Thromboembolism peaking 3 months after starting testosterone therapy: testosterone–thrombophilia interactions

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ABSTRACT

We assessed time of thrombotic events (venous thromboembolism (VTE)) after starting testosterone therapy (TT) in 21 men who sustained 23 VTE. The density of thrombotic events was greatest at 3 months after starting TT, with a rapid decline in events by 10 months. The 21 cases with VTE on TT differed from 110 patient controls with unprovoked VTE, not taking TT (VTE-no TT) for Factor V Leiden heterozygosity (FVL) (33 per cent vs 13 per cent, P=0.037), for high lipoprotein (a) (Lp(a)) (55 per cent vs 17 per cent, P=0.012), and for the lupus anticoagulant (33 per cent vs 4 per cent, P=0.003). These differences between cases and VTE-no TT controls were independent of age and gender. TT can interact with underlying thrombophilia–hypofibrinolysis promoting VTE. We suggest that TT should not be started in subjects with known thrombophilia. Coagulation screening, particularly for the FVL , Lp(a), and the lupus anticoagulant should be considered before starting TT, to identify men at high VTE risk who have an adverse risk/benefit ratio for TT.

Significance of this study

What is already known about this subject?

- In June 2014, based on postmarketing reports, both the Food and Drug Administration (FDA) and Canada Health published a warning outlining the risks of venous thromboembolism (VTE) to be added to all testosterone product labels.
- The FDA has subsequently emphasized the importance of a prospective, placebo-blinded controlled clinical trial to assess cardiovascular and thrombotic safety of testosterone therapy (TT).
- We previously compared thrombophilia in 67 cases who developed VTE after starting TT versus 111 patient controls who had unprovoked VTE without TT. VTE occurred 6 months (median) after starting TT. Compared with controls, cases were more likely to have Factor V Leiden heterozygosity (FVL) (24 per cent vs 12 per cent, P=0.038) and the lupus anticoagulant (14 per cent vs 4 per cent, P=0.019).
- After a first VTE while taking TT, and then continuing TT, 11 of 67 cases had a second VTE despite adequate anticoagulation, and 6 of these 11, remaining on TT and still anticoagulated, had a third VTE.

What are the new findings?

- The density of thrombotic events was greatest at 3 months after starting TT, with a rapid decline in events by 10 months.
- Twenty-one new cases with VTE on TT were more likely than 110 patient controls with unprovoked VTE, not taking TT, to have FVL (33 per cent vs 13 per cent, P = 0.037), high lipoprotein (a) (Lp(a)) (55 per cent vs 17 per cent, P = 0.012), and the lupus anticoagulant (33 per cent vs 4 per cent, P = 0.003).
- These differences between cases and VTE-no TT controls were independent of age and gender.

INTRODUCTION

Testosterone has been approved in the USA since the 1950s as hormone replacement therapy (HRT) for male hypogonadism,[1] and its use has been carefully evaluated.[2 3] Ultimately the risk/benefit ratio of testosterone therapy (TT) can optimally be better defined by a placebo-controlled clinical trial, comparable to the Women's Health Initiative trials,[4] which were required to resolve conflicting epidemiological and clinical data.

In June 2014, based on postmarketing surveillance reports, both the US Food and Drug Administration (FDA)[5] and Canada Health[6] added a warning to labeling of testosterone products that focused on risks of venous thromboembolism (VTE). VTE, especially pulmonary embolism (PE), is a major mortality risk,[7] is an important component of hospital expense,[8] and is associated with a 52 per cent increased risk of subsequent work disability.[9] The FDA has subsequently emphasized the importance of a prospective, blinded placebo-controlled clinical trial to assess cardiovascular and thrombotic safety of TT.[10] In January 2016, the FDA released a warning, of 'possible increased cardiovascular risk associated with testosterone use … we are requiring labeling changes for all prescription

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Significance of this study

How might these results change the focus of research or clinical practice?

