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Platelet aggregation in Klinefelter syndrome is not aggravated by testosterone replacement therapy: A longitudinal follow-up study

Simon Chang1,2 | Ole Halfdan Larsen3 | Anne-Mette Hvas4,5 | Anne Skakkebæk6 | Claus Højbjerg Gravholt2,3 | Anna-Marie Bloch Münster1

Abstract

Background: Men with Klinefelter syndrome (KS) are routinely offered testosterone replacement therapy (TRT) suggested to potentially promote platelet aggregation and increase cardiovascular risk.

Objective: We investigated platelet aggregation in men with KS before and during TRT.

Materials and methods: Forty-one adult men with KS participated, of which 20 had no history of TRT at baseline, with 15 completing follow-up after 18 months TRT. Further, we included 21 adult men with KS on long-term TRT (>10 years) and a male reference population. We assessed platelet impedance aggregometry using adenosine diphosphate (6.5 μM), thrombin-receptor-activating-peptide-6 (TRAP 32 μM), and arachidonic acid (ASPI 0.5 mM) as agonists in KS compared to a male reference population and stratified by route of TRT administration.

Results: Platelet aggregation among men with KS at baseline or during TRT was not increased compared with the male reference population. For all three agonist, no change was seen in platelet aggregation in KS at follow-up compared with baseline \( (p \geq 0.2) \). Platelet aggregation was not associated with total testosterone and furthermore, platelet count was not affected by treatment with testosterone. Men with KS treated with testosterone gel showed slightly increased TRAP- and ASPI-induced platelet aggregation compared with those treated with testosterone injection \( (p = 0.02 \) and \( p = 0.04 \), respectively).

Discussion and conclusions: We observed normal platelet aggregation in men with KS before TRT and following both short and long term treatment. Our findings do not support an independent role of platelets in driving the cardiovascular risk in KS.

Keywords: clinical study, Klinefelter syndrome, platelets, testosterone

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INTRODUCTION

Klinefelter syndrome (KS), characterized by the presence of a supernumerary X-chromosome in a man, results in primary testicular failure and hypogonadotropic hypogonadism.\(^1\) Mortality due to cardiovascular disease is increased in KS,\(^2\) and KS is associated with a higher prevalence of numerous diseases associable to cardiovascular risk such as hypercholesterolemia, hypertension, the metabolic syndrome, and type 2 diabetes as well as a four times increased risk of venous thromboembolism.\(^1,^2\)

Due to the many negative effects of male hypogonadism on physical health and overall well-being,\(^3\) men with KS are routinely offered testosterone replacement therapy (TRT). However, very little data are available describing the safety of TRT in men with KS, and a possible association between TRT and increased cardiovascular risk has been much debated.\(^4\)

Rupture of a vulnerable artherosclerotic plaque leads to activation and aggregation of platelets causing occlusion of a coronary vessel.\(^5,^6\) The ability of a single parameter such as platelet aggregation measured by impedance aggregometry to predict thrombotic events is limited.\(^7\) However, impedance aggregometry has been widely applied to evaluate potential platelet contributions to the risk of cardiovascular events. This includes, for example, cardiac transplant patients\(^8\) and essential thrombocytosis where increased aggregation with effect sizes of more than 10% was suggested to contribute to the increased thrombembolic risk in the patient group.\(^9\) In addition, platelet aggregation seems to play a role in venous thromboembolism.\(^10\) Platelets might contribute to the formation of venous blood clots through several mechanisms. For instance, the activation of platelets is capable of inhibiting fibrinolysis by orchestrating clot contraction causing formation of tightly bundled fibrin clots that are less accessible for fibrinolysis, while simultaneously releasing plasminogen activator inhibitor 1 (PAI-1), a potent fibrinolysis inhibitor, from its α-granules.\(^11\) Also, microparticles derived from platelets are able to promote coagulation activation and thrombin generation through binding of activated coagulation factors to the phospholipid surface of the vesicle.\(^10\)

