Cardiovascular risk and mortality in men receiving testosterone replacement therapy for Klinefelter syndrome in Denmark: a retrospective cohort study

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Summary

Background Men with Klinefelter syndrome (KS) have hypogonadism, increased morbidity, and excess mortality. Testosterone replacement therapy (TRT) has the potential to alleviate this burden. We assessed the risk of major cardiovascular events (MACE) and mortality in KS according to TRT exposure.

Methods We performed a nationwide registry based matched cohort study. We compared incidences of MACE and mortality between TRT exposed (KS-TRT) or unexposed KS (KS-non-TRT), and a male background population comparison cohort. The study period was from 1 January 1994 to 31 December 2022.

Findings We identified 557 KS-TRT, and matched these with unexposed men with KS born the same year (total KS n = 950). We similarly identified a comparison cohort of 50,150 men from the background population matched on month and year of birth. Median age at entry for KS-TRT was 31.1 years (interquartile range; 19.9–40.0) and median follow-up time was 12.9 years (interquartile range; 7.5–20.7). KS-TRT was associated with lower all-cause mortality (adjusted hazard ratio (95% CI); 0.56 (0.37–0.85)), with mortality in KS-TRT comparable to the comparison cohort (hazard ratio (95% CI); 1.27 (0.91–1.79)). Incidence of MACE was comparable between KS-TRT and KS-non-TRT.

Interpretation TRT could alleviate excess mortality in KS and appears safe regarding cardiovascular risk. Today, most men with KS go undiagnosed, missing proper medical attention. There is a dire need for a policy change to ensure timely diagnosis and treatment in all men with KS.

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Introduction

Klinefelter syndrome (47,XXY; KS) is the most common sex chromosomal abnormality, affecting approximately one in 600 males.[1] The condition leads to hypergonadotropic hypogonadism[1] and an unfavorable trajectory characterized by obesity, disruption of normal metabolism, and high risk of diabetes and cardiovascular disease.[1] KS is further associated with increased mortality and a median loss of more than five years.[1]

Testosterone replacement therapy (TRT) in men with KS is recommended to alleviate the comorbidity burden and elevate quality of life.[1,2] Although the currently available longitudinal data does not indicate any adverse effects of TRT in men with KS,[3–6] issues regarding safety of TRT in hypogonadism has not been resolved.[7] The TRAVERSE study, to date the largest randomized controlled study on TRT, found no increased risk of major cardiovascular events (MACE) following TRT in

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Research in context

Evidence before this study

Klinefelter syndrome is a congenital condition that leads to hypogonadism, increased morbidity, and elevated mortality. The current clinical recommendation is to offer testosterone replacement therapy to alleviate hypogonadal symptoms and improve quality of life. However, a search of the entire Pubmed database up until August 31 2024 without language restrictions and including search terms "Klinefelter syndrome" and "testosterone replacement therapy" yields no randomized evidence supporting the cardiovascular safety of testosterone supplementation specifically in men with Klinefelter syndrome. Evidence from randomized trials of testosterone replacement therapy in elderly hypogonadal men without Klinefelter syndrome (e.g. TRAVERSE) are not directly applicable to Klinefelter syndrome, among other things due to the early onset and often lifelong exposure to testosterone replacement therapy in Klinefelter syndrome.

Added value of this study

Given that conducting a long-term randomized controlled trial of testosterone replacement therapy in Klinefelter syndrome is impractical and unethical, we conducted a national registry-based matched case–control study to emulate such a trial. We identified 557 men with Klinefelter syndrome who were exposed to testosterone replacement therapy and calculated hazard ratios for major adverse cardiovascular events and mortality, comparing them with men with Klinefelter syndrome who had no prior testosterone treatment. Our data show, for the first time, that testosterone replacement therapy in men with Klinefelter syndrome is associated with nearly a 50% reduction in mortality rate (adjusted hazard ratio (95% CI): 0.56 (0.37–0.85)) and no increased incidence of major cardiovascular events.