- TT can interact with underlying thrombophilia–hypofibrinolysis resulting in VTE.
- We suggest that TT should not be started in patients with known thrombophilia and should not be continued after an initial VTE on TT.
- Coagulation screening, particularly for the FVL, Lp(a), and the lupus anticoagulant, should be considered before starting TT, to identify patients at high risk for VTE with an adverse risk/benefit ratio for TT.

testosterone products to reflect the possible increased risk of heart attacks and strokes associated with testosterone use'.[11]

The sole currently available placebo-controlled clinical trial is the National Institutes of Health–supported prospective-placebo-controlled Testosterone Trial,[12] where >51,000 men were screened to enroll 790 men over age 65 with low serum T levels (<275 ng/dL), and evidence of sexual dysfunction, physical dysfunction, or reduced vitality. Placebo controlled, the intervention arm received 12 months of testosterone gel therapy to increase serum T to the mid-normal range for men aged 19–40. Compared with placebo, there were small increases in sexual activity, desire, erectile function, and slightly improved mood in the testosterone group.[12] However, there were no differences in 6 min walking distance or vitality.[12] In four subsequent publications, the Testosterone Trial investigators assessed coronary atherosclerosis, cognitive function, bone density, and unexplained anemia.[13–16] Testosterone had no effect on memory impairment, but it increased non-calcified coronary plaque volume on CT angiography and increased bone mineral density and hemoglobin levels. The follow-up period was not long enough or powered to measure differences in cardiovascular events, fractures, or mortality.

Prothrombotic testosterone supplementation interacts[17–23] with familial and acquired thrombophilia and hypofibrinolysis,[19 24–27] which are common in the general population, leading to VTE events that peak at 3 months[28] and then rapidly decline. We and others speculate that VTE events peaking around 3 months,[28] with a subsequent sharp decline, may reflect TT-induced depletion of susceptible[29] thrombophilic patients from the population, leaving a thrombophilia-winnowed residual group with progressively fewer VTE events over time despite continuation of testosterone supplementation.

After our publication of 67 patients who developed VTE after starting TT,[27] we evaluated 21 new, not previously reported men with VTE after starting TT. Our specific aim in this study of 21 men was to assess interaction between thrombophilia and TT in the occurrence of VTE, the timing of occurrence of VTE after administration of TT, and the prevalence of thrombophilia in cases with VTE after TT compared with patient controls with unprovoked VTE, not taking exogenous TT.

MATERIALS AND METHODS

Patients

Signed informed consent

The study followed a protocol approved by the institutional review board (IRB) of the Jewish Hospital, Cincinnati, Ohio, with signed informed consent of each participant.

Exclusions from the study included patients and controls whose VTE was provoked, associated with cancer, polycythemia vera, recent soft tissue trauma, bone fracture, hip–knee–foot surgery, airline flights>8 hours, or immobilization for >2 weeks.

The 21 patients with TT–VTE (all men) were studied in the temporal order of referral to our center over the last 1.5 years because of thrombotic events. TT had been prescribed by the referring physicians. If not already stopped, TT was discontinued by us before measures of coagulation factors. From referring MDs and patient history, time elapsed from starting TT to development of a thrombotic event was recorded. Coagulation measures were made ≥2 months after the cases' initial thrombotic event.[27]

We obtained a detailed history[27 30 31] for VTE, deep venous thrombosis (DVT), PE, osteonecrosis, ocular thrombosis, recurrent miscarriage, pre-eclampsia, eclampsia, and hemolysis-liver abnormality-low platelet count (HELLP) syndrome for all of the patients with TT–VTE and VTE-no TT controls. Cases with osteonecrosis were documented as a venous thrombosis outcome equivalent by history, physical examination, imaging, and thrombophilia–hypofibrinolysis testing as previously reported.[32–35]

We relied on patient history of compliance to previously prescribed TT, without a systematic way to measure compliance.

Controls with VTE not taking testosterone

Since unprovoked VTE is associated with thrombophilia,[27] to determine whether there was an enrichment of thrombophilia in patients with VTE after starting testosterone, a control group of 110 subjects, not taking TT, referred to our center for evaluation of unprovoked VTE, was assessed (no-TT VTE controls). The controls were not age-gender matched to cases. We studied the no-TT VTE controls in the temporal sequence of their referral to our specialized thrombosis center. These patients had been referred to us for evaluation of unprovoked thrombotic events, either DVT-PE, or osteonecrosis, or ocular thrombosis.