Platelets are seemingly sensitive to testosterone. Both platelets and their precursors, megakaryocytes, express androgen receptors,\(^12\) and platelet aggregation have been shown to be reduced in castrated men\(^13\) and conversely stimulated by testosterone supplementation in healthy men.\(^14\) Specifically, testosterone has been shown to regulate platelet aggregation by increasing platelet thromboxane A2 receptor density.\(^13,^14\)

Only few reports on platelet function in men with KS exist. Both platelet hyperaggregability and normal platelet aggregability have been demonstrated in blood samples from two individual cases of KS, applying adenosine diphosphate (ADP) as agonist.\(^15,^16\) More recently, a cross-sectional study found increased platelet aggregability in a group of 23 testosterone-treated men with KS compared with 46 age-matched controls evaluated in platelet-rich plasma samples by light transmission aggregometry following platelet activation by arachidonic acid.\(^17\) This suggests that testosterone treatment in KS could increase platelet aggregation and thereby potentially increase cardiovascular risk. On the contrary, a recent study did not find an association between testosterone and thrombin generation performed in platelet rich plasma from 52 men with KS compared with 43 age-matched controls.\(^18\) As such, the role of platelets in relations to cardiovascular risk in men with KS is still controversial.

We applied a longitudinal design to investigate platelet aggregation in men with KS before and during testosterone treatment and compared to a normal reference population. We wanted to elucidate whether KS in itself was associated with hyperaggregability, and secondly we aimed to assess any associations between platelets aggregation, hypogonadism, and testosterone treatment in men with KS. We hypothesized that platelet aggregation in untreated KS would be low to normal and that TRT would increase platelet aggregation in KS to some extent, but without causing a hyperaggregable state.

MATERIALS AND METHODS

Participants and study design

Men, 18 years of age or older, with newly diagnosed 47,XXY KS and no history of testosterone supplementation therapy were recruited between 2015 and 2018 from endocrinology and fertility clinics in Denmark and in corporation with the Danish Klinefelter Association as previously described.\(^19\) Similarly, a second group of men with 47,XXY KS on long-term TRT was included. Exclusion criteria were self-reported prior thrombosis, current anticoagulation therapy or use of platelet inhibitors, current use of narcotics (e.g., marihuana, amphetamine, cocaine etc.), diabetes mellitus, and prior severe head trauma.

At baseline, untreated men with KS initiated TRT when presenting with carefully evaluated hypergonadotropic hypogonadism. This was in accordance with the standard treatment protocol for men with KS at our outpatient clinic.\(^1\) Patients were free to choose between TRT with injectable testosterone or testosterone gel. Study follow-up was after 18 months of TRT having to be continued for the entire period.

To allow evaluation of platelet aggregation in KS compared with non-KS individuals, data from a healthy male reference population established in the clinical research laboratory at the Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus were applied.\(^9,^20\)

Ethics

The overall study was approved by the Central Denmark Regional Committees on Health Research Ethics (1-10-72-131-15), and the Danish Data Protection Agency (1-16-02-472-15). Informed consent was obtained from all participants, and the study was registered with Clinicaltrials.gov (NCT02526628). Other data have been previously published.\(^19\)
2.2.1 | Assessment of platelet aggregation

Morning blood samples were collected from an antecubital vein after overnight fasting. Blood samples for hematology, including platelet count, were anticoagulated with ethylenediaminetetraacetic acid (Becton Dickinson Biosience, CA) and then assessed using a hematology analyzer (Sysmex XE-5000/XN9000, Sysmex Europe, Norderstedt, Germany). Whole blood for platelet aggregation was collected in hirudin tubes (Roche, Basel, Switzerland) rested for a minimum of 30 min after collection and was analyzed within 2 h using the Multi-plate Analyzer (Roche, Basel, Switzerland). Hirudinized blood incubated for 3 min after dilution 1:1 with 37°C isotonic saline. Agonists applied to induce platelet aggregation were ADP (ADPtest 6.5 μM), thrombin-receptor-activating-peptide-6 (TRAP) (TRAP-6test 32 μM), and arachidonic acid (ASPI) (ASPItest 0.5 mM) (all from Roche, Basel, Switzerland). Platelet aggregation was quantified as the area under the curve (AUC, aggregation units [AU]) x minutes). The aggregometer performs two measurements in parallel and when these varied with more than 20% from the mean, the experiment was repeated. The coefficient of variation (CV%) established in our laboratory is <15% for all the applied agonists.

