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ORIGINAL ARTICLE

Male Health

Testosterone replacement therapy and vascular thromboembolic events: a systematic review and meta-analysis

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To evaluate the relationship between testosterone replacement therapy (TRT) and arterial and/or venous thrombosis in patients with pre-treatment total testosterone (TT) <12 nmol l^{-1}, we performed a meta-analysis following the Population Intervention Comparison Outcome model. Population: men with TT <12 nmol l^{-1} or clear mention of hypogonadism in the inclusion criteria of patients; intervention: TRT; comparison: placebo or no therapy; outcomes: arterial thrombotic events (stroke, myocardial infarction [MI], upper limbs, and lower limbs), VTE (deep vein thrombosis [DVT], portal vein thrombosis, splenic thrombosis, and pulmonary embolism), and mortality. A total of 2423 abstracts were assessed for eligibility. Twenty-four studies, including 14 randomized controlled trials (RCTs), were finally included, with a total of 4027 and 310 288 hypotestosteronemic male patients, from RCTs and from observational studies, respectively. Based on RCT-derived data, TRT did not influence the risk of arterial thrombosis (odds ratio [OR] = 1.27, 95% confidence interval [CI]: 0.47–3.43, *P* = 0.64), stroke (OR = 1.34, 95% CI: 0.09–18.97, *P* = 0.83), MI (OR = 0.51, 95% CI: 0.11–2.31, *P* = 0.39), VTE (OR = 1.42, 95% CI: 0.22–9.03, *P* = 0.71), pulmonary embolism (OR = 1.38, 95% CI: 0.27–7.04, *P* = 0.70), and mortality (OR = 0.70, 95% CI: 0.20–2.38, *P* = 0.56). Meanwhile, when only observational studies are considered, a significant reduction in the risk of developing arterial thrombotic events, MI, venous thromboembolism, and mortality was observed. The risk for DVT remains uncertain, due to the paucity of RCT-based data. TRT in men with TT <12 nmol l^{-1} is safe from the risk of adverse cardiovascular events. Further studies specifically assessing the risk of DVT in men on TRT are needed.

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INTRODUCTION

Hypogonadism, defined as impaired spermatogenesis and/or impaired secretion of testosterone (T), is a congenital or acquired disease, divided into primary (testicular cause), secondary (hypothalamic/pituitary cause), or functional (intact hypothalamic–pituitary–testicular [HPT] axis, activity reduced in relation to extrinsic cause) forms.[1] Organic hypogonadism refers to diseases of the hypothalamus, pituitary, or testes, resulting in clinical syndromes of infertility and/or androgen deficiency.[1,2] Some authors described late-onset hypogonadism (LOH) as symptoms of hypogonadism and a low T concentration in older men, and its management (in the absence of organic hypogonadism) is unclear and subject to debate.[3–5] Some authors suggested categorizing men in this manner if symptoms suggestive of hypogonadism are present and serum total T (TT) levels are <12 nmol l^{-1} (<3.5 ng ml^{-1}) confirmed on at least two morning blood samples.[5] When not reversible, T replacement therapy (TRT) is the primary medical intervention for the treatment of male hypogonadism, except when contraindications coexist or patients try to have a child.[4,5]

On January 31, 2014, the Food and Drug Administration (FDA) of the USA issued a safety warning on the risk of stroke, heart attack, and death in men taking previously FDA-approved T products. The announcement was made because two previous studies suggested an increased risk of cardiovascular events among patients on T therapy.[6,7] As a result, prescriptions of T have dropped significantly[8] and the scientific community has begun to further evaluate this issue. Numerous studies and meta-analyses on this topic have been published since 2014, revealing that TRT is safe when prescribed to patients with hypogonadism.[9,10] When it comes to the safety of TRT, the guidelines of the European Academy of Andrology (EAA)[11] made a clear distinction between cardiovascular (CV) events and venous thromboembolism (VTE). In particular, while there is no clear evidence of increased TRT-related CV risk,[10] the association between endogenous T levels may be associated with VTE,[12] possibly due to a pre-existing state of thrombophilia-hypofibrinolysis.[13,14] Therefore, before prescribing TRT, the EAA guidelines suggest collecting personal and family history of VTE, as well as evaluating the presence of risk factors for VTE (recommendation No. 27; level 2 ⊕◯◯◯ [low]). Similar conclusions have been proposed very recently in a joint position statement by the Italian Society of Andrology and Medical Sexology and the Italian Society of Endocrinology.[5]

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This evidence prompted some authors to investigate further the relationship between TRT and VTE. A meta-analysis that included 13 randomized controlled trials (RCTs) and assessed the risk of VTE in patients on TRT found no increase in the treated arm compared to the one that received placebo. However, baseline serum T levels were not fully detailed in all included studies. Furthermore, the presence of hypogonadism was not fully specified among the inclusion criteria. These two aspects represent important limitations that could likely have influenced the results of this meta-analysis.[15] In contrast, a meta-analysis performed on 27 RCTs found an increased risk of VTE in patients treated with T compared to those treated with placebo, although only 14 of the 27 studies included in the meta-analysis showed a pretreatment serum T level <12 nmol l^{-1}.[16] Finally, a more recent meta-analysis investigated the occurrence of CV events and mortality in patients on TRT, suggesting the absence of an increased risk in the short and medium term.[17]

Therefore, the present systematic review and meta-analysis aimed to evaluate the relationship between TRT and the risk of arterial and/or venous thrombosis (including VTE) in hypotestosteronemic patients. To accomplish this, we carefully selected the studies that enrolled patients with serum TT levels <12 nmol l^{-1}. We also evaluated the association between the endpoint measures and TT levels using a meta-regression model for all the outcomes.

MATERIALS AND METHODS

Search strategy

The present meta-analysis was performed according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines for Meta-analyses and Systematic Reviews of Observational Studies[18] and the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P).[19] The MOOSE and PRISMA-P checklists have been included in **Supplementary Table 1** and **2**, respectively.

The following search strategy was used: "(TITLE-ABS-KEY (trt) AND TITLE-ABS-KEY (thromb*) OR TITLE-ABS-KEY (testosterone) AND TITLE-ABS-KEY (thromb*) OR TITLE-ABS-KEY (trt) AND TITLE-ABS-KEY (embol*) OR TITLE-ABS-KEY (testosterone*) AND TITLE-ABS-KEY (embol*)) (ALL (trt) AND ALL (thromb*) OR ALL (testosterone) AND ALL (thromb*) OR ALL (trt) AND ALL (embol*) OR ALL (testosterone*) AND ALL (embol*)) (ALL (testosterone) AND ALL (thromb*) OR ALL (testosterone) AND ALL (embol*))".

These searches were performed in the Scopus, PubMed, Cochrane, and Embase databases, from their inception through November 2022. The search was limited to original articles, studies on humans only, and without language restrictions. After the duplicates were eliminated, the identified abstracts were screened for eligibility by the researchers.

Selection criteria

Full-text articles of eligible abstracts, including non-English abstracts, were downloaded and translated into English if needed. They were assessed for eligibility using the Population, Intervention, Comparison/Comparator, Outcome, Study type (PICOS) model system[20] (**Supplementary Table 3**). The selection of eligible studies was carried out by 3 researchers (CG, CL, and VG). For each article, the eligibility assessment was performed by two reviewers independently in a non-blinded manner. The titles and abstracts of the studies were first independently screened for inclusion. In cases of uncertainty, each researcher reviewed the full text to determine inclusion. Any disagreement between the reviewers was resolved by discussion between the two reviewers. However, if a consensus was not reached, another reviewer made the final decision (RC). The selected articles were finally subjected to data extraction.

Data extraction

The following data were collected: study design, the timing when the event took place, presence of thrombophilia, D-dimer levels, fibrinogen, partial thromboplastin time (PTT), international normalized ratio (INR), hematocrit, TT levels before and during treatment, levels of 17β-estradiol (E_2), and presence of Klinefelter syndrome. The number of events and population size was collected for each outcome in the treated and untreated groups.

Quality assessment

The quality of evidence (QoE) of the studies was assessed by 3 investigators (CG, CL, and VG). In detail, all studies were assessed using Cambridge Quality Checklists.[21] Further assessment of QoE was performed for RCTs using the Cochrane Risk of Bias scale.[22]

Statistical analyses

Quantitative data analysis was performed using the Comprehensive Meta-Analysis Software (version 3; Biostat Inc., Englewood, NJ, USA) and the Review Manager (RevMan) version 5.4 (The Cochrane Collaboration, 2020; https://community.cochrane.org/organizational-info/resources/policies/policies-all-members-and-supporters/cochrane-privacy-policy#aboutus) for meta-analysis of quantitative data. The odds ratio (OR) was used as the effect size for statistical comparison between cases and controls, and $P \leq 0.05$ was considered statistically significant. Cochran's Q test and heterogeneity index (I^2) were used to assess heterogeneity across pooled studies, with $P < 0.10$ considered statistically significant. I^2 is an indicator of heterogeneity and lies between 0 and 100%, where <25% suggests low heterogeneity, 50% suggests moderate heterogeneity, and 75% suggests high heterogeneity. The pooled effect size was calculated using both the fixed effect and random effect models, depending on the level of heterogeneity. In the case of low heterogeneity, the fixed effect model was adopted, while in the case of significant heterogeneity, the random effects model was adopted. For each outcome, sub-analysis was performed based on the study design (namely, RCTs and observational studies). Cumulative analysis was performed to assess the chronological trend of statistical significance over a period. For this, the effect size and the corresponding CI were calculated after the addition of each new study in chronological order. The trend of the P-value and the statistical inference were used to draw inferences regarding the strength of the association, its vulnerability to variations, and the history of variations. The pooled effect size and the corresponding confidence interval (CI) were calculated after the exclusion of one study at a time. A study resulting in the change of inference upon its exclusion was labeled as a "sensitive study". Publication bias was qualitatively analyzed by the asymmetry of the funnel plot, which suggested some missing studies on one side of the graph. For quantitative analysis of publication bias, we employed Egger's intercept test, which evaluated the statistical significance of publication bias. In case of the presence of publication bias, unbiased estimates were calculated using the "trim and fill" method.[23]

RESULTS

Using the aforementioned search strategy, 2320 abstracts were extracted using the Scopus database. Further research was carried out using PubMed, Cochrane and Embase (n=1345), and manually sifting the bibliographic citations of the most recent meta-analyses on the subject (n = 100). After duplicates removal, a total of 2423 records were screened, based on the main title and abstract examination. After

the exclusion of 2273 abstracts, 150 full-text articles were assessed for eligibility. Of those, 126 were unsuitable for our study. Particularly, 87 full-text articles were excluded due to a lack of extractable data. Finally, 24 studies were included in the quantitative synthesis (**Figure 1**). A complete list of the studies excluded with reason is shown in **Supplementary Table 4**.