Laboratory assessment of thrombophilia and hypofibrinolysis

PCR measures of thrombophilia (G1691A Factor V Leiden, G20210A prothrombin, methylenetetrahydrofolate reductase (MTHFR C677T/A198C), and hypofibrinolysis (plasminogen activator inhibitor (PAI-1 SERPINE 1) gene polymorphism 4G/5G) were performed using previously published methods.[27 34 35]

Serological thrombophilia measures included Factors VIII and XI, homocysteine, antigenic proteins antithrombin III, C, S and free S, and the APL syndrome (anticardiolipin antibodies (ACLA IgG and IgM), the lupus anticoagulant, anti-beta2-glycoprotein). The three components of the antiphospholipid antibody syndrome were quantitated by ELISA using previously published methods.[36–38] If ACLA

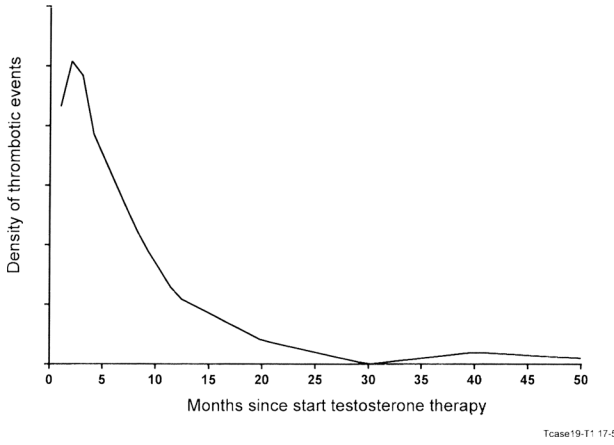

Figure 1 Quadratic spline curve illustrating the density of venous thromboembolism events over time since starting testosterone therapy.

IgG or IgM was high, or the lupus anticoagulant was present, repeat testing was done, usually within 3 months, to reconfirm the abnormality. High homocysteine was characterized by levels greater than the laboratory 95th percentile.[39]

Hypofibrinolytic lipoprotein (a) (Lp(a)) was measured,[34 35] but plasminogen activator inhibitor 1 activity levels, associated with hypofibrinolysis, were not measured.

Statistical methods

SAS V.9.4 was used for all statistical analyses.

Fisher's exact test was used to compare thrombophilia–hypofibrinolysis in cases versus VTE-no TT controls.

Stepwise logistic regression (SLE=0.20, SLS=0.05) was used to assess the association of Factor V Leiden, high Lp(a), and the lupus anticoagulant with age, gender, and the case–control group status. Also logistic regression was used to assess the association of Factor V Leiden, high Lp(a), and the lupus anticoagulant with age in cases.

The timing of VTE events was graphed; a quadratic spline curve was fitted to illustrate VTE density along the time since starting TT (figure 1).

RESULTS

The 21 TT cases were all male, age 52±14 years, median 53. Of the 21 cases, 8 had DVT alone, 5 had DVT and PE, 3 had PE alone, 2 had ischemic stroke, 2 had idiopathic osteonecrosis, and 1 had central retinal artery thrombosis. One patient had three separate PE events.

Of the 21 men, 10 had had been given TT intramuscularly, 7 used TT Gel (50 mg/day), 3 clomiphene (25, 25, and 50 mg), and 1 human chorionic gonadotropin (HCG) along with the TT. The mean±SD intramuscular TT dose was 180±128 mg/week, median 150 mg/week.

As displayed in figure 1, the peak density of thrombotic events in the 21 cases was at 3 months, with a rapid fall in events by 10 months. Seventy-six per cent of the thrombotic events in the 21 cases occurred in a time frame ranging from <1 month to 8 months after starting TT. Of the 14 patients having Factor V Leiden heterogeneity and/or high Lp(a), and/or the lupus anticoagulant, thrombotic events occurred by 3 months in 7, by 6 months in 10, by 8 months in 12, and by 12 months in all 14.

The 21 TT cases differed from the 110 VTE-no TT controls, having more Factor V Leiden heterozygosity (33 per cent vs 13 per cent, P=0.037), had more frequent high Lp(a) (55 per cent vs 17 per cent, P=0.012), and were more likely to have the lupus anticoagulant (33 per cent vs 4 per cent, P=0.003) (table 1).