2.3 | Reference data

For ADP and ASPI, reference data were available from 60 men (ADP, mean ± standard deviation [SD], 746 ± 162 AUC; ASPI, mean ± SD, 951 ± 174 AUC; age, median interquartile range [IQR], 43 [28–53] years; platelet count, median [IQR], 214 [195–240] × 10^9/l). For TRAP, reference data were available from 88 men (TRAP, mean ± SD, 1215 ± 174 AUC; age, median [IQR], 39 [27–53] years; platelet count, median [IQR], 239 [207–281] × 10^9/l). Individuals included for the reference data were not treated with anticoagulation therapy or platelet inhibitors.

2.4 | Sex hormones

Testosterone and estradiol were measured by liquid chromatography tandem mass spectrometry. The limit of detection was 0.1 nmol/L, and the working range was 0.2–100 nmol/L with a coefficient of variation of <10%. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were quantitatively determined by immunoassay employing the Cobas e601 electrochemiluminescence measuring unit (Cobas, Roche Diagnostics Limited, Rotkreuz, Switzerland).

2.4.1 | Metabolism

Body composition was assessed by dual-energy X-ray 129 absorptiometry (DXA) using a Hologic QDR2000/w osteodensitometer (Hologic, Inc., Waltham, 130 MA, USA). Metabolic syndrome was assessed according to NCEP ATPIII criteria as the presence of three or more risk factors: large waistline, high triglyceride levels, low high-density lipoprotein, high blood pressure, or high fasting blood sugar.

2.5 | Statistical analysis

Data from the reference population were known a priori, and based on these conditions, we calculated a sample size of n = 7 and n = 14 participants in the KS group to respectively detect a 20% or 15% change in ASPI aggregation (α = 0.05, β = 0.20). Distribution of variables was evaluated by histograms and quantile-quantile plots. Data are presented as mean ± SD or median (IQR). Comparisons of continuous variables between baseline and follow-up in normally distributed variables were compared applying a paired t-test. Non-normally distributed variables were compared applying Wilcoxon signed rank test. Group comparisons between the KS follow-up group and either the KS long-term treated group or the reference population were performed applying an unpaired t-test or Wilcoxon rank sum test according to distribution. Comparisons between different types of treatment were performed in a similar way. Additionally, multivariate regression with treatment type as factor variable and adjustment for testosterone, FSH, and LH was performed comparing treatment types. Association between testosterone levels and platelet aggregation was evaluated by univariate linear regression. Evaluation of associations between changes in platelet aggregation and hormone-related variables from baseline to follow-up among men with KS was performed by multilevel mixed-effects linear regression. Stata version 17.0 (StataCorp, College Station, Texas, USA) was used for statistical analysis. Graphs were created in GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, California, USA).

3 | RESULTS

3.1 | Participant characteristics

Characteristics of the participants at baseline and follow-up are given in Table 1. Twenty men with newly diagnosed KS were included at baseline. Of these, five declined to participate in the follow-up consultation. Among the 15 KS men completing follow-up, six were treated with injectable testosterone (Nebido, Bayer), and nine were treated with testosterone gel (1 with Tostran, Kyowa Kirin; 8 with Testogel, Besins). As expected, higher levels of sex hormones as well as hematocrit and hemoglobin were seen post-treatment. Similarly a significant reduction in total body fat was seen at follow-up (Table 1). Platelet count was not affected by TRT. Twenty-one KS men with long-term TRT were included. Of these, 17 were treated with injectable testosterone (Nebido, Bayer), and four were treated with testosterone gel (two with Tostran; two with Testogel). Median (IQR) duration of testosterone treatment in the long-term treated KS group was 10.5 (9.5–19.5) years.
Table 1  Age, hormone characteristics, and hematology in men with KS evaluated at baseline and follow-up and in men with KS evaluated after long-term testosterone replacement therapy

