficiency: recommendations from the fourth International Consultation for Sexual Medicine (ICSM 2015). *J Sex Med*. 2016;**13**(12):1787-1804.
26. Zitzmann M, Aksglaede L, Corona G, et al. European Academy of Andrology guidelines on Klinefelter syndrome: endorsing organization: European Society of Endocrinology. *Andrology*. Published online September 22, 2020. doi: 10.1111/andr.12909
27. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;**33**(1):4-15.
28. Chantarangkul V, Clerici M, Bressi C, Giesen PL, Tripodi A. Thrombin generation assessed as endogenous thrombin potential in patients with hyper- or hypo-coagulability. *Haematologica*. 2003;**88**(5):547-554.
29. Tripodi A, Primignani M, Chantarangkul V, et al. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology*. 2009;**137**(6):2105-2111.
30. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood*. 2014;**123**(18):2768-2776.
31. Shaydakov ME, Sigmon DF, Blebea J. *Thromboelastography (TEG)—StatPearls*. StatPearls Publishing; 2020. Accessed May 10, 2020. https//www.ncbi.nlm.nih.gov/books/NBK537061
32. Tripodi A, Ammollo CT, Semeraro F, et al. Hypercoagulability in patients with cushing disease detected by thrombin generation assay is associated with increased levels of neutrophil extracellular trap-related factors. *Endocrine*. 2017;**56**(2):298-307.
33. Mount GR, Roebuck JD. Antiphospholipid syndrome in a 21-year-old with Klinefelter syndrome. *J Clin Rheumatol*. 2009;**15**(1):27-28.
34. Seth A, Rajpal S, Penn RL. Klinefelter's syndrome and venous thrombosis. *Am J Med Sci*. 2013;**346**(2):164-165.
35. Angel JR, Parker S, Sells RE, Atallah E. Recurrent deep vein thrombosis and pulmonary embolism in a young man with Klinefelter's syndrome and heterozygous mutation of MTHFR-677C > T and 1298A > C. *Blood Coagul Fibrinolysis*. 2010;**21**(4):372-375.
36. Glueck CJ, Jetty V, Goldenberg N, Shah P, Wang P. Thrombophilia in Klinefelter syndrome with deep venous thrombosis, pulmonary embolism, and mesenteric artery thrombosis on testosterone therapy: a pilot study. *Clin Appl Thromb Hemost*. 2017;**23**(8):973-979.

37. Lapecorella M, Marino R, De Pergola G, Scaraggi FA, Speciale V, De Mitrio V. Severe venous thromboembolism in a young man with Klinefelter's syndrome and heterozygosis for both G20210A prothrombin and factor V Leiden mutations. *Blood Coagul Fibrinolysis*. 2003;**14**(1):95-98.
38. Shanmugam VK, Tsagaris KC, Attinger CE. Leg ulcers associated with Klinefelter's syndrome: a case report and review of the literature. *Int Wound J*. 2012;**9**(1):104-107.
39. Hussein TM, Abd Elmoaty Elneily D, Mohamed Abdelfattah Elsayed F, El-Attar LM. Genetic risk factors for venous thromboembolism among infertile men with Klinefelter syndrome. *J Clin Transl Endocrinol*. 2020;**20**:100228.
40. Erkal B, Kalayci Yigin A, Palanduz S, Dasdemir S, Seven M. The effect of PAI-1 gene variants and PAI-1 plasma levels on development of thrombophilia in patients with Klinefelter syndrome. *Am J Mens Health*. 2018;**12**(6):2152-2156.
41. Chang S, Biltoft D, Skakkebæk A, et al. Testosterone treatment and association with thrombin generation and coagulation inhibition in Klinefelter syndrome: a cross-sectional study. *Thromb Res*. 2019;**182**:175-181.
42. Boffa MC, Karmochkine M. Thrombomodulin: an overview and potential implications in vascular disorders. *Lupus*. 1998;**7**(Suppl 2):S120-S125.
43. Dissemond J, Knab J, Lehnen M, Goos M. Increased activity of factor VIII coagulant associated with venous ulcer in a patient with Klinefelter's syndrome. *J Eur Acad Dermatol Venereol*. 2005;**19**(2):240-242.
44. Bojesen A, Kristensen K, Birkebaek NH, et al. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. *Diabetes Care*. 2006;**29**(7):1591-1598.
45. Manotas MC, Calderón JC, López-Kleine L, Suárez-Obando F, Moreno OM, Rojas A. Identification of common differentially expressed genes in Turner (45,X) and Klinefelter (47,XXY) syndromes using bioinformatics analysis. *Mol Genet Genomic Med*. 2020;**8**:e1503.
46. Salemi M, Cannarella R, Condorelli RA, et al. Evidence for long noncoding RNA GAS5 up-regulationin patients with Klinefelter syndrome. *BMC Med Genet*. 2019;**20**(1):4.
47. Houghton DE, Alsawas M, Barrioneuvo P, et al. Testosterone therapy and venous thromboembolism: a systematic review and meta-analysis. *Thromb Res*. 2018;**172**:94-103.
48. Caron P, Bennet A, Camare R, Louvet JP, Boneu B, Sié P. Plasminogen activator inhibitor in plasma is related to testosterone in men. *Metabolism*. 1989;**38**(10):1010-1015.
49. Erem C, Kocak M, Hacihasanoglu A, Yilmaz M. Blood coagulation and fibrinolysis in male patient with hypogonadotropic hypogonadism: plasma factor V and factor X activities increase in hypogonadotropic hypogonadism. *J Endocrinol Invest*. 2008;**31**(6):537-541.
50. Jin H, Lin J, Fu L, et al. Physiological testosterone stimulates tissue plasminogen activator and tissue factor pathway inhibitor and inhibits plasminogen activator inhibitor type 1 release in endothelial cells. *Biochem Cell Biol*. 2007;**85**(2):246-251.
51. Li S, Li X, Li J, Deng X, Li Y, Cong Y. Experimental arterial thrombosis regulated by androgen and its receptor via modulation of platelet activation. *Thromb Res*. 2007;**121**(1):127-134.
52. Norris PG, Rivers JK, Machin S, Dowd PM, Griffiths WAD. Platelet hyperaggregability in a patient with Klinefelter's syndrome and leg ulcers. *Br J Dermatol*. 1987;**117**(1):107-109.
53. Chang S, Larsen OH, Skakkebæk A, et al. Aggregation is not increased in testosterone treatment naive Klinefelter syndrome. Poster presented at: ENDO 2017: the Endocrine Society annual meeting; April 1-4, 2017; Orlando, FL, USA.
54. Owens AP III, Mackman N. Microparticles in hemostasis and thrombosis. *Circ Res*. 2011;**108**(10):1284-1297.
55. Horstman LL, McCauley RF, Jy W, Ahn YS. Tissue factor-negative cell-derived microparticles play a distinctive role in hemostasis: a viewpoint review. *Semin Thromb Hemost*. 2019;**45**(5):509-513.
56. Bucciarelli P, Martinelli I, Artoni A, et al. Circulating microparticles and risk of venous thromboembolism. *Thromb Res*. 2012;**129**(5):591-597.