For the 21 TT cases and 110 VTE-no TT controls, a logistic regression model was constructed for Factor V (Leiden vs normal), by stepwise selection from age, gender and group (cases vs controls), SLE=0.15 and SLS=0.05. Age and gender did not enter into the model, only group entered. Factor V Leiden heterozygosity was more common in cases, OR 3.43, 95 per cent CI 1.11 to 10.60.

For the 21 TT cases and 110 VTE-no TT controls, a logistic regression model was constructed for the Lp(a) (≥35 mg/dL vs <35), by stepwise selection from age, gender and group (cases vs controls), SLE=0.15 and SLS=0.05. Age and gender did not enter into the model, only group entered. Cases were more likely to have Lp(a)≥35 mg/dL, OR 5.90, 95 per cent CI 1.55 to 22.52.

For the 21 TT cases and 110 VTE-no TT controls, a logistic regression model was constructed for the lupus anticoagulant (positive vs negative), by stepwise selection from age, gender and group (cases vs controls), SLE=0.15 and SLS=0.05. Age and gender did not enter into the model, only group entered. Cases were more likely to have the lupus anticoagulant positive, OR 10.88, 95 per cent CI 2.50 to 47.23.

In the 21 TT cases, Factor V heterozygosity, Lp(a) and the lupus anticoagulant did not associate with age (P=0.76, 0.87 and 0.57, respectively, by logistic regression).

DISCUSSION

Congruent with our recent description of 67 cases with VTE after starting TT,[27] the 21 cases in the current report were more likely to be heterozygous for the Factor V Leiden mutation (33 per cent vs 13 per cent, P=037), and to have the lupus anticoagulant (33 per cent vs 4 per cent, P=0.003). A new finding was that they were also more likely to have hypofibrinolytic high Lp(a) (55 per cent vs 17 per cent, P=0.012). These differences were independent of age.

An important new finding in our current study, congruent with Martinez *et al*,[28] was that VTE events peaked at 3 months, with a subsequent rapid decline in VTE events by 10 months. Seventy-six per cent of the thrombotic events occurred in a time frame ranging from <1 month to 8 months after starting TT. Martinez *et al*[28] recently examined relationships of TT and risk of VTE. Participants included 19,215 patients with confirmed VTE (DVT and PE) and 909,530 age-matched controls from a source population including >2.22 million men.[28] Rate ratios of VTE in association with current TT were compared with no treatment by conditional logistic regression, adjusted for comorbidities and all matching factors.[28] In the first six months of TT, the rate ratio of VTE was 1.63 (95 per cent CI 1.12 to 2.37) corresponding to 10.0 (1.9–21.6) additional VTE events above the base rate of 15.8/10,000 person-years.[28] After more than 6 months on TT, the rate ratio was 1.00

Table 1 Coagulation disorders in 21 patients who had deep venous thrombosis (DVT), or pulmonary embolism (PE), or ocular thrombosis, or osteonecrosis (ON) or ischemic stroke on testosterone therapy compared with 110 patients who had DVT–PE–ON without TT (VTE-controls)

	Factor V Leiden/ low *RAPC	PTG	PAI-1	MTHFR	Factor VIII	Factor XI	ACLA IgG	ACLA IgM	Homocysteine
Abnormal range	GA, AA	GA, AA	4G4G	AA	>150%	>150%	Dated†	Dated‡	Dated§
21 TT cases	6/18 (33%)	0/12 (0%)	3/13 (23%)	6/15 (40%)	7/15 (47%)	3/15 (20%)	0/14 (0%)	3/14 (21%)	7/15 (47%)
110 VTE-controls	14/110 (13%) P=0.037	7/108 (6%) NS	33/100 (33%) NS	30/100 (30%) NS	39/106 (37%) NS	22/108 (20%) NS	4/108 (4%) NS	14/108 (13%) NS	30/105 (29%) NS

	Protein C	Protein S	Free S	Antithrombin III	Lp(a)	Lupus anticoagulant
Abnormal range	<73%	<63%	<66%	<80	≥35	positive
19 TT cases	2/13 (15%)	0/11 (0%)	0/9 (0%)	1/13 (8%)	6/11 (55%)	5/15 (33%)
110 VTE-controls	21/105 (20%) NS	12/104 (12%) NS	24/85 (28%) NS	3/102 (3%) NS	12/71 (17%) P=0.012	4/91 (4%) P=0.003