<table>
<thead>
<tr>
<th></th>
<th>Baseline n = 15</th>
<th>Follow-up n = 15</th>
<th>p-Value</th>
<th>Long-term treated n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.8 ± 9.2</td>
<td>40.5 ± 9.2</td>
<td></td>
<td>44.4 ± 8.0</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of testosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment (days)</td>
<td>557 (539–578)</td>
<td>3850 (3461–7128)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>6.2 (5.1–12.0)</td>
<td>23.0 (14.3–33.0)</td>
<td>0.0007*</td>
<td>22.0 (12.0–28.0)</td>
</tr>
<tr>
<td>Free testosterone (nmol/l)</td>
<td>0.11 (0.08–0.24)</td>
<td>0.43 (0.30–0.65)</td>
<td>0.0007*</td>
<td>0.37 (0.26–0.74)</td>
</tr>
<tr>
<td>Total estradiol (pmol/l)</td>
<td>57.0 (46.6–81.0)</td>
<td>89.1 (65.9–111.0)</td>
<td>0.009*</td>
<td>73.8 (50.9–148.0)</td>
</tr>
<tr>
<td>Free estradiol (pmol/l)</td>
<td>1.2 (1.0–1.6)</td>
<td>1.8 (1.2–2.5)</td>
<td>0.03*</td>
<td>1.8 (1.0–3.1)</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>26.5 ± 9.0</td>
<td>13.1 ± 9.8</td>
<td>0.001</td>
<td>1.6 (0.2–12.8)</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>39.1 ± 13.5</td>
<td>21.7 ± 15.6</td>
<td>0.002</td>
<td>1.6 (0.4–15.9)</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>33.7 ± 8.5</td>
<td>27.2 ± 5.6</td>
<td>0.005</td>
<td>27.9 ± 4.9</td>
</tr>
<tr>
<td>Metabolic syndrome (n [%])</td>
<td>6 (40)</td>
<td>8 (53)</td>
<td></td>
<td>13 (62)</td>
</tr>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>216 ± 35</td>
<td>230 ± 59</td>
<td>0.2</td>
<td>212 ± 39</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.43 ± 0.03</td>
<td>0.47 ± 0.02</td>
<td>0.0004</td>
<td>0.49 ± 0.05</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>8.9 (8.6–9.3)</td>
<td>9.8 (8.8–10.1)</td>
<td>0.02*</td>
<td>10.3 ± 1.0</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SD or median (IQR) except for metabolic syndrome, which is given as absolute numbers with percent of population. Comparison at baseline and follow-up by paired T-test unless otherwise stated. Abbreviations: FSH, follicle stimulating hormone; LH, luteinizing hormone. *Wilcoxon signed-rank test.

3.2 Platelet aggregation

For all three agonists, no change in platelet aggregation was seen among men with KS at follow-up compared with baseline (p ≥ 0.2, Figure 1). Platelet aggregation was not different comparing the untreated group of men with KS evaluated at baseline or at follow-up with the second group of men with KS with long-term testosterone treatment (p ≥ 0.2, Figure 1). Both TRAP- and ASPI-induced aggregation was reduced in KS at base-line compared with the reference population, while no difference was observed regarding ADP induced aggregation (Figure 1). No change in platelet aggregation was seen comparing either of the treated KS groups and the reference population. Compiling data from all testosterone treated men with KS (n = 36), both TRAP- and ASPI-induced aggregation was reduced in testosterone-treated KS compared with the reference population (p = 0.0007 and p = 0.02, respectively), while no difference was observed for ADP induced aggregation (p = 0.56).