Implications of all the available evidence

Klinefelter syndrome affects approximately one in 600 male births, yet only about one in four individuals are ever diagnosed. As a result, the vast majority of boys and men with Klinefelter syndrome may suffer from not receiving appropriate healthcare interventions. Our findings suggest that proper treatment of hypogonadism in men with Klinefelter syndrome could reduce mortality. These results highlight the need for policy changes to ensure timely diagnosis and treatment for all individuals born with Klinefelter syndrome.

men with symptomatic hypogonadism and high cardiovascular risk,[8] but evidence from studies investigating relative hypogonadism due to old age or obesity, cannot be directly transferred to the lifelong exposure to hypogonadism in KS.[9] Unfortunately, large-scale randomized trials in KS are notoriously difficult to conduct. This is in part due to the rarity of the syndrome and the low detection rates,[1] but also due to the need for very long follow-up time, as ideally TRT in KS is initiated when signs of hypergonadotropic hypogonadism first emerges, most often during puberty,[1,2,10] and sustained life-long. In addition, there is a general consensus that it would be unethical to administer a placebo for prolonged periods to hypogonadal males with KS. The perhaps only feasible approach to investigate the impact of long-term TRT on health outcomes is by utilizing data from large health registries. We present a population-based matched cohort study based on Danish health registry data, addressing effects of TRT exposure on mortality and the incidence of MACE in men with KS.

Methods

Design and setting

We performed a Danish national registry-based study approved by The Danish Data Protection Agency (record number: 1-16-02-568-15). The Danish registries encompass the entire population and are linked at the individual level.[11] Diagnoses were retrieved from The Danish National Patient Registry[12] according to the International Classification of Diseases (ICD) tenth revision used in Denmark from 1993 and onwards. Death occurrences were retrieved from the Civil Registration System[13] and the cause of death was retrieved from The Danish Registry of Causes of Death[14] according to the ICD-10 classification. Information regarding all redeemed prescriptions, from 1994 and onwards, are recorded in the Danish National Prescription Registry,[15] and were identified according to Anatomical Therapeutic Chemical classification (ATC) codes. For each redeemed prescription, the product number can be used to assess a standardized Defined Daily Dose (DDD) as defined by the World Health Organization.[15] Individual laboratory analyses, from 2013 and onwards, were retrieved from the Register of Laboratory Results for Research administered by the Danish Health Data Authority according to the Nomenclature for Properties and Units (NPU) Laboratory Terminology. The observation period was from 1 January 1994 until 31 December 2022.

Case identification and TRT exposure

Men with KS were identified in the Danish Cytogenetic Central Registry 16 (Fig. 1). KS was defined as any 47,XXY, 46,XY/47,XXY mosaic or other 47,XXY mosaic karyotypes. We excluded men with KS who died before the start of our observation period (n = 81), men with KS who did not have a minimum of 1 year follow-up (n = 4) and boys with KS (n = 53) who did not reach an age of 15 years before emigrating, dying or end of study. Lastly,