Among the 24 studies included in this meta-analysis, we found 5 retrospective cohort studies, 4 observational studies, one prospective controlled registry study, and 14 RCTs (**Table 1** and **2**).

Quality of evidence of included studies

All 24 included studies were assessed using the Cambridge Quality Checklist. Although this scale does not establish a precise threshold for differentiating between high- and low-quality studies, out of a total score of 15, seventeen studies scored >10, six studies scored 6 to 10, and one study scored <6. Fourteen RCT studies were also evaluated using the Cochrane risk bias for RCTs (**Supplementary Table 5**).

Arterial thrombotic events (all districts)

Eight RCTs[24–31] including 1309 men (697 cases *vs* 612 controls), and 5 observational studies[32–36] including 329 536 men (84 139 cases *vs* 245 397 controls) evaluated arterial thrombotic events in any district. The analysis showed the presence of inter-study heterogeneity, as demonstrated by the *Q*-test (*Q*-value = 27.6; $P = 0.010$) and $I^2 = 53\%$. In RCTs, TRT did not influence the risk of arterial thrombosis (OR = 1.27, 95% CI: 0.47–3.43, $P = 0.64$). According to the evidence coming from observational studies, the risk of arterial thrombosis was significantly reduced in patients compared to controls (OR = 0.56, 95% CI: 0.50–0.63, $P < 0.00001$; **Figure 2**).

There was no evidence of publication bias as shown by Egger's test ($P = 0.50$) and the symmetry of the funnel plots (**Supplementary Figure 1a**) of arterial thrombotic events. No study was sensitive enough to alter the above-reported results (**Supplementary Figure 1b**).

The cumulative analysis showed that the reduced risk of arterial thrombosis in patients with TRT remains significant with the addition of each study (**Supplementary Figure 2**).

Meta-regression analysis showed no significant association between the risk of developing an arterial thrombosis and TT levels (either measured before or after treatment) both in the hypotestosteronemic patients while undergoing TRT (patient group) and in the untreated hypotestosteronemic patients (control group), as shown in **Supplementary Figure 3**.

Stroke

Two RCTs[37,38] including 1021 men (513 cases *vs* 508 controls) and 6 observational studies[6,32,33,35,36,39] including 381 924 men (93 810 cases *vs* 288 114 controls) evaluated the risk of stroke. The analysis showed the presence of significant inter-study heterogeneity, as demonstrated by the *Q*-test (*Q*-value = 581.4; $P < 0.0001$) and $I^2 = 99\%$. According to the RCTs, TRT did not influence the risk of stroke (OR = 1.34, 95% CI: 0.09–18.97, $P = 0.83$). Similarly, considering the evidence coming only from the observational studies, the risk of stroke did not significantly differ between patients and controls (OR = 0.62, 95% CI: 0.26–1.46, $P = 0.27$; **Figure 3**).

There was no evidence of publication bias as shown by Egger's test ($P = 0.86$) and the symmetry of the funnel plots (**Supplementary Figure 4a**). No study was sensitive enough to alter

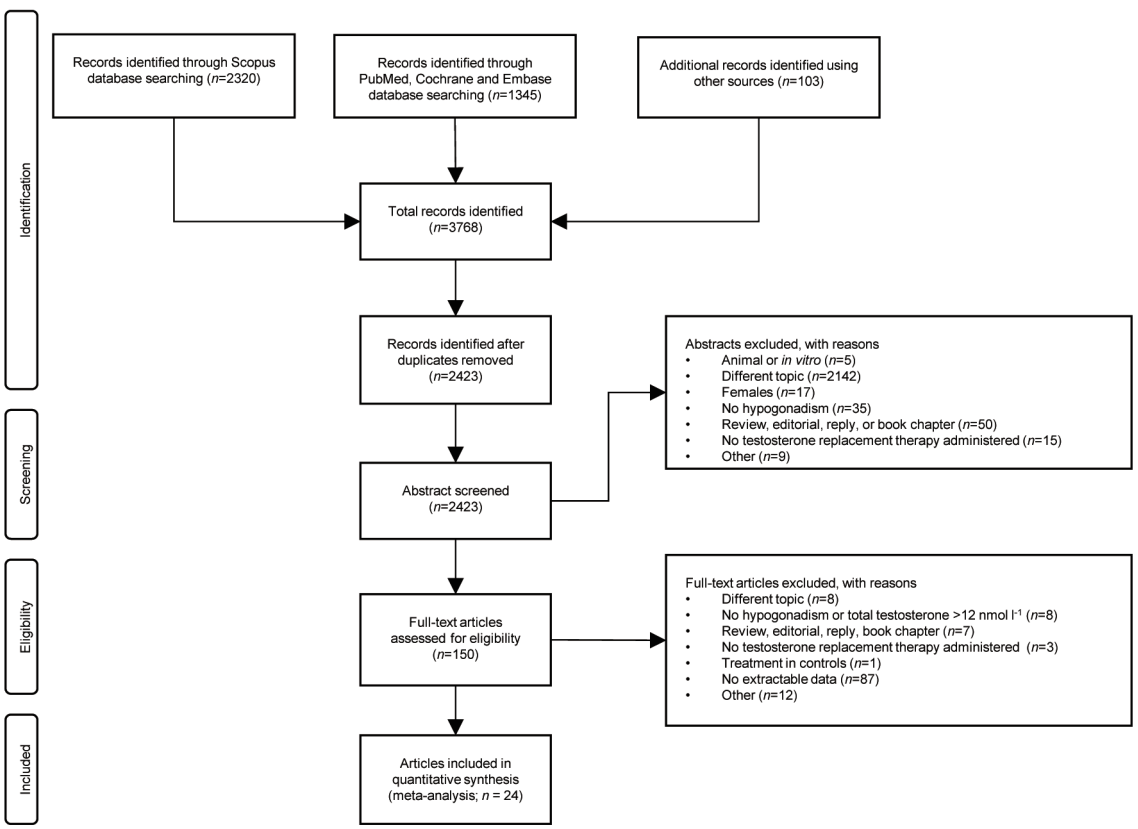

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

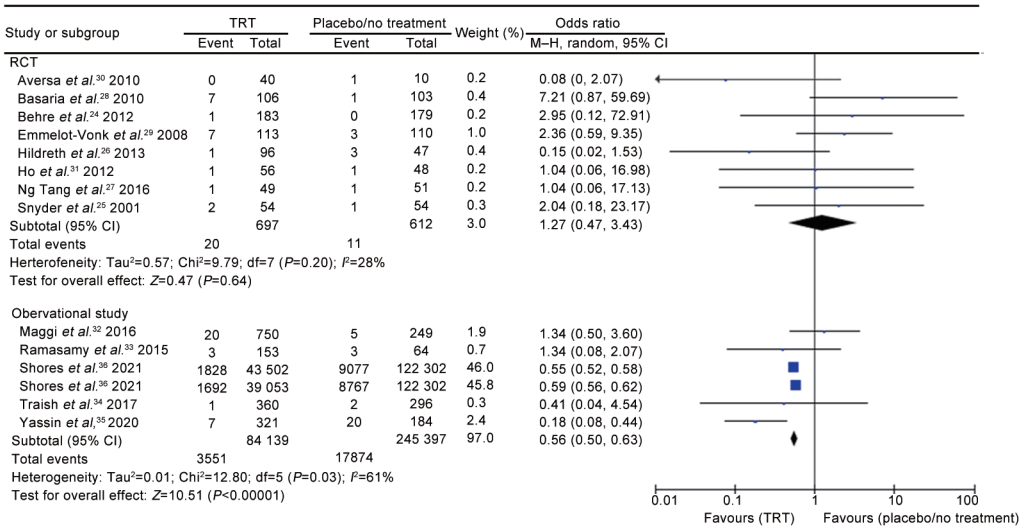

Figure 2: Risk of arterial thrombotic events in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. TRT: testosterone replacement therapy; RCT: randomized controlled trial; df: degree of freedom; CI: confidence interval; M–H: Mantel–Haenszel.

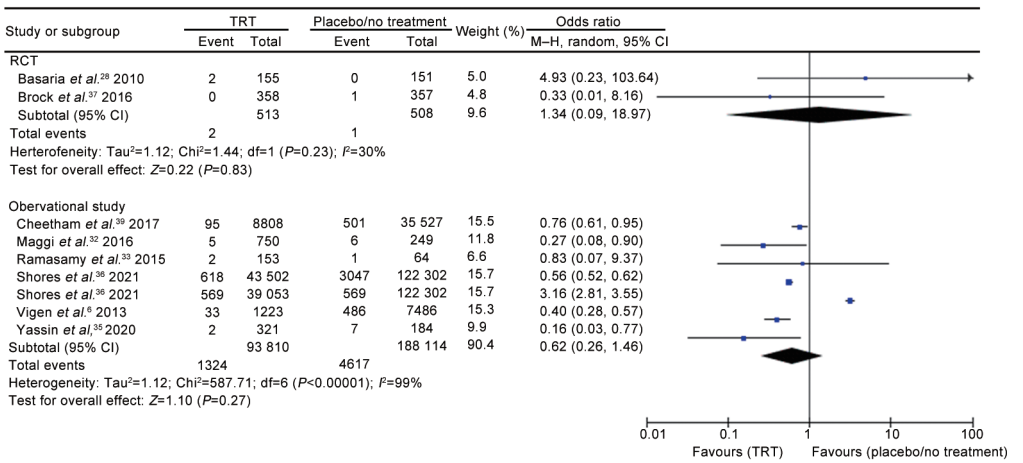

Figure 3: Risk of stroke in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. TRT: testosterone replacement therapy; RCT: randomized controlled trial; df: degree of freedom; CI: confidence interval; M–H: Mantel–Haenszel.

the above-reported results (**Supplementary Figure 4b**). The cumulative analysis showed that the risk of stroke reached a non-significant value with the addition of the study by Shores *et al.*,[36] to the pool and remained non-significant after that (**Supplementary Figure 5**). There were no enough studies to perform the meta-regression analysis.