3.2.1 Platelet aggregation and hormones in KS

Platelet aggregation at baseline was not associated with total testosterone, estradiol to testosterone ratio, hematocrit or hemoglobin (p ≥ 0.27). At baseline, total estradiol was inversely associated with both ADP- and TRAP-initiated platelet aggregation (β [95% CI] = −2.1 [−4.0; −0.2], p = 0.03) and β [95% CI] = −2.4 [−4.7; −0.2], p = 0.04, respectively) while ASPI-initiated platelet aggregation was inversely associated with LH (β [95% CI] = −11.7 [−22.3; −1.1], p = 0.03). At follow-up these associations were lost, and the only association between platelet aggregation and the aforementioned hormone-related variables was an inverse association between ADP initiated platelet aggregation and hematocrit (β [95% CI] = −3397 [−6713; −82], p = 0.045). When adjusting the regression models for platelet count, only the inverse association between TRAP and total estradiol and ASPI and LH at baseline remained significant (p = 0.046 and p = 0.02, respectively).

In long-term treated KS, we observed an inverse association between ADP-initiated platelet aggregation and hemoglobin (p = 0.02), an inverse association between TRAP initiated platelet aggregation and hematocrit and hemoglobin (p = 0.01), a positive association between TRAP-initiated platelet aggregation and LH (p = 0.01), and an inverse association between ASPI-initiated platelet aggregation and hematocrit (p = 0.04). However, all these associations were lost when adjusting the regression models for platelet count.

Applying multilevel mixed-effects linear regression, the changes in both ASPI- and TRAP-initiated platelet aggregation over the cause of follow-up were inversely associated with the concomitant changes in LH (β [95% CI] = −7.9 [−13.8; −2.0], p = 0.01 and β [95% CI] = −5.9 [−11.6; −0.1], p = 0.044, respectively) and continued to be so when adjusting for changes in platelet count. On the other hand, changes in ADP-initiated platelet aggregation was associated with changes in platelet count (β [95% CI] = 1.1 [0.2; 1.9],
3.2.2 Effect of testosterone formulation

Studying all testosterone treated men with KS, both TRAP- and ASPI-initiated platelet aggregation was increased in those treated with testosterone gel compared with those treated with injection testosterone (Table 2). Also, ADP (AUC) was numerically increased in the testosterone gel-treated group (Table 2). Total testosterone was not different between gel treated and injectable testosterone treated groups, but gonadotropin levels were higher in the gel treated group than in men treated with injectable testosterone. However, adjusting for hormone levels in a multivariate regression analysis only strengthened the association, suggesting lower platelet aggregation in men with KS treated with injection testosterone compared with gel preparations (Table 2).

4 DISCUSSION

We present the first longitudinal assessment of platelet aggregation in KS evaluated in the hypogonadal state and following both short-term and chronic long-term TRT. Compared with data from sizeable reference groups, we saw no indications of increased platelet aggregation among men with KS. In fact, KS in the hypogonadal state was associated with reduced levels of platelet aggregation. In spite of an almost four-fold increase in testosterone levels posttreatment, we saw no significant effects of testosterone supplementation on platelet aggregation in KS.

We saw reduced TRAP- and ASPI-induced platelet aggregation at baseline and when comparing all testosterone treated men with KS and the reference population, and we did not see any association between platelet aggregation and testosterone levels in any of the KS groups. Collectively, our data indicate that irrespective of testosterone treatment status, KS is not associated with platelet hyperaggregability, and thus platelet aggregation seems less likely as a driver of the excess thrombotic risk in KS. Our findings further substantiate that testosterone treatment in KS, in addition to many well-known beneficial effects, seems safe in relation to cardiovascular risk, reassuring KS patients and health care professionals.

ASPI-induced platelet aggregation in the hypogonadal state was significantly lower than the male reference population. It has been proposed that increasing testosterone levels are associated with a higher density of platelet thromboxane A2 receptors and increased aggregation. The ASPI test applies arachidonic acid to induce thromboxane A2-dependent platelet aggregation, and thus, hypogonadism in KS could affect density of platelet thromboxane A2 receptors. However, from our data, the effect size of substantially increasing testosterone levels in KS on thromboxane A2-mediated platelet aggregation is negligible and probably not clinically relevant, considering also the significant finding of overall lower ASPI-induced platelet aggregation in even the testosterone-treated KS compared with the reference population.