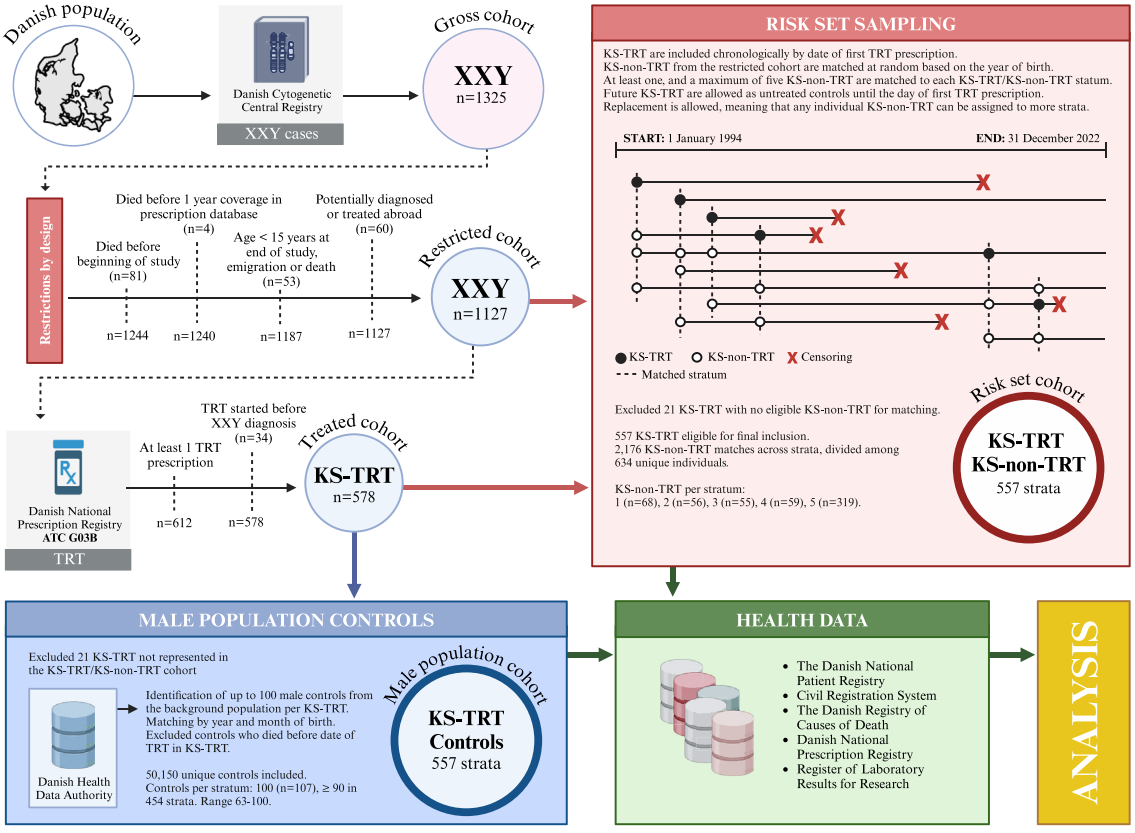

Fig. 1: Flow-diagram depicting formation of study cohorts through utilization of Danish health registries and application of risk set sampling. Abbreviations: XXY: Klinefelter syndrome, TRT: testosterone replacement therapy, KS-TRT: TRT exposed men with Klinefelter syndrome, KS-non-TRT: Non-TRT exposed men with Klinefelter syndrome.

we excluded men with KS who had emigrated and subsequentially reentered Denmark, as we have no knowledge about potential diagnosis or treatment while abroad (n = 60). Exposure to TRT was defined as redemption of any prescription adhering to ATC G03B.[16] We excluded men with KS receiving TRT before being diagnosed with KS (n = 34).

KS comparison cohort with risk set sampling

We designed a matched cohort study applying risk set sampling with replacement based on timing of TRT exposure in an intention-to-treat analysis (Fig. 1).[17] Strata were formed according to chronological exposure to TRT, with the man with KS with the earliest date of first TRT prescription matched first and the last man with KS to be exposed matched last. Each man with KS exposed to TRT (KS-TRT) was matched on the day of the first prescription to as many as five men with KS born the same year, with a known KS diagnosis and no prior TRT (KS-non-TRT). A man with KS eventually being exposed to TRT was eligible for inclusion as an untreated control at any time until the day of the first TRT prescription. Unexposed controls for each stratum were sampled randomly among all eligible participants with replacement, meaning that any unexposed man with KS could potentially be reselected for different strata.[17]

Comparison cohort from background population

We included a comparison cohort of men without known KS from the Danish background population (Fig. 1). Each KS-TRT was matched with up to 100 control men by year and month of birth. Construction of the comparison cohort was undertaken by the Danish Health Data Authority and was blinded to the authors. Controls were selected completely at random from the entire Danish male population with no exclusion criteria applied and without distinction to future morbidities e.g. hypogonadism or treatments e.g. TRT.

Outcomes

For all outcomes, the date of event was set as the first occurrence of the outcome. A complete list of ATC, ICD and NPU codes applied for this study is supplied in the Supplementum (Supplemental Table S1).

We evaluated blood testosterone levels in relation to TRT. Several different assays with different reference

ranges have been used over time. To allow comparison of data over time the individual testosterone measurements were normalized using the location-scale model as proposed by Chuang-Stein.[18] We also assessed luteinizing hormone (LH) in relation to TRT. Due to a very high number of different assays applied over the course of the study, we evaluated LH levels as the percentage in relation to the upper reference limit for each assay.