*Dated cut point for RAPC low: < 2.0 (before January 1, 1999); < 1.94 (January 1, 19 99 to January 23, 2001); < 2.2 (January 23, 2001 to March 25, 2004); < 2.1 (after March 25, 2004).
†Dated cut point for IgG high: ≥ 23 GPL (anticardiolipin G units) (before October 31, 2012); ≥ 15 (after November 1, 12).
‡Dated cut point for IgM high: ≥ 10 MPL (anticardiolipin M units) (before April 30, 2012); ≥ 13 (after May 1, 12).
§Dated cut point for homocysteine high: ≥ 13.5 µmol/L (before March 20, 2005); ≥ 12 (March 21, 2005 to March 27, 2006); ≥ 10.4 (March 28, 2006 to April 14, 2008); ≥ 11.4 (April 15, 2008 to November 14, 2008); ≥ 15 (November 15, 2008 to December 2, 2014); ≥ 10.4 (after December 3, 2014).
ACLA, anticardiolipin antibody; CC, wild type normal; Factor V, Factor V Leiden mutation; Lp(a), lipoprotein (a); MTHFR C677T, A1298C, methylenetetrahydrofolate reductore; PAIG, plasminogen activator inhibitor gene; PAI-1, plasminogen activator inhibitor activity; Pro C, protein C antigen; Pro S, protein S antigen; PTG, prothrombin gene mutation; RAPC, resistance to activated protein C; TC, heterozygote mutant; TT, homozygous mutant; VTE, venous thromboembolism.

(0.68–1.47) and after treatment cessation it was 0.68 (0.43–1.07).[28] The early increase in VTE in the first six months on TT[27 28] is also similar to that seen with oral estrogens.[40]

Martinez *et al*[28] commented that the pattern of VTE events peaking rapidly in the first three months and declining gradually thereafter may have accounted for underestimation of the association between TT use and VTE, as in a recent case–control study.[41] In our current report, like Martinez *et al*,[28] our peak VTE event rate was at 3 months after starting TT. We speculate that the rapid peak of VTE events around 3 months[28] and subsequent sharp decline reflects depletion of susceptible[29] patients with thrombophilia–hypofibrinolysis where TT interacts with procoagulants to produce VTE,[27] leaving over time a procoagulant-winnowed residual group with progressively fewer VTE events over time.

To facilitate VTE prevention, before starting TT, we recommend considering measurement of the Factor V Leiden mutation,[27] the lupus anticoagulant, and Lp(a). Our findings may have important clinical implications since VTE risk is an important determinant of the benefit/risk ratio of TT. In a parallel fashion, pretreatment thrombophilia screening before starting exogenous estrogens appears to be important in women.[42–45] In women, PE accounts for about one-third of the incidence of potentially fatal VTE events associated with HRT, and HRT increases the risk of VTE twofold to threefold.[46]

Does adequate evidence exist in the area of thrombophilia testing to evaluate risk for VTE either before starting TT or after VTE associated with TT therapy? Corona *et al*[47] have recommended an anamnestic screening for thrombophilia before starting TT, paralleling screening before use of oral contraceptives. We have made the same recommendation, based on our recent publication of 67 patients with VTE 6 months after starting TT.[27] However, It has been stated that there is little to no value based on moderate-quality evidence for thrombophilia testing among patients with a history of thromboembolism.[48 49] Hicks *et al*[49] and Stevens *et al*[48] have recommended that thrombophilia testing not be performed in most situations. Within this frame of reference, a placebo-controlled randomized trial of TT with entry thrombophilia testing would be needed. Without large randomized trials of men, a 'Mens Health Initiative', powered to assess risk cardiovascular disease (CVD), VTE, prostate cancer, and all-cause mortality of testosterone replacement therapy (TRT), there will be persistent controversy whether TT is good, bad, or indifferent. Paralleling the WHI studies in women,[4 50] an optimal prospective study would be placebo-controlled, and double-blind,[10] with thrombotic and CVD endpoints, and include assessment of elevated hemoglobin, hypertension, prostate cancer, and osteoporosis.[5 10] Such a trial would be difficult to fund and carry out since Onasanya *et al*[51] concluded that any randomized controlled clinical trial aimed at detecting a difference in cardiovascular risk between TT and placebo group would require at least 17,664 participants in each trial group. However, since, as in our current study and in the report by Martinez *et al*,[28] the peak in VTE events was 3 months after starting TT, a placebo-controlled TT study might have to be only 1 year long, to capture most of the VTE events[17–21 27 28] and, probably, cardiovascular events, the majority of which occur within the first year of starting TT.[52–55]