It can only be speculated whether testosterone in a similar fashion is modulating the density of the platelet thrombin receptor PAR-1,
causing the lower TRAP-induced platelet aggregation seen in hypogonadal men with KS compared with the reference population.

Our findings are in contrast with a previous cross-sectional report by Di Minno et al. finding increased thromboxane A2-mediated platelet aggregation in 23 testosterone-treated men with KS. Platelets have been shown to partake in complex interactions with both erythrocytes and leukocytes during activation, recruitment, and aggregation, and the use of plasma-based light transmission aggregometry in that study neglects any potential regulation of platelet aggregation by erythrocytes or leukocytes. Testosterone, as an anabolic agent, promotes erythropoiesis and by that affects the balance between formed elements of the blood. It can be speculated that in KS, testosterone might indirectly counterbalance any direct effects on platelet aggregation via modulation of secondary regulatory mechanisms related to erythrocyte and leukocyte function. Also, concordance between platelet aggregation-induced by arachidonic acid measured by either light transmission aggregometry or, as in our case, multiple electrode aggregometry via modulation of secondary regulatory mechanisms related to erythrocyte and leukocyte function. Also, concordance between platelet aggregation-induced by arachidonic acid measured by either light transmission aggregometry or, as in our case, multiple electrode aggregometry has been found to be around 80%, with overall moderate agreement between measurements (Kappa statistics = 0.48). This further hinders direct comparison between the two studies. Compared with our follow-up group, participants in the study by Di Minno et al. were on average 9 years younger and presented with lower levels of total testosterone (14.1 ± 9.5 nmol/L) and higher levels of estradiol (106.4 ± 14.6 pmol/L). This variation in age and hormonal status between the two study populations could affect platelet aggregation, although neither study saw any association between platelet aggregation and hormone levels. Also, in the study by Di Minno et al. platelet aggregation was lower in controls even though the men in the control group expressed higher levels of testosterone than the testosterone-treated KS group. This could further indicate that testosterone might not be the most prominent regulator of platelet aggregation in KS.

In a recent study by Indirli et al., the contribution of platelets did not significantly alter the respective coagulability in 52 men with KS compared with 43 healthy age-matched male controls, neither when platelet activity was inhibited during thromboelastographic evaluation of coagulation nor when comparing thrombin generation in platelet-poor or platelet-rich plasma. Platelet aggregation was not directly evaluated in that study, but the finding supports our notion that platelets may not play a prominent role in driving the excess thrombotic risk in KS.

The absence of an association between testosterone and platelet aggregability seen among our groups of men with KS, who are treated to obtain physiological levels of testosterone, is in line with previous studies on effects of exogenous testosterone applied to achieve physiological testosterone levels, while the administration of exogenous testosterone to obtain supraphysiological levels of testosterone is widely believed to have detrimental effects on normal platelet function. This underlines the need for careful monitoring of testosterone treatment in men with KS.

The association between estrogens and platelet aggregability seen among our groups of men with KS, who are treated to obtain physiological levels of testosterone, is in line with previous studies on effects of exogenous testosterone applied to achieve physiological testosterone levels, while the administration of exogenous testosterone to obtain supraphysiological levels of testosterone is widely believed to have detrimental effects on normal platelet function. This underlines the need for careful monitoring of testosterone treatment in men with KS.
TRAP-initiated platelet aggregation and estradiol observed in the untreated state at baseline, this effect is lost following TRT. Controversially, we found that the insignificant increase in platelet aggregation during the cause of follow-up was in fact associated with the halving of LH, seen as a consequence of negative feedback by improved androgenisation with increased testosterone and estrogen levels.