The primary endpoints were all-cause mortality and first-time diagnosis of MACE. There is no consensus on the definition of MACE or cardiovascular death.[19] We defined MACE as the composite of non-fatal myocardial infarction (ICD) or non-fatal stroke (ICD) and cardiovascular death. A non-fatal event was defined by the absence of death within 30 days of that event. We defined cardiovascular death as death due to diagnoses in the cardiovascular chapter ranging from ICD-10 code I000 to I789. Secondary endpoints included single components of the MACE definitions as described above. Tertiary endpoints included rates of atrial arrhythmia, pulmonary embolism and heart failure. Also, as the diagnosis of hypertension and hypercholesterolemia are not sufficiently coded in the hospital registries, we accessed these entities by evaluation of new prescriptions for antihypertensive medicine and statins. We also included new prescriptions of platelet inhibitors as a marker for secondary prevention of cardiovascular disease.

Statistical analysis

Survival analysis

To compare incidences of morbidities and mortality, we computed hazard ratios (HR) using stratified Cox regression with cluster-robust variance estimates. Entry in the study was at the day of TRT exposure in each stratum and follow-up was until censoring or first occurrence of the specific outcome under investigation, death, emigration, or end of study. KS-non-TRT were censored on the day of TRT exposure if exposed themselves during follow-up as non-exposed controls. We excluded Individuals with first occurrence of the outcome under investigation prior to study entry from the analysis of that specific outcome.

Supplementary analyses

A linear mixed-effects model was employed to compare levels of total testosterone and LH observed from one year before until three years after TRT exposure with the individual as a random effect and the timing relative to TRT exposure (before/after) as a fixed effect.

Sensitivity analysis

We computed a secondary adjusted hazard estimate for each of the primary and secondary outcomes, considering use of antihypertensives, statins and platelet inhibitors, as well as presence of diabetes, myocardial infarction, or stroke prior to study entry.

Analyses were performed using Stata 18 (StataCorp LLC, College Station, TX, USA).

Role of the funding source

The funders of the study had no role in any aspects of study design, data collection, data analysis, data interpretation, writing of the manuscript, or decision to submit for publication.

Results

A total of 950 men with KS were included, with 557 (59%) exposed to TRT (KS-TRT) (Fig. 1). The risk set sampling procedure yielded 2176 individual matches divided among 634 non-TRT exposed men with KS (KS-non-TRT) and forming 557 strata in the KS-TRT/KS-non-TRT cohort. Similarly, 50,150 unique male controls from the background population were identified, yielding 557 strata in the KS-TRT/Controls cohort.

Evaluation of testosterone replacement therapy

The median (IQR) total testosterone levels in KS-TRT prior to TRT was 7.7 (3.5–11.4) nmol/L increasing to 13.9 (9.1–20.0) nmol/L after TRT ($p < 0.001$, Fig. 2A). Similarly, median (IQR) LH, expressed as the relative level in relation to the upper limits of the applied assays, was 177 (110–264) % prior to TRT and 65 (3–159) % after TRT ($p < 0.001$, Fig. 2A).

The mean annual DDD's testosterone redeemed were relatively stable throughout the observation period, with a small increase around 2006 coinciding with the introduction of Nebido injections (Fig. 2B).

The route of TRT administration can be either oral, transdermal or parenteral. During the course of the study, 24.9% of prescriptions were for oral testosterone, 18.6% for transdermal testosterone, and 56.5% were for parenteral testosterone (Supplemental Figure S1), with Nebido (testosterone undecanoate) accounting for 39.4% of all testosterone prescriptions. Over time, an increasing favoring of transdermal and parenteral administration over oral administration was seen.

All-cause mortality and major cardiovascular events

The prevalence of cardiovascular comorbidities at baseline was comparable between KS-TRT and KS-non-TRT (Table 1). Similarly, there was no significant difference in the use of medications between KS-TRT and KS-non-TRT (Table 1). Cardiovascular comorbidities were increased in men with KS compared with men in the background population at baseline (Table 1).

TRT was associated with a reduced incidence of all-cause mortality in men with KS (Table 2, Fig. 3). Furthermore, while all-cause mortality in KS-non-TRT was increased in comparison to controls (HR 1.77 (1.31–2.40)), this was not the case when comparing