Myocardial infarction

Five RCTs[24,25,30,31,38] including 940 men (488 cases *vs* 452 controls) and six observational studies[6,32,33,35,36,39] including 381 924 men (93 810 cases *vs* 288 114 controls) evaluated the risk of myocardial infarction. The analysis showed the presence of inter-study heterogeneity, as demonstrated by the *Q*-test (*Q*-value = 47.1; $P < 0.0001$) and $I^2 = 77\%$. In the RCTs, TRT did not influence the risk of myocardial infarction (OR = 0.51, 95% CI: 0.11–2.31, $P = 0.39$). According to the evidence coming from the observational studies, TRT was associated with a reduced risk of myocardial infarction (OR = 0.59, 95% CI: 0.49 –0.70, $P < 0.00001$; **Figure 4**).

There was no evidence of publication bias as shown by Egger's test ($P = 0.93$) and the symmetry of the funnel plots (**Supplementary Figure 6a**) of myocardial infarction. No study was sensitive enough to alter the above-reported results (**Supplementary Figure 6b**). The cumulative analysis showed that the reduced risk of myocardial infarction in patients with TRT remains significant with the addition of each study (**Supplementary Figure 7**).

Meta-regression analysis showed that the risk of developing myocardial infarction in hypotestosteronemic patients was significantly correlated with serum TT levels measured during the treatment (**Supplementary Figure 8**).

Venous thromboembolism (all districts)

Three RCTs[24,37,40] (824 cases *vs* 811 controls) and five observational studies[32,33,35,36,41] (122 141 cases *vs* 255 955 controls) evaluated the risk of venous thrombosis. The analysis showed the presence of inter-study heterogeneity, as demonstrated by the *Q*-test (*Q*-value = 26.3; $P = 0.001$) and $I^2 = 70\%$. Evidence coming from RCTs showed no significantly different risk in patients versus controls (OR = 1.42, 95% CI: 0.22–9.03, $P = 0.71$). According to observational studies, the risk of venous thrombosis was significantly reduced in patients

Table 1: Main characteristics of the randomized controlled trials included in the analysis

Study	*Control group (n)*	*Patients for each TRT formulation (n)*			*Event timing (week)*	*Age (year), mean±s.d.*		*Hematocrit (%), mean±s.d.*		*Total testosterone (ng ml^{-1}), mean±s.d.*				*Symptoms of hypoandrogenism*	*E_2 (pg ml^{-1}), mean±s.d.*		*Klinefelter among cases (yes/no)*	
		Transdermal	*Injective*	*Oral*		*Case*	*Control*	*Case*	*Control*	*Baseline (case)*	*Baseline (control)*	*TRT (case)*	*Placebo (control)*		*Case*	*Control*	*Case*	*Control*
Barnouin *et al.*[24]	Placebo (41)		42		26	73.6±3.7	72.2±3.2	42.0±0.5	40.6±0.6	2.1±0.1	2.2±0.1	3.1±0.2	0.6±0.2	Not mention	61.5±4.3	54.2±4.8	-	-
Behre *et al.*[42]	Placebo (179)		183		24	61.9±6.6	62.1±6.3	-	-	3.0±0.7	3.1±0.7	5.8±4.4	3.1±1.0	Yes	-	-	No	No
Brock *et al.*[40]	Placebo (275)		283		36	54.7±10.6	55.9±11.4	-	-	2.0±0.7	2.0±0.7	-	-	Yes	-	-	No	No
Brock *et al.*[37]	Placebo (357)	358	-	-	12	53.9±10.8	55.4±11.1	-	-	2.0±0.7	2.0±0.7	-	-	Yes	-	-		
Snyder *et al.*[25]	Placebo (394)	394	-	-	52	≥65[a]	≥65[a]	-	-	<2.8[b]	<2.8[b]	-	-	Yes	-	-	No	No
Tan *et al.*[43]	Placebo (58)	-	56	-	48	53.8±6.9[c]	53.1±8.3[c]	43.1±3.4[c]	43.4±2.9[c]	2.6±0.6[c]	2.6±0.5[c]	6.9±1.7	3.2±1.0	Yes	-	-	No	No
Srinivas-Shankar *et al.*[44]	Placebo (132)	130	-	-	24	73.7±5.7	73.9±6.4	44.0±3.0	42.0±4.0	3.2±0.9	3.1±0.9	5.3±2.7	3.1±1.0	Not mention	-	-	No	No
Basaria *et al.*[38]	Placebo (151)	155	-	-	156	66.9±5.0	68.3±5.3	43.7±3.7	43.6±3.6	3.1±0.6	3.1±0.7	5.7±2.5	3.3±1.0	Not mention	21.8±14.9	18.5±10.0	No	No
Hildreth *et al.*[26]	Placebo (47)	96	-	-	52	66.5±5.8	66.5±5.2	46.3±3.0	46.7±3.0	3.0±0.4	2.9±0.4	5.3±2.9	2.9±0.7	Not mention	-	-	No	No
Ng Tang Fui *et al.*[27]	Placebo (51)	-	49	-	56	54.3 (47.3–59.8)[d]	52.8 (47.6–60.1)[d]	43.0±2.0	44.0±2.0	2.4±0.7	2.4±0.7	4.1[e]	2.9[e]	Not mention	-	-	No	No
Basaria *et al.*[28]	Placebo (103)	106	-	-	24	74.0±6.0	74.0±5.0	-	-	2.5±0.6	2.4±0.7	5.7±4.0	2.9±1.6	Not mention	-	-	No	No
Emmelot-Vonk *et al.*[29]	Placebo (110)	-	-	113	27	67.1±5.0	67.4±4.9	46.0±3.0	45.0±2.0	3.2±0.5	3.0±0.5	-	-	Not mention	-	-	-	-
Aversa *et al.*[30]	Placebo (10)	-	40	-	52	58.0±10.0	57.0±8.0	43.0±3.5	44.0±3.0	2.4±0.7	2.6±0.5	4.1±0.4	0.1±0.4	Yes	-	-	-	-
Ho *et al.*[31]	Placebo (58)	-	56	-	48	53.4±7.4	53.0±8.2	-	-	2.6±0.6	2.6±0.5	6.8[e]	3.2[e]	Yes	-	-	-	-

[a]Minimum. [b]Maximum. [c]Median±s.d.. [d]Median (IQR). [e]Mean. E_2: 17β-estradiol; TRT: testosterone replacement therapy; s.d.: standard deviation; -: not available

Table 2: Main characteristics of the observational studies included in the analysis

Study	*Type of study*	*Control group (n)*	*Patients for each TRT formulation (n)*			*Event timing (week)*	*Age (year), mean±s.d.*		*Hematocrit (%), mean±s.d.*		*Total testosterone (ng ml^{-1}), mean±s.d.*				*Symptoms of hypoandrogenism*	*E_2 (pg ml^{-1}), mean±s.d.*		*Klinefelter among cases (yes/no)*	
			Transdermal	*Injective*	*Oral*		*Case*	*Control*	*Case*	*Control*	*Baseline (case)*	*Baseline (control)*	*TRT (case)*	*Placebo (control)*		*Case*	*Control*	*Case*	*Control*
Sharma *et al.*[41]	Retrospective cohort study	No treatment (10 854)	38 362			-	64.0±11.2	66.6±13.1	-	-	-	-	-	-	Not mention	-	-	-	-
Maggi *et al.*[32]	Retrospective study	No treatment (249)	510	225	15	165	58.9±10.3	59.7±11.1	-	-	2.4±1.1	2.7±1.1	4.4[a]	3.3[a]	Not mention	-	-	-	-
Ramasamy *et al.*[33]	Retrospective study	No treatment (64)	47	53	-	187	74.0±6.3	75.0±6.0	45.0±5.0	42.0±5.0	-	-	4.8±3.4	2.4±0.5	Yes	37.0±21.0	26.0±7.0	-	-
Vigen *et al.*[6]	Retrospective cohort study	No treatment (7486)	1223			75	60.6±7.6	63.8±9.0	-	-	1.8±0.6	2.1±0.7	-	-	Not mention	-	-	No	No
Cheetham *et al.*[39]	Retrospective cohort study	No treatment (35 527)	8808			132	58.4[a]	59.8[a]	-	-	3.18[a]	-	-	-	Not mention	-	-	No	No
Traish *et al.*[34]	Observational study	No treatment (296)	-	360	-	416	57.4±7.3	64.8±4.3	-	-	2.8±0.4	2.8±0.4	4.8±0.5	2.6±0.4	Yes	-	-	Yes	Yes
Yassin *et al.*[35]	Prospective controlled registry study	No treatment (184)	-	321	-	624	59.0±9.5	66.1±7.6	-	-	2.2±0.6	2.7±0.7	4.6[a]	2.7[a]	Yes	-	-	-	-
Muraleedharan *et al.*[45]	Observational study	No treatment (174)	60	1	3	187	60.9±11.8	58.5±10.4	-	-	1.9±0.7	2.2±0.5	-	-	Not mention	-	-	-	-
Eisenberg *et al.*[46]	Observational study	No treatment (225)	204	80	-	-	54.1±8.7	54.9±11.1	-	-	3.3±2.0	3.6±1.4	-	-	Not mention	-	-	-	-
Shores *et al.*[36]	Observational cohort study	No treatment (122 302)	43 502	39 053	-	223.6	61.7±10.2	59.8±9.4	-	-	2.1±1.3	1.7±0.7	-	-	Not mention	-	-	-	-