We have previously demonstrated a four-fold increased risk of venous thromboembolism and non-differential rates of arterial thrombosis among 1155 men with KS compared to a large age-matched population-based comparison cohort.² Our current finding of normal platelet aggregation, irrespective of testosterone treatment, is in line with these findings considering the classical concept of platelets mainly being involved in the pathology leading up to arterial thrombosis.¹⁰ Also, our findings do not support an independent role of platelets in driving the observed excess risk of venous thromboembolism among men with KS. It seems likely that the excess thrombotic risk observed in men with KS is a result of complex interactions caused by the presence of a supernumerary X chromosome transcending all aspects of physiology by causing differentiated expression of genes and proteins, inducing clinical hypogonadism and an unfavorable metabolic profile with skewing of the hemostatic balance. Importantly, however, so far no study has demonstrated any detrimental effects of TRT on clinical cardiovascular risk in men with KS, with results from an epidemiological study rather pointing toward a protective effect of TRT.²

We find higher platelet aggregation in men with KS treated with testosterone gel compared with testosterone injection. Testosterone injection is commonly believed to carry the highest risk cardiovascular impact due to relatively high testosterone levels immediately after injections compared to the more steady delivery of testosterone using gel formulations.³² In our recent epidemiological study,² we did not see an effect of testosterone formulation on the risk of venous thromboembolism or arterial thrombosis in KS. Also, among available cohort studies in non-KS populations, only one large study found higher cardiovascular risk associated with testosterone injection,³³ while most other studies have not been able to demonstrate any effect of testosterone formulation on rates of cardiovascular outcomes.³³ A larger cohort study is needed to clarify whether route of testosterone administration is a modifier of cardiovascular risk in KS, and based on the current study, we have not been able to demonstrate what causes this difference in platelet aggregation.

We applied a longitudinal design and a standard of care regimen of testosterone treatment, but the relatively low number of participants could be affecting our estimates and could limit the external validity of our findings. Due to the exploratory nature of the current study, we did not correct or adjust for multiple inferences. In addition, a starter effect of testosterone treatment has been proposed with increased cardiovascular risk during the first 3–6 months of treatment.³⁴ Having more data points closer to the onset of treatment would have been preferable, but this was not feasible here. Previously, from our epidemiological studies, we showed that the shortest time from the initiation of testosterone treatment in a man with KS and first thrombosis was 1.2 years,⁷ speaking against such a starter effect in KS. We did not adjust for the timing of treatment in those men with KS treated with injectable testosterone. It can be speculated that platelet aggregation might differ immediately before and after injections. However, as stated, we did not find an association between platelet aggregation and testosterone levels. In the current study, participants were relatively young men with KS, and it cannot be ruled out that platelet aggregation in KS with increasing age will follow a different path than in controls. Also, the included men with KS were free of comorbidities such as diabetes and previous cardiovascular diseases. Again, it can only be speculated whether platelet aggregation would be affected differently in KS compared with controls with an increasing comorbidity burden. The KS males on long term TRT and the KS males on short term TRT resembled each other concerning most measured variables, except FSH and LH, which were somewhat higher among short term KS, likely because more in this group received transdermal TRT. However, we cannot exclude the possibility that there were still discrete differences in relation to unmeasured androgen-dependent variables such as body composition, bone, and brain function.

In conclusion, we demonstrated normal platelet aggregation in men with KS both in a paired design following 18 months of testosterone treatment and in a cross-sectional design following long-term testosterone treatment. Our findings support that testosterone treatment is safe in regard to cardiovascular risk in men with KS.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS
SC helped with the research design, acquisition, analysis, and interpretation of the data, drafted the paper, and revised it critically. OHL helped with the research design, analysis, and interpretation of the data, drafted the paper, and revised it critically. AMH helped with the interpretation of the data and revised the manuscript critically. AS helped with the research design and revised the manuscript critically; CHG helped with the research design and interpretation of the data and revised the manuscript critically. AMBM helped with the research design and interpretation of the data and revised the manuscript critically.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request. cd_value_code=text

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REFERENCES