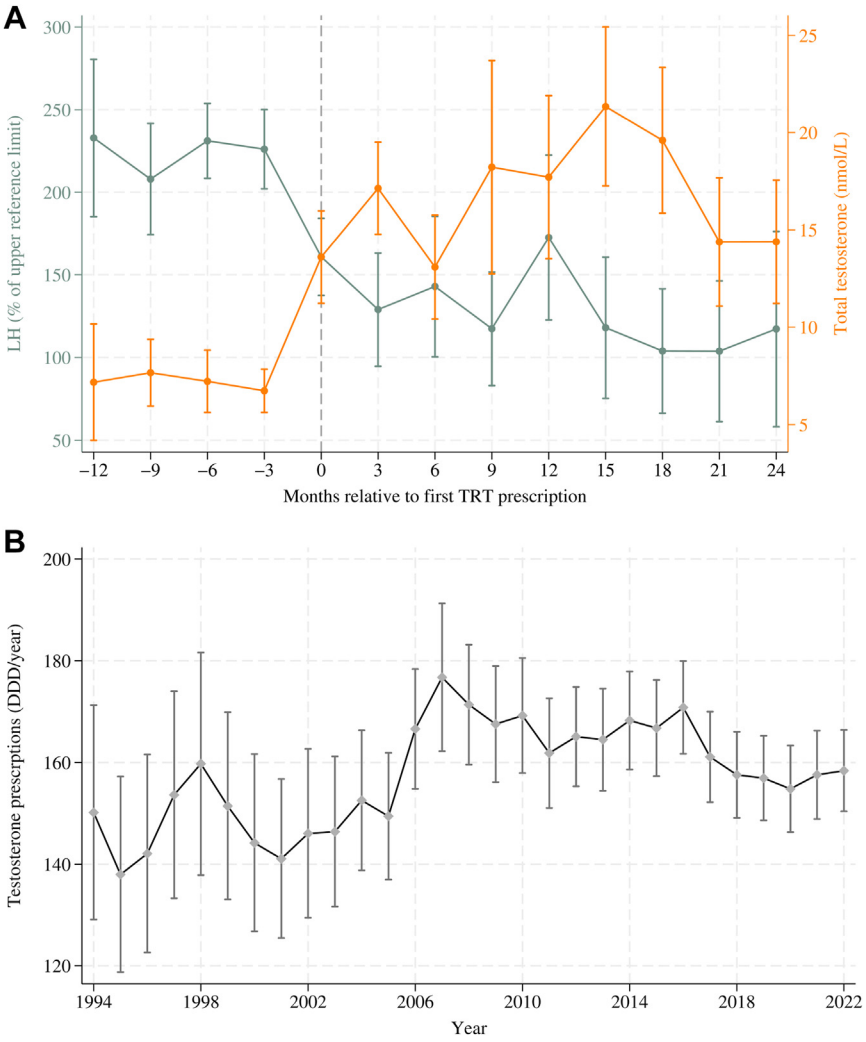

Fig. 2: Evaluation of testosterone replacement therapy (TRT) in men with Klinefelter syndrome. A) Luteinizing hormone (LH) and total testosterone in testosterone treated men with Klinefelter syndrome. Data are given as the averaged mean ± SD for each three-month period in relation to initiation of testosterone replacement therapy. B) Annual number of World Health Organization (WHO) Defined Daily Dose (DDD) redeemed per man with Klinefelter syndrome exposed to testosterone replacement therapy.

KS-TRT with controls (HR 1.27 (0.91–1.79), Table 2, Fig. 3). A similar pattern was observed for cardiovascular mortality, although the estimates did not reach statistical significance (Table 2, Fig. 4).

Causes of death among men with KS are given in Supplemental Figure S2. There was an indication of fewer deaths due to neoplasms among KS-TRT compared with KS-non-TRT (Adjusted HR 0.38 (0.11–1.35). Age at all-cause mortality was 58.6 ± 14.6 years in KS-TRT and 58.5 ± 12.5 in KS-non-TRT (Student t-test, p = 0.97).

The overall incidence of MACE was comparable between KS-TRT and KS-non-TRT (Table 2, Fig. 4). MACE occurrences were evenly distributed throughout the observation period. Mean ± SD duration from start of TRT to MACE in KS-TRT was 14.9 ± 7.8 years, with the earliest event occurring 3.03 years after initiation of TRT. Equally, the rates of MACE in KS-TRT was not different compared with controls (Table 2, Fig. 4).

Other cardiovascular endpoints

TRT exposure in men with KS did not significantly affect the incidence of myocardial infarction. The incidence of stroke in KS-TRT was not different compared with controls, but an increased incidence of stroke was seen in the unadjusted model comparing KS-TRT with KS-non-TRT. In the adjusted model, TRT was no longer significantly associated with increased incidence of stroke (Table 2).