[a]Mean. E_2: 17β-estradiol; TRT: testosterone replacement therapy; s.d.: standard deviation; -: not available

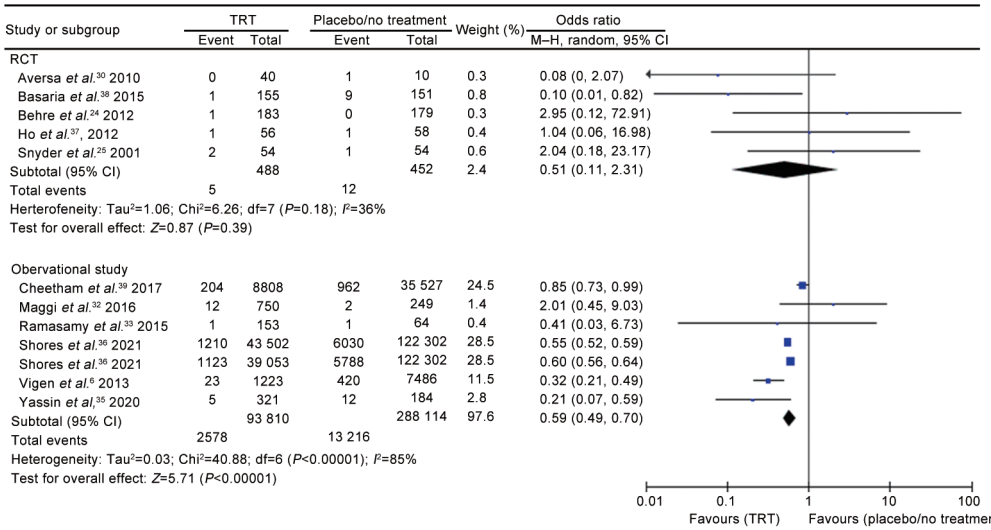

Figure 4: Risk of myocardial infarction in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. TRT: testosterone replacement therapy; RCT: randomized controlled trial; df: degree of freedom; CI: confidence interval; M–H: Mantel–Haenszel.

compared to controls (OR = 0.78, 95% CI: 0.61–1.00, P = 0.05; **Supplementary Figure 9**).

There was no evidence of publication bias as shown by Egger's test (P = 0.125) and the symmetry of the funnel plots (**Supplementary Figure 10a**). Three studies[35,36,40] were sensitive enough to alter the above-reported results. Their removal resulted in the loss of significance (**Supplementary Figure 10b**). The cumulative analysis showed the achievement of statistical significance after the addition of the study by Yassin *et al.*[35] (**Supplementary Figure 11**). There were no enough studies to perform the meta-regression analysis.

Deep vein thrombosis (DVT)

Only one RCT[24] and two observational studies[32,41] (39 112 cases *vs* 11 103 controls) evaluated the risk of DVT. Due to the absence of heterogeneity, as demonstrated by the Q-test (Q-value = 0.2; P = 0.893) and I^2 = 0, the fixed effect model was used. According the observational studies, TRT was associated with an increased risk of DVT (OR = 1.44, 95% CI: 1.04–2.00, P = 0.03). Only one RCT was included.[24] which reported no difference in the DVT risk (**Supplementary Figure 12**).

There was no evidence of publication bias as shown by Egger's test (P=0.095) and the symmetry of the funnel plots (**Supplementary Figure 13a**) of DVT. The study by Sharma *et al.*[41] was sensitive enough to alter the above-reported results (OR = 1.854, 95% CI: 0.468–7.348, P = 0.38; **Supplementary Figure 13b**). The cumulative analysis showed no change in the results with the addition of any of the studies (**Supplementary Figure 14**). There were no enough studies to perform the meta-regression analysis.

Pulmonary embolism

Three RCTs[37,40,42] including 683 cases and 673 controls and four observational studies[32,33,35,41] including 1431 cases and 538 controls evaluated pulmonary embolism. Due to the absence of significant heterogeneity, as demonstrated by the Q-test (Q-value = 2.4; P = 0.881) and I^2 = 0, the fixed effect model was used. In RCTs, TRT did not influence the risk of pulmonary embolism (OR = 1.38, 95% CI: 0.27–7.04, P = 0.70). Similarly, the evidence coming from observational studies showed no significantly different risk in patients versus controls (OR = 0.76, 95% CI: 0.35–1.75, P = 0.67; **Supplementary Figure 15**).

There was no evidence of publication bias as shown by Egger's test (P = 0.41) and the symmetry of the funnel plots (**Supplementary Figure 16a**). No study was sensitive enough to alter the above-reported results (**Supplementary Figure 16b**). The cumulative analysis showed no change in the results with the addition of any of the studies (**Supplementary Figure 17**). There were no enough studies to perform the meta-regression analysis.

Mortality

Four RCTs[38,40,43,44] including 624 cases and 614 controls and seven observational studies[6,33,35,39,45,46] including 11 213 cases and 43 956 controls, evaluated mortality in association with TRT. The analysis showed the presence of heterogeneity among the studies, as demonstrated by the Q-test (Q-value = 27.8; P = 0.002) and I^2 = 64%. In RCTs, TRT did not influence the risk of mortality (OR = 0.70, 95% CI: 0.20–2.38, P = 0.56). According to observational studies, the risk of mortality was significantly reduced in patients versus controls (OR = 0.51, 95% CI: 0.35–0.76, P = 0.0001; **Supplementary Figure 18**).

There was evidence of publication bias as shown by Egger's test (P = 0.021) and the asymmetry of the funnel plots (**Supplementary Figure 19a**) of mortality. No study was sensitive enough to alter the final result (**Supplementary Figure 19b**). The cumulative analysis showed that the risk of overall mortality reached a non-significant value with the addition of two studies.[28,44] It returned to reach significance after the addition of the other studies (**Supplementary Figure 20**). There were no enough studies to perform the meta-regression analysis.

DISCUSSION

Key findings

This systematic review and meta-analysis aimed to assess the association between TRT, arterial thrombosis, and VTE in men with TT <12 nmol l^{-1}. This task was accomplished by selecting and analyzing data from several studies on this topic. Overall, 24 studies were selected and included. The data analyzed concerned serum TT levels before and after TRT, mean hematocrit value during treatment with T or placebo, mean serum E_2 values, information on thrombophilia, and the risk of developing arterial or venous thromboembolic events. The outcomes

analyzed were arterial thrombosis, stroke, myocardial infarction, VTE, DVT, pulmonary embolism, and mortality.

Data analyses showed that TRT does not influence the risk of arterial events, including stroke and pulmonary embolism, of developing arterial and venous thrombosis and myocardial infarction, and the risk of mortality. Meanwhile, when only observational studies are considered, a significant reduction in the risk of developing arterial thrombotic events, MI, venous thromboembolism and mortality was observed. The risk of developing DVT remains uncertain, due to limited RCT-derived evidence.

The sensitive analysis of three outcomes (arterial thrombosis, stroke, and venous thrombosis) showed that the study by Shores *et al.*[36] was sensitive enough to skew the results. One possible cause could be the way T exposure was calculated. Contrary to many studies comparing patients using T with patients not receiving therapy or receiving a placebo, Shores *et al.*[36] compared current users of T with previous ones. This was done to evaluate the association between current T exposure and cardiovascular events. In addition, the databases used in the study did not contain any information on signs or symptoms of T deficiency or indications for T treatment. Furthermore, patients were assumed to have started treatment at the date of compilation and were compliant with the treatment. Removal of this study resulted in a significant reduction in the risk of vascular events.

Importance of the topic

The value of our data lies in the presence of controversies in the literature regarding TRT, endogenous serum T levels, and CV/thromboembolic risk.

In January 2014, the FDA launched a safety warning for T-containing drugs as two studies had shown a correlation between T administration and adverse CV effects, such as heart attack and stroke. This led to a notable reduction in TRT prescriptions.[8] TRT is the primary treatment of hypogonadism, a clinical syndrome resulting from the failure of the testis to produce physiological testosterone concentrations,[1,2] and which, if left untreated, leads to poor quality of life causing loss of muscle mass and strength, sexual dysfunctions, osteoporosis, and, according to some studies, even an increased risk of CV events.[47,48] Given the importance of treating hypogonadism, several studies have focused on the presumed association between TRT, CV events, and VTE. On the other hand, authors also investigated the relationship between endogenous T and CV/thromboembolic risk. Therefore, the present systematic review and meta-analysis aimed to probe the current literature on the association between TRT and CV/thromboembolic risk to assess whether or not this association is real in patients with hypotestosteronemia so that specialists can prescribe T with greater awareness with a benefit for patients.

Some studies reported an association between TRT and adverse CV events. Particularly, the studies that led the FDA to issue the aforementioned warning on TRT found a higher risk of death, heart attack, and stroke in patients who underwent TRT, compared to the untreated group,[6] and of myocardial infarction in the 90 days after the T prescription.[7] Similarly, in a parallel-group, randomized, placebo-controlled, double-blind trial conducted on 209 patients, Basaria *et al.*[28] found more CV-related adverse events in patients receiving T than in the placebo group.