Thrombotic events peaked at 3 months in the current study, congruent with those in Martinez *et al*,[28] and peaked at median 4.5 months in other studies,[17–20 22] similar to CVD events peaking ~3 months.[54] The short 3-month interval between starting TT and development of thrombotic and CVD events[52–55] may, speculatively, reflect a shared thrombotic pathophysiology since CVD events ~3 months after starting TT probably do not reflect a conventional arterial atherosclerotic event.

Colburn *et al* have described recurrent renal infarctions in a patient taking both testosterone and anabolic steroids despite anticoagulation with apixaban.[56] If TT is continued in thrombophilic patients after an initial thrombotic event, concomitant adequate anticoagulation does not appear to prevent recurrent thrombotic events.[22 23 27]

In contrast to the positive association of TT with VTE reported by Martinez *et al*.,[28] Li *et al*[57] retrospectively assessed 102,650 patients treated with exogenous testosterone and 102,650 untreated patients. No significant association was found between TT and idiopathic or overall VTE events. However, there were discrepant findings for injectable formulations and the risk of overall VTE. Ramasamy *et al*[58] carried out a retrospective chart review of 217 hypogonadal men >65 years, comparing those receiving TT (n=153) to those without TT. There were only four thrombotic events, all occurring after ≥2 years of follow-up, with no difference between TT-treated men and untreated hypogonadal men for myocardial infarction (MI), trensient ischemic atttack (TIA)/ cerebrovascular accident (CVA), or PE.[58] Sharma *et al*[59] retrospectively studied 10,854 veterans not receiving TRT and 60,553 receiving TRT. Sharma *et al*[59] did not detect a significant association between TRT and risk of DVT/PE in adult men with low serum T, who were at low to moderate baseline risk of DVT/PE.

Beyond interacting with familial and acquired thrombophilia,[17–20 22 23 25 27 60 61] TT is associated with prothrombotic changes including hypertension,[62] elevated hemoglobin and hematocrit,[63] low high-density lipoprotein cholesterol,[63] platelet aggregation,[64] and transient (~4 months) hyperviscosity.[65] TT increases circulating estrogens[66] that subsequently play a role in thrombotic events.[22] Since testosterone is aromatized to E2,[67] it may be prothrombotic by the same mechanism as reported in women, where HRT interacts with the Factor V Leiden mutation to increase risk of VTE.[27]

Limitations of the current study include the following: we do not know the denominator of men taking TT, to be able to assess the rate ratio of VTE[28] in a cohort taking TT compared with controls not taking TT. However, in 19,215 men with VTE and 909,530 age-matched controls, Martinez *et al*[28] reported '… In the first six months of testosterone treatment, the rate ratio of venous thromboembolism was 1.63 (1.12 to 2.37)'. Our current and previous studies[27] of only morbid VTE may be a limitation since mortal VTE might be characterized by a larger percentage of subjects with thrombophilia.

CONCLUSIONS

Our current study of thrombotic events in 21 men, peaking 3 months after starting TT, is congruent with the population study of Martinez *et al*[28] and provides new evidence relative to the 2014 FDA warning on the TT-associated risks of VTE,[5] based in part on our previous reports.[17–23] We suggest that TT should not be started in subjects with known thrombophilia and recommend consideration of screening for thrombophilia, particularly Factor V Leiden, the lupus anticoagulant, and Lp(a) before starting TT, to identify men at high risk for VTE with an adverse risk–benefit ratio for TT. When TT is given to patients with thrombophilia, thrombosis may occur and recur[27] despite adequate anticoagulation.

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Competing interests None declared.

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