Recently, the TRAVERSE study, demonstrated a possible association between TRT and increased risk of atrial arrhythmia and pulmonary embolism.[8] Here, TRT

	KS-TRT	KS-non-TRT	Controls
	n = 557	n = 2176[a]	n = 50,150
Age at entry (years)	31.1 (19.9–40.0)	31.1 (20.0–40.0)	30.7 (19.2–39.3)
Observation time per person (years)	12.9 (7.5–20.7)	9.8 (3.8–17.9)	13.0 (7.2–21.2)
Comorbidities			
Heart failure (%(n))	0.4 (2)	0.3 (7)	0.2 (112)
Atrial arrhythmia (%(n))	0.7 (4)	2.2 (25)	0.5 (256)
Myocardial infarction (%(n))	0.9 (5)	0.2 (4)	0.2 (1052)
Pulmonary embolism (%(n))	0.7 (4)	0.8 (17)	0.1 (51)
Stroke (%(n))	0.5 (3)	0.3 (6)	0.5 (259)
Diabetes (any type) (%(n))	4.3 (24)	4.2 (91)	1.6 (807)
Type 2 diabetes (%(n))	3.6 (20)	4.1 (89)	1.1 (560)
Medication			
Statins (%(n))	3.8 (21)	4.5 (97)	2.9 (1492)
Antihypertensives (%(n))	11.8 (66)	10.1 (220)	6.8 (3416)
Platelet inhibitors (%(n))	3.6 (20)	3.4 (75)	2.0 (1013)

Continuous data are median with interquartile range. Abbreviations: KS; Klinefelter syndrome, TRT: testosterone replacement therapy. [a]The total number of KS-non-TRT matches, divided among 634 individual participants.

Table 1: **Demographics of participants at entry in study and according to exposure to testosterone replacement therapy.**

in men with KS was not associated with an increased incidence of either of these conditions (Table 2). Also, often heart failure is considered a component of MACE.[19] KS-TRT had a two-fold increased incidence of heart failure compared with KS-non-TRT and controls (Table 2). The incidence of starting treatment with statins, antihypertensive medications or platelet inhibition was not different between KS-TRT and KS-non-TRT, but the incidence of starting antihypertensive medications was increased in KS-TRT compared with controls (Table 2).

Discussion

Men with KS have routinely been offered TRT based on the assumption that replenishment of testosterone levels would be beneficial. However, evidence describing the efficacy and safety of TRT in KS has been lacking. Here, we present real world data for the first time demonstrating an almost halving of mortality incidence in KS-TRT vs KS-non-TR. Furthermore, all-cause mortality in KS-TRT was comparable with men in the background population, indicating that TRT has the potential for alleviating the increased mortality among men with KS.[1] Today, as much as 75% of men with KS are never diagnosed, despite suffering from the same comorbidity pattern as those receiving a diagnosis.[20,21] Further, diagnosis of KS is commonly delayed well into adulthood and many men with diagnosed KS are never treated despite being hypogonadal.[3,21] The potential reduction of excess mortality in KS by TRT is a quintessential finding regarding health care in KS and calls for a change in policy to ensure timely diagnosis and treatment of hypogonadism in all men with KS.

We present data covering a period of almost 30 years with a mean observation time in KS-TRT of 13 years. The data supports the long-term safety of TRT in KS, an important perspective since most often signs of hypergonadotropic hypogonadism are present in puberty, indicating a need for treatment.[1,2,10]

The indication for TRT in men with KS differs from treatment in other groups of men presenting with later onset of hypogonadism. Still, these results are in line with some previous observational studies among non-KS cohorts of primarily elderly men, finding reduced mortality rates with TRT and higher endogenous testosterone.[22,23]