Even the association between TRT and venous thromboembolism has been investigated but contrasting results have emerged. Baillargeon *et al.*[49] did not find any association between TRT and VTE in middle-aged and older men in their case-control study. Ayele *et al.*[15] also found no association between TRT and VTE. Conversely, a case-control study showed an association between TRT and the risk of VTE within the first six months after prescription. After this time, the association was no longer significant.[50]

Regarding the association between endogenous T levels and CV/thromboembolic risk, there is contrasting evidence in the literature. According to some studies, low endogenous levels of T appear to be associated with an increased risk of CV diseases, CV death, and all-cause of death.[47,48] Yeap *et al.*[48] found that lower TT levels predict an increased incidence of stroke and transient ischemic attack (TIA). In particular, the hazard ratio (HR) for incidence of stroke or TIA was 1.39 for men with TT <8 nmol l^{-1}, and 2.08 for men with TT ≥8 nmol l^{-1} and <11.7 nmol l^{-1}, compared with men with TT ≥11.7 nmol l^{-1}.[48] Notably, the risk was not significantly higher in men with TT <8 nmol l^{-1}, but authors attributed this to the small number of men and events in this group. Anyway, men with TT levels <11.7 nmol l^{-1}, have shown a significantly higher risk. Khaw *et al.*[47] found an inverse correlation between endogenous T levels and mortality due to CV diseases and all causes. In contrast, Corona *et al.*[51] suggested hypogonadism as a protective factor in men with a high CV risk burden, since in their study, T <12 nmol l^{-1} were associated with a lower incidence of CV disease in patients with high CV risk. Studies have also analyzed the association between endogenous T and arterial thrombosis. For example, Lou *et al.*,[12] based on a two-sample Mendelian randomization study, suggest that endogenous T could be considered as a modifiable risk factor for thromboembolism and heart failure since they found a positive association between endogenous T levels and thromboembolism, heart failure, and myocardial infarction in men. As for VTE, Svartberg *et al.*[52] did not find any association between endogenous T and a 10-year risk of VTE in middle-aged and older men. Similar results emerged from the study by Holmegard *et al.*[53] in which T levels were not associated with the risk of VTE, pulmonary embolism, and DVT.

Finally, there are contrasting data regarding both the association between endogenous T levels and CV/thromboembolic risk and also between TRT and CV/thromboembolic risk. Our purpose was to clarify the second association, using a meta-regression approach.

Comparison with previous meta-analyses

Since T approval in medical use in 1939, association studies between TRT and major CV events have been published from 2000 onward. The main meta-analyses are briefly described in **Supplementary Table 6**, with details on population, studies included, time of observation, and arterial or venous thrombosis outcomes. Most of them have not found an increased risk of CV events in patients treated with T, either in the venous or arterial districts. On the contrary, the single meta-analysis that showed an association between TRT and arterial CV events was published in 2014, referring specifically to the oral formulation and the authors did not find an increased risk with the cutaneous and parenteral formulation.[54]

Current meta-analyses are limited by the lack of RCT-based evidence,[55] and the inclusion of patients with serum TT within the normal range.[5,10,15,53,56] Conversely, the present study strictly included only patient with TT <12 nmol l^{-1}, and analyzed a well-sized population, which increases the statistical power of our study.

Limitations

This study had several limitations. The temporal range of observation is short-to-medium ranging from 12 weeks to 624 weeks, with a median of 36 weeks for RCTs and 165 weeks for observational studies. No long-term studies assessing the correlation between TRT and thromboembolism are currently available. This conclusion is also

shared by another meta-analysis on adverse CV events and mortality in men during T treatment,[17] where data evaluating the CV safety of T beyond a 12-month duration of administration are scarce.

Klinefelter syndrome (KS) is a frequent chromosome disorder causing male infertility and hypogonadism. Patients with KS have a predisposition to the development of thromboembolic events.[57] The inclusion of these patients could be a limitation. However, in our meta-analysis, only one study included KS patients.[34] No studies reviewed in this meta-analysis reported data on the presence or absence of thrombophilia in hypotestosteronemic patients treated with T. Patients with thrombophilia could potentially have been included. Nevertheless, the data support the absence of an increased thromboembolic risk in patients undergoing TRT. Our meta-analysis also considered observational studies,[34,45,46] which reduce the quality of evidence. That data could not be extracted from as many as 87 studies represents an additional limitation. Finally, several endogenous factors (sex hormone binding globulin and sensitivity of androgen receptor), the different dosages and route of administration, and the timing when the blood examination was performed, can influence the serum testosterone levels.

Wider implications

The main endocrinological, andrological, and urological associations/societies have drawn up their guidelines for the treatment of hypogonadism, also addressing the issue of CV risk.

The Endocrine Society (ES), American Urological Association (AUA), EAA, European Association of Urology (EAU), American Association of Clinical Endocrinology (AACE), British Society for Sexual Medicine (BSSM), Società Italiana di Endocrinologia (SIE), Società Italiana di Andrologia e Medicina della Sessualità (SIAMS), International Consultation on Sexual Medicine (ICSM), and International Society for the Study of the Aging Male (ISSAM) substantially agree on the importance of hematocrit evaluation because of the association between elevated hematocrit and the risk of CV disease.[58] In particular, they recommend against starting TRT in patients with elevated hematocrit and they also suggest its monitoring to reduce or discontinue TRT if it exceeds 54% (50% according to AACE and ICSM),[1,5,11,59–63] as shown in **Supplementary Table 7**.

None of them places adverse CV events among those certainly related to TRT, but most of them conclude that there is no substantive evidence. Some authors suggest investigating the patients' CV risk and risk of VTE. Others suggest performing an electrocardiogram before TRT, especially in men with previous CV disease. Some others indicate not initiating TRT in patients with recent acute CV events.

Our study contributes to increasing awareness of the association between TRT and CV/thromboembolic risk in patients with hypotestosteronemia. Based on the largest population analyzed so far, we found no association between TRT and an increased risk of arterial events, including stroke and pulmonary embolism, of developing arterial and venous thrombosis and myocardial infarction, according to both RCT- and observational study-based evidence. The risk of developing DVT remains uncertain, due to limited RCT-derived evidence, thus suggesting that further studies, specifically assessing the risk of DVT in hypotestosteronemic patients on TRT, are needed.

CONCLUSION

This systematic review and meta-analysis examined a large cohort, and provides a high level of evidence in favor of the safety of TRT, due to the absence of association between TRT and increased risk of arterial thrombotic events. The risk for DVT remains uncertain, due to the paucity of RCT-based data. Therefore, the present study reinforces the concept that TRT in hypotestosteronemic patients is safe from the risk of CV adverse events, and indicates the need for further studies that specifically assess the risk of DVT in hypotestosteronemic patients on TRT.

AUTHOR CONTRIBUTIONS

RC and AEC are the project managers and conceived the study. RC made the literature search and analyzed the data. CG, CL, and VG extracted the data. CG, CL, VG, and RC wrote the draft. FB, AC, and AEC reviewed the article. SLV and RAC supervised the study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols 2020 checklist

PRISMA 2020 checklist section and topic			*Location where item is reported*
		Title	
Title	1	Identify the report as a systematic review	Page 1
		Abstract	
Abstract	2	See the PRISMA 2020 for abstracts checklist	Page 2–3
		Introduction	
Rationale	3	Describe the rationale for the review in the context of existing knowledge	Page 4–5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses	Page 5
		Methods	
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses	**Table 1**, page 6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted	Page 5–6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used	Page 5–6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process	Page 5–6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process	Page 5–6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (*e.g.,* for all measures, time points, analyses), and if not, the methods used to decide which results to collect	Page 5–7
	10b	List and define all other variables for which data were sought (*e.g.,* participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information	Page 5–7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool (s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process	Page 7–8
Effect measures	12	Specify for each outcome the effect measure (s) (*e.g.,* risk ratio, mean difference) used in the synthesis or presentation of results	Page 7–8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (*e.g.,* tabulating the study intervention characteristics and comparing against the planned groups for each synthesis [item #5])	Page 7–8
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions	Page 7–8
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses	Page 7–8
	13d	Describe any methods used to synthesize results and provide a rationale for the choice (s). If meta-analysis was performed, describe the model (s), method (s) to identify the presence and extent of statistical heterogeneity, and software package (s) used	Page 7–8
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (*e.g.,* subgroup analysis, meta-regression)	Page 7–8
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results	Page 7–8
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases)	Page 7–8
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome	Page 7–8
		Results	
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram	Page 8–9
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded	Page 8–9, **Figure 1** and **Supplementary Table 3**
Study characteristics	17	Cite each included study and present its characteristics	Page 8–10
Risk of bias in studies	18	Present assessments of risk of bias for each included study	Page 11–12
Results of individual studies	19	For all outcomes, present, for each study: (a) Summary statistics for each group (where appropriate) and (b) An effect estimate and its precision (*e.g.,* confidence/credible interval), ideally using structured tables or plots	Page 12–16

Contd...

Supplementary Table 1: Contd...

PRISMA 2020 checklist section and topic			Location where item is reported
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies	Page 12–16
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (*e.g.,* confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect	Page 12–16
	20c	Present results of all investigations of possible causes of heterogeneity among study results	Page 12–16
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results	Page 12–16
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed	Page 12–16
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed	Page 12–16
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence	Page 16
	23b	Discuss any limitations of the evidence included in the review	Page 22
	23c	Discuss any limitations of the review processes used	Page 22
	23d	Discuss implications of the results for practice, policy, and future research	Page 22–23
Other information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered	-
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared	-
	24c	Describe and explain any amendments to information provided at registration or in the protocol	-
Support	25	Describe sources of financial or nonfinancial support for the review, and the role of the funders or sponsors in the review	-
Competing interests	26	Declare any competing interests of review authors	-
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review	-

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; 372: n71. For more information, visit: PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols. Available from: https://www.prisma-statement.org/ (last accessed on 15 March, 2023)

Supplementary Table 2: Meta-Analysis of Observational Studies in Epidemiology checklist for meta-analyses of observational studies

Item number	*Recommendation*	*Reported on page number*
	Reporting of background should include	
1	Problem definition	4
2	Hypothesis statement	5
3	Description of study outcome(s)	7
4	Type of exposure or intervention used	5–6
5	Type of study designs used	5–6
6	Study population	5–6
	Reporting of search strategy should include	
7	Qualifications of searchers (*e.g.,* librarians and investigators)	5–7
8	Search strategy, including time period included in the synthesis and key words	5–7
9	Effort to include all available studies, including contact with authors	6
10	Databases and registries searched	5
11	Search software used, name and version, including special features used (*e.g.,* explosion)	6
12	Use of hand searching (*e.g.,* reference lists of obtained articles)	6
13	List of citations located and those excluded, including justification	**Supplementary Table 3**
14	Method of addressing articles published in languages other than English	5–6
15	Method of handling abstracts and unpublished studies	5–6
16	Description of any contact with authors	5–6
	Reporting of methods should include	
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	8–16
18	Rationale for the selection and coding of data (*e.g.,* sound clinical principles or convenience)	8–16
19	Documentation of how data were classified and coded (*e.g.,* multiple raters, blinding and interrater reliability)	8–16
20	Assessment of confounding (*e.g.,* comparability of cases and controls in studies where appropriate)	8–16
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	8–16
22	Assessment of heterogeneity	8–16
23	Description of statistical methods (*e.g.,* complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	8–16
24	Provision of appropriate tables and graphics	**Figure 2–7** and **Supplementary Figure 1–16**
	Reporting of results should include	
25	Graphic summarizing individual study estimates and overall estimate	**Figure 2–7** and **Supplementary Figure 1–16**
26	Table giving descriptive information for each study included	**Table 2**
27	Results of sensitivity testing (*e.g.,* subgroup analysis)	**Supplementary Figure 1–16**
28	Indication of statistical uncertainty of findings	8–16
	Reporting of discussion should include	
29	Quantitative assessment of bias (*e.g.,* publication bias)	22–23
30	Justification for exclusion (*e.g.,* exclusion of non-English language citations)	22–23
31	Assessment of quality of included studies	22–23
	Reporting of conclusions should include	
32	Consideration of alternative explanations for observed results	17–19
33	Generalization of the conclusions (*i.e.*, appropriate for the data presented and within the domain of the literature review)	22–24
34	Guidelines for future research	22–24
35	Disclosure of funding source	-

From: Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, *et al*. Meta-analysis of observational studies in epidemiology (MOOSE) group. Meta-analysis of observational studies in epidemiology. A proposal for reporting. *JAMA* 2000; 283: 2008–12

Supplementary Table 3: Inclusion and exclusion criteria according to the population, intervention, comparison/comparator, outcomes, study type model[20]

	Inclusion criteria	*Exclusion criteria*
Population	Patients with TT levels T <12 nmol l^{-1}	Eugonadal patients, women
Intervention	TRT	Other treatments (*e.g.,* SERMs, AIs) alone or in combination with testosterone
Comparison	Placebo or no therapy	Other treatments (*e.g.,* SERMs, AIs)
Outcomes	Arterial thrombotic events (all districts, stroke, myocardial infarction, upper limbs, lower limbs) VTE (all districts, DVT, portal vein thrombosis, splenic thrombosis, pulmonary embolism) Mortality	/
Study type	Observational studies, randomized controlled studies, case-control studies	Animal studies, *in vitro* studies, review and meta-analyses, case reports, book chapters, editorials

AIs: aromatase inhibitors; DVT: deep vein thrombosis; SERMs: selective estrogen receptor modulators; VTE: venous thromboembolism; TRT: testosterone replacement therapy; TT: total testosterone

Supplementary Table 4: Studies excluded with reasons

Study	*Exclusion reason*
Yarnell *et al.*[64]	Females
Pastuszak *et al.*[65]	Different outcomes
Abbe *et al.*[66]	No original research
Walker *et al.*[14]	No available data
Glueck *et al.*[67]	Case report
Glueck *et al.*[68]	Females
Li *et al.*[69]	No numbers of events
Martinez *et al.*[50]	No number of events
Glueck *et al.*[70]	No available data
Baillargeon *et al.*[49]	No number of events
Glueck *et al.*[71]	Female
Haider *et al.*[72]	Previous cardiovascular events
Kaufman *et al.*[73]	No available data
Kalinchenko *et al.*[74]	Different outcomes
Etminan *et al.*[75]	No data available
Sharma *et al.*[76]	No data available
Oni *et al.*[77]	No data available
Baillargeon *et al.*[78]	No hypogonadism
Kenny *et al.*[79]	Total testosterone >12 nmol l^{-1}
Nair *et al.*[80]	Total testosterone >12 nmol l^{-1}
Snyder *et al.*[81]	Total testosterone >12 nmol l^{-1}

Supplementary Table 5: Quality of evidence assessment of the included studies (results of the Cambridge Quality Checklist,[21] Cochrane risk of bias for randomized controlled trials[22])

Study	*Type of study*	*Cambridge Quality Checklists*			*Cochrane risk of bias for RCTs (risk of bias)*
		Checklist for correlates	*Checklist for risk factors*	*Checklist for causal risk factors*	
Sharma *et al*.[41]	Retrospective cohort study	3	2	4	-
Barnouin *et al*.[42]	RCT	4	3	7	High
Shores *et al*.[36]	Retrospective cohort study	3	2	4	-
Maggi *et al*.[32]	Retrospective study	3	3	6	-
Ramasamy *et al*.[33]	Retrospective study	0	2	4	-
Behre *et al*.[24]	RCT	2	3	7	High
Brock *et al*.[40]	RCT	3	3	7	High
Brock *et al*.[37]	RCT	2	3	7	Some concerns
Snyder *et al*.[25]	RCT	3	3	7	High
Tan *et al*.[43]	RCT	2	3	7	High
Srinivas-Shankar *et al*.[44]	RCT	4	3	7	High
Basaria *et al*.[38]	RCT	4	3	7	High
Hildreth *et al*.[26]	RCT	3	3	7	High
Ng Tang Fui *et al*.[27]	RCT	3	3	7	High
Basaria *et al*.[28]	RCT	3	3	7	High
Vigen *et al*.[6]	Retrospective cohort study	2	2	5	-
Cheetham *et al*.[39]	Retrospective cohort study	2	2	5	-
Traish *et al*.[34]	Observational study	3	2	5	-
Emmelot-Vonk *et al*.[29]	RCT	2	3	7	High
Aversa *et al*.[30]	RCT	1	3	1	High
Ho *et al*.[31]	RCT	1	3	7	High
Yassin *et al*.[35]	Prospective controlled registry study	2	3	5	-
Muraleedharan *et al*.[45]	Observational study	1	2	5	-
Eisenberg *et al*.[46]	Observational study	3	3	5	-

RCT: randomized controlled trial

Supplementary Table 6: Main published meta-analyses on testosterone-replacing therapy and cardiovascular events

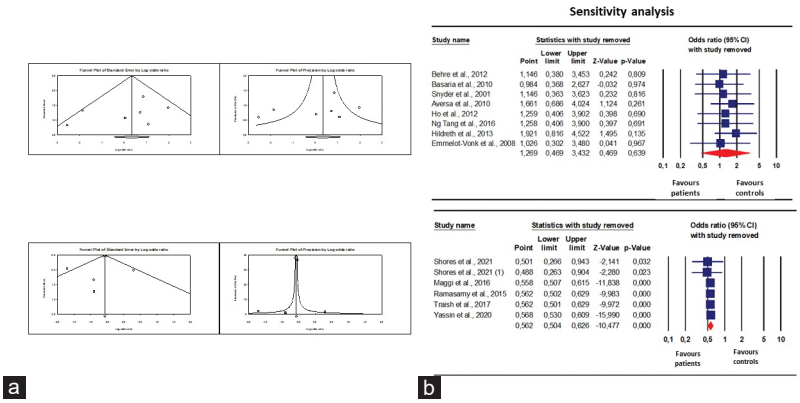
Author	*Year of publication*	*Issue*	*Studies included*	*Number of studies analyzed*	*Intervention*	*Number of patients, total (case; control)*	*Female patients (yes/no)*	*Klinefelter patients*	*Thrombophilic patients*	*Hypogonadism (yes/no)*	*Observation time (minimum–maximum), months*	*Result: Increased risk (yes/no)*
Fallara *et al*.[82]	2022	Mortality, CV events (arterial)	RCT; retrospective registry based	10	TRT *vs* placebo	179,631 (89,515; 90,116)	No	NR	NR	Yes	6–74.4	No
Hudson *et al*.[17]	2022	Mortality, CV and cerebrobascular events (arterial)	Treated-placebo	17	TRT *vs* placebo	3431 (1750; 1681)	No	NR	NR	Yes	4–36	No
Cannarella *et al*.[83]	2022	Heart failure	RCT	7	TRT *vs* placebo or no treatment	140 (71; 69)	No	NR	NR	Yes	2–12	No
Ayele *et al*.[15]	2021	Venous TE	RCT	13	TRT *vs* placebo or active-comparator	5050 (2636; 2414)	No	NR	NR	NT	3–36	No
Sansone *et al*.[84]	2020	Endothelial function (flow-mediated dilation)	RCT, observational	6	TRT *vs* placebo	Random effects model (86)	No	NR	NR	Yes	0–12	No
Corona *et al*.[10]	2018	Major adverse CV events	RCT, pharmaco-epidemiological studies	93	TRT *vs* placebo	8479 (4653; 3826)	No	NR	NR	NT	11 (mean)	No
Borst *et al*.[54]	2014	CV events (arterial)	RCT	35	TRT *vs* placebo	3703 (2114; 1589)	No	NR	NR	NT	3–62 (mean: 11.9)	Yes (only oral)
Corona *et al*.[85]	2011	CV morbidity and mortality	Cross-sectional studies, longitudinal studies, RCT	70	TRT *vs* placebo	25,299 (total)	No	NR	NR	Yes	3–36	ND
Calof *et al*.[56]	2005	CV events (arterial)	Clinical trials	19	TRT *vs* placebo	1084 (651; 433)	No	NR	NR	NT	3–36	No

RCT: randomized controlled trials; ND: not determined; NR: not reported; NT: not totally; TE: thromboembolism; TRT: testosterone replacement therapy; CV: cardiovascular

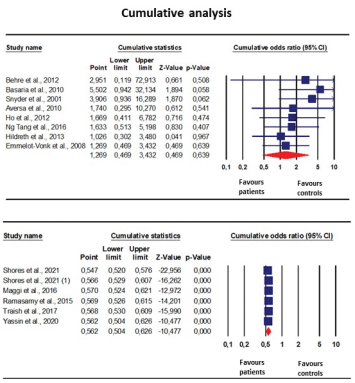
Supplementary Table 7: Main society/association guidelines/consensus statement's recommendation on the association between testosterone-replacing therapy and cardiovascular/thromboembolic risk

Society/association	*CV/thromboembolic risk*	*Recommendation against TRT*	*Prior to TRT*
AUA	At this time, it cannot be stated definitively whether testosterone therapy increases or decreases the risk of CV events	Recent (3–6 months) CV event Hematocrit >54%	Hematocrit evaluation Inform patients that there is no substantive evidence of the increased risk of CV events with TRT
AACE	No specific recommendations on this issue are possible until further research clarifies the potential risks and benefits of therapy	Hematocrit >50%	Hematocrit evaluation
BSSM	Undefined	Hematocrit >54%	Hematocrit evaluation
EAA	When hypogonadism is properly diagnosed and managed, there is currently no consistent evidence of an increased risk of CV disease during TRT	Recent major acute CV event Severe heart failure Polycythemia Hematocrit >48%–50%	Hematocrit evaluation Obtain a detailed family and personal history of VTE and associated risk factors
ES	Undefined	Hematocrit<48%	Hematocrit evaluation
EAU	There is no substantive evidence that testosterone treatment, when replaced to the normal physiological range, is related to the development of major adverse CV events	Hematocrit >54%	Hematocrit evaluation Electrocardiogram
SIAMS/SIE	Evidence on the safety of TRT on the CV profile is of moderate quality	Recent CV event	Hematocrit evaluation Evaluate global CV risk and associated morbidities, including hematocrit levels Collect a detailed family, personal and clinical history of VTE, thrombophilia-hypofibrinolysis
ICSM	The weight of evidence indicates that TRT is not associated with increased CV risk Preliminary evidence suggests the possibility of beneficial effects of TRT on the CV function	Hematocrit >50%	Hematocrit evaluation
ISAAM	NR	Hematocrit >52% Untreated severe congestive heart failure	Hematocrit evaluation

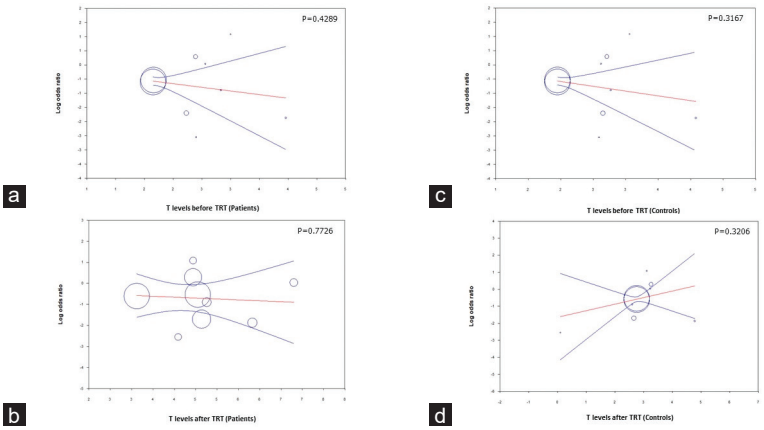
AUA: American Urological Association; CV: cardiovascular; TRT: testosterone replacement therapy; AACE: American Association of Clinical Endocrinology; BSSM: British Society for Sexual Medicine; EAA: European Academy of Andrology; ES: Endocrine Society; EAU: European Association of Urology; SIAMS: Società Italiana di Andrologia e Medicina della Sessualità; SIE: Società Italiana di Endocrinologia; ICSM: International Consultation on Sexual Medicine; NR: not reported; VTE: venous thromboembolism; ISAAM: International Society for the Study of the Aging Male



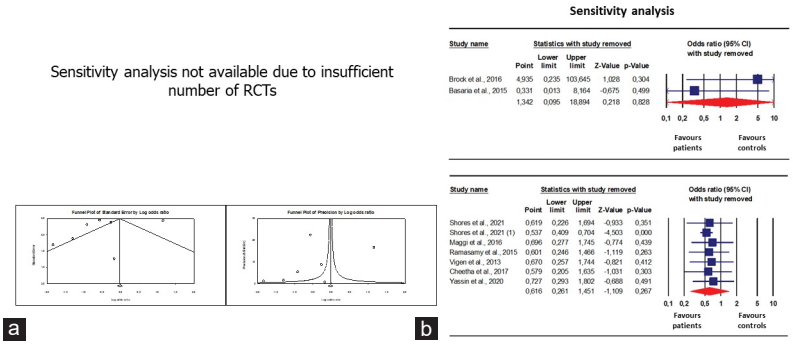
Supplementary Figure 1: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of arterial thrombotic events in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. RCTs are in the upper panel, observational studies in the lower panel.



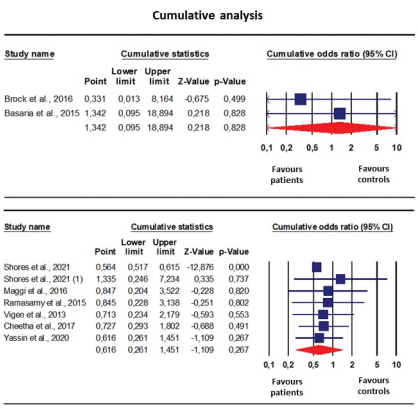
Supplementary Figure 2: Cumulative analysis of the risk of arterial thrombotic events in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. RCTs are in the upper panel, observational studies in the lower panel.



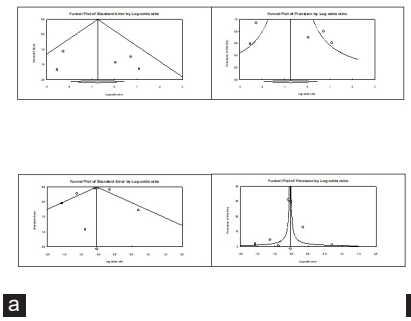
Supplementary Figure 3: Meta-regression analysis on the effect of testosterone levels in patients (**a**) before and (**b**) after and in controls (**c**) before and (**d**) after testosterone replacement therapy (TRT) on the risk of arterial thrombotic events.



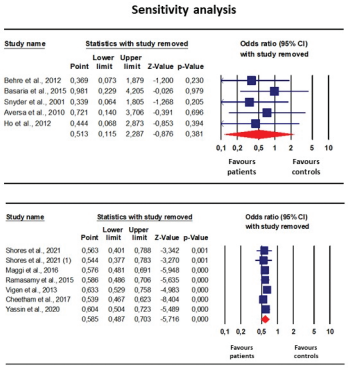
Supplementary Figure 4: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of stroke in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. RCTs are in the upper panel, observational studies in the lower panel.



Supplementary Figure 5: Cumulative analysis of the risk of stroke in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. RCTs are in the upper panel, observational studies in the lower panel.

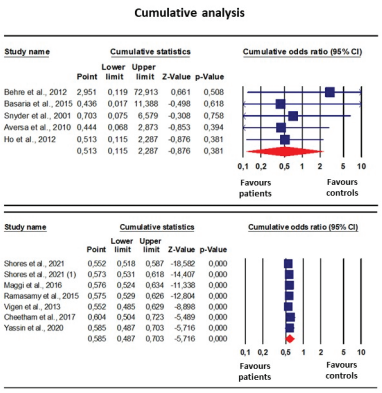


a

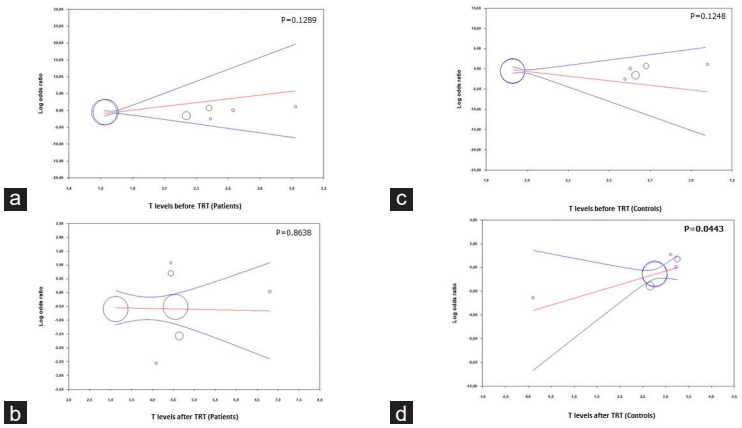


b

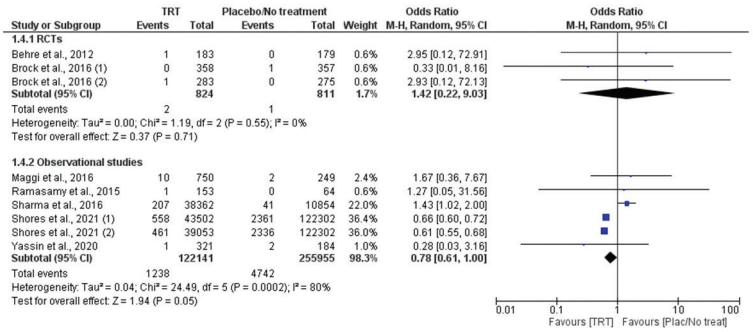
Supplementary Figure 6: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of myocardial infarction in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. RCTs are in the upper panel, observational studies in the lower panel.



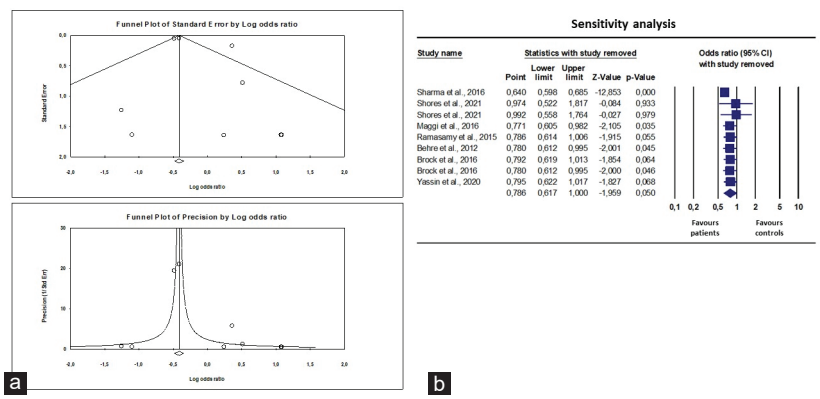
Supplementary Figure 7: Cumulative analysis of the risk of myocardial infarction in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment. RCTs are in the upper panel, observational studies in the lower panel.



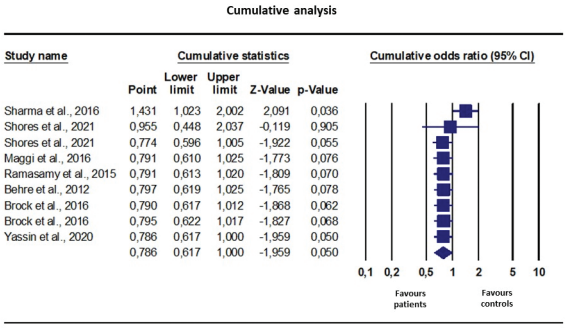
Supplementary Figure 8: Meta-regression analysis on the effect of testosterone levels in patients (**a**) before and (**b**) after and in controls (**c**) before and (**d**) after testosterone replacement therapy (TRT) on the risk of myocardial infarction.



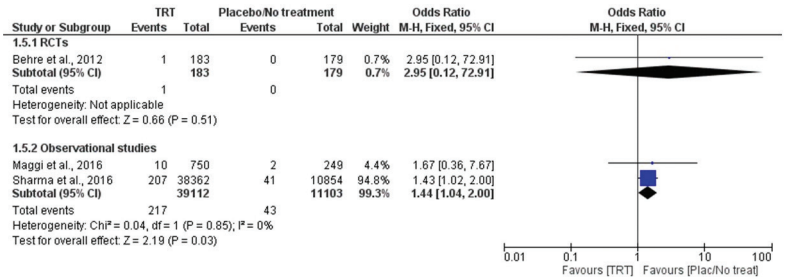
Supplementary Figure 9: Risk of venous thrombosis in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



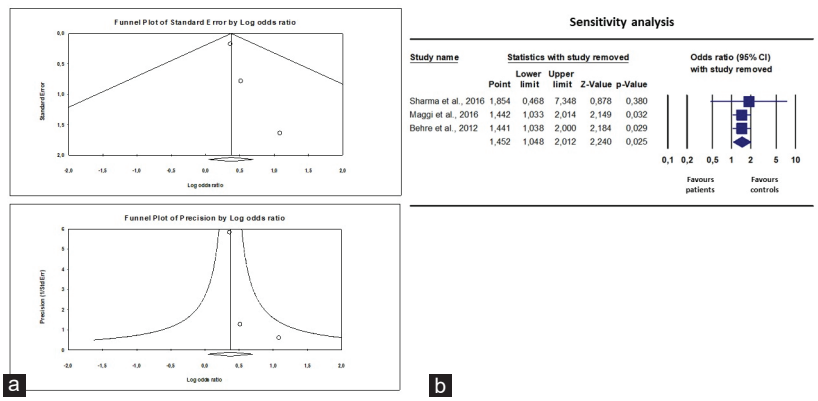
Supplementary Figure 10: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of venous thrombosis in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



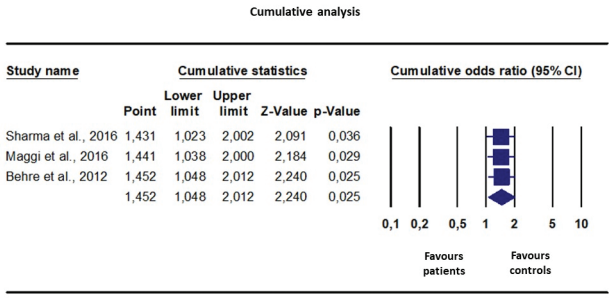
Supplementary Figure 11: Cumulative analysis of the risk of venous thrombosis in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



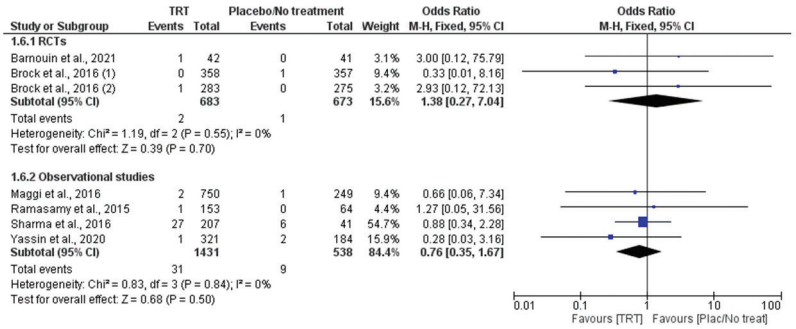
Supplementary Figure 12: Risk of deep vein thrombosis in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



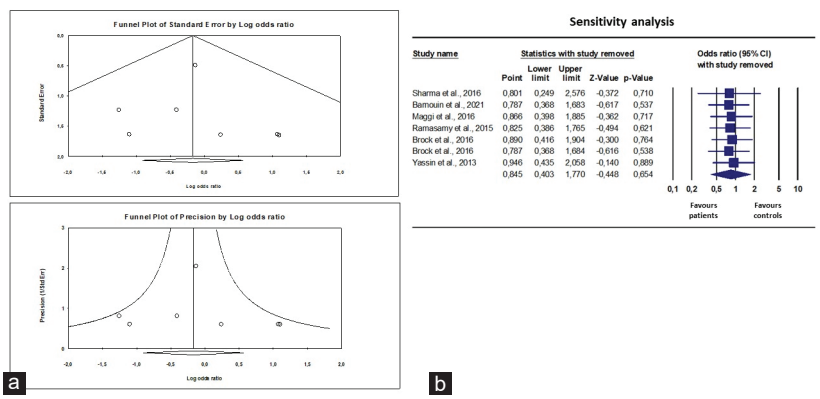
Supplementary Figure 13: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of deep vein thrombosis in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



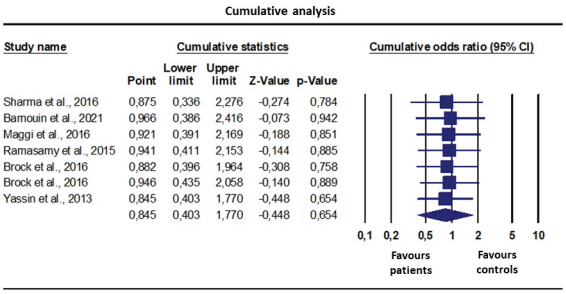
Supplementary Figure 14: Cumulative analysis of the risk of deep vein thrombosis in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



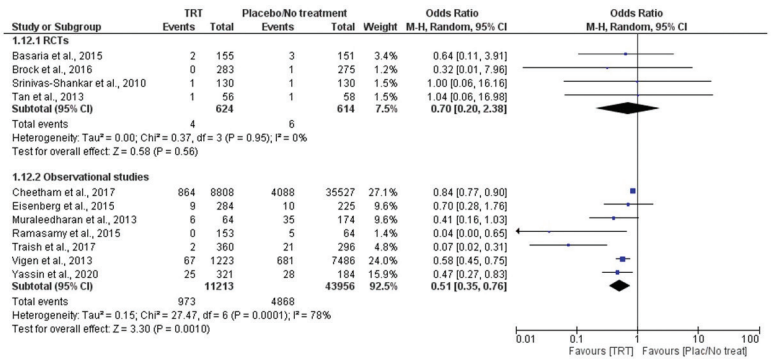
Supplementary Figure 15: Risk of pulmonary embolism in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



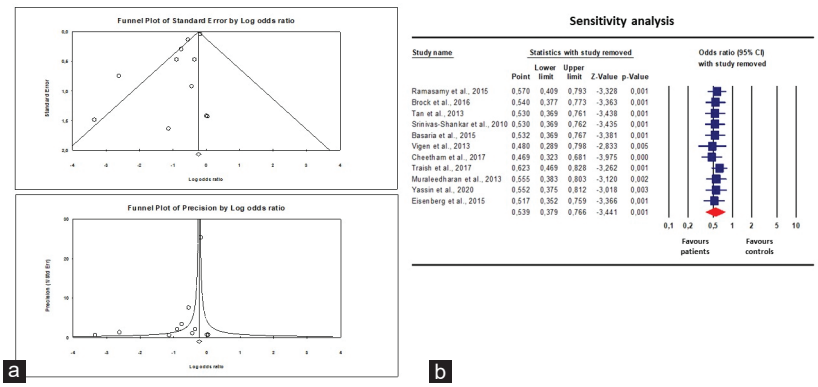
Supplementary Figure 16: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of pulmonary embolism in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



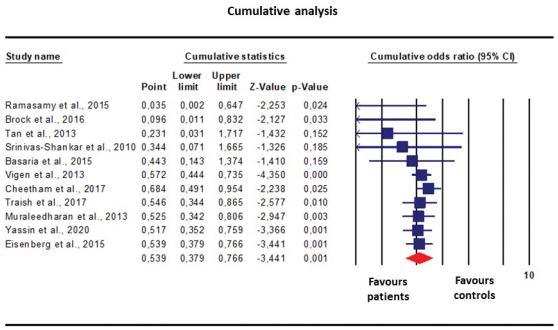
Supplementary Figure 17: Cumulative analysis of the risk of pulmonary embolism in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



Supplementary Figure 18: Risk of mortality in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



Supplementary Figure 19: (**a**) Funnel plot and (**b**) sensitivity analysis of the risk of mortality in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.



Supplementary Figure 20: Cumulative analysis of the risk of mortality in hypogonadal patients on testosterone replacement therapy compared to hypogonadal controls on placebo or no treatment.