	KS-TRT	KS-non-TRT	Controls	HR	HR (adjusted)[b]	HR
	n = 557	n = 2176[a]	n = 50,150	KS-TRT vs KS-non-TRT	KS-TRT vs KS-non-TRT	KS-TRT vs control
All-cause mortality (%(n))	7.0 (39)	8.6 (188)	6.5 (3261)	0.60 (0.40–0.89)	0.56 (0.37–0.85)	1.27 (0.91–1.79)
MACE (%(n))	5.3 (30)	5.2 (115)	5.0 (2528)	1.03 (0.71–1.49)	1.05 (0.73–1.52)	1.16 (0.82–1.64)
Myocardial infarction (%(n))	1.4 (8)	1.6 (35)	2.1 (1052)	0.82 (0.39–1.69)	0.80 (0.38–1.68)	0.69 (0.36–1.36)
Stroke (%(n))	3.4 (19)	2.0 (43)	2.4 (1198)	1.72 (1.06–2.78)	1.57 (0.98–2.52)	1.56 (0.997–2.45)
Cardiovascular death (%(n))	1.4 (8)	2.3 (51)	1.4 (708)	0.56 (0.26–1.22)	0.53 (0.20–1.38)	0.94 (0.47–1.87)
Other cardiovascular endpoints						
Atrial arrhythmia (%(n))	5.2 (29)	5.9 (129)	3.0 (1517)	0.86 (0.56–1.30)	0.82 (0.54–1.26)	2.12 (1.45–3.08)
Pulmonary embolism (%(n))	3.1 (17)	2.6 (56)	0.8 (399)	1.06 (0.56–2.01)	1.01 (0.52–1.96)	4.05 (2.51–6.54)
Heart failure (%(n))	4.1 (23)	2.1 (46)	1.8 (916)	1.98 (1.22–3.21)	2.16 (1.23–3.81)	2.42 (1.58–3.70)
Medications						
Statins (%(n))	22.4 (125)	22.5 (490)	14.1 (7071)	1.02 (0.82–1.27)	0.98 (0.78–1.22)	2.06 (1.71–2.48)
Antihypertensives (%(n))	28.0 (156)	28.4 (618)	18.8 (9425)	1.11 (0.92–1.33)	2.07 (1.73–2.48)	2.09 (1.77–2.47)
Platelet inhibitors (%(n))	14.0 (78)	13.5 (293)	8.8 (4398)	1.13 (0.92–1.38)	1.04 (0.79–1.35)	2.12 (1.67–2.68)

Abbreviations: HR; hazard ratio, KS; Klinefelter syndrome, MACE; Major Adverse Cardiovascular Events. [a]The total number of KS-non-TRT matches, divided among 634 individual participants. [b]Adjustment applied for prior use of antihypertensives, statins, and platelet inhibitors, as well as previous diabetes, myocardial infarction, or stroke.

Table 2: **Cardiovascular outcomes according to exposure to testosterone replacement therapy.**

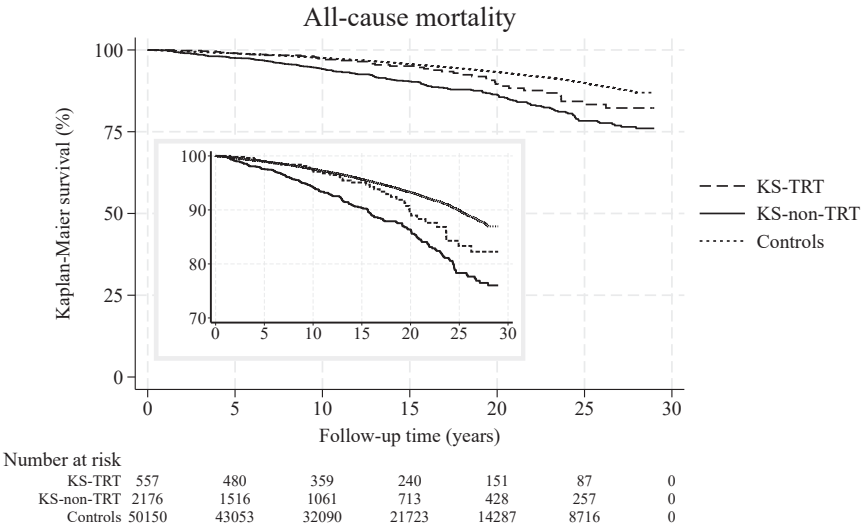

Fig. 3: All-cause mortality in men with Klinefelter syndrome according to testosterone replacement therapy (TRT) status.

Similarly, rates of MACE among KS-TRT were comparable to the background population, supporting that TRT in KS is safe regarding major cardiovascular risk, which is further supported by similar findings in non-KS cohorts receiving TRT.[8,24] The current study adds to our previous published registry study advocating that TRT in men with KS is also safe regarding the risk for venous thromboembolism.[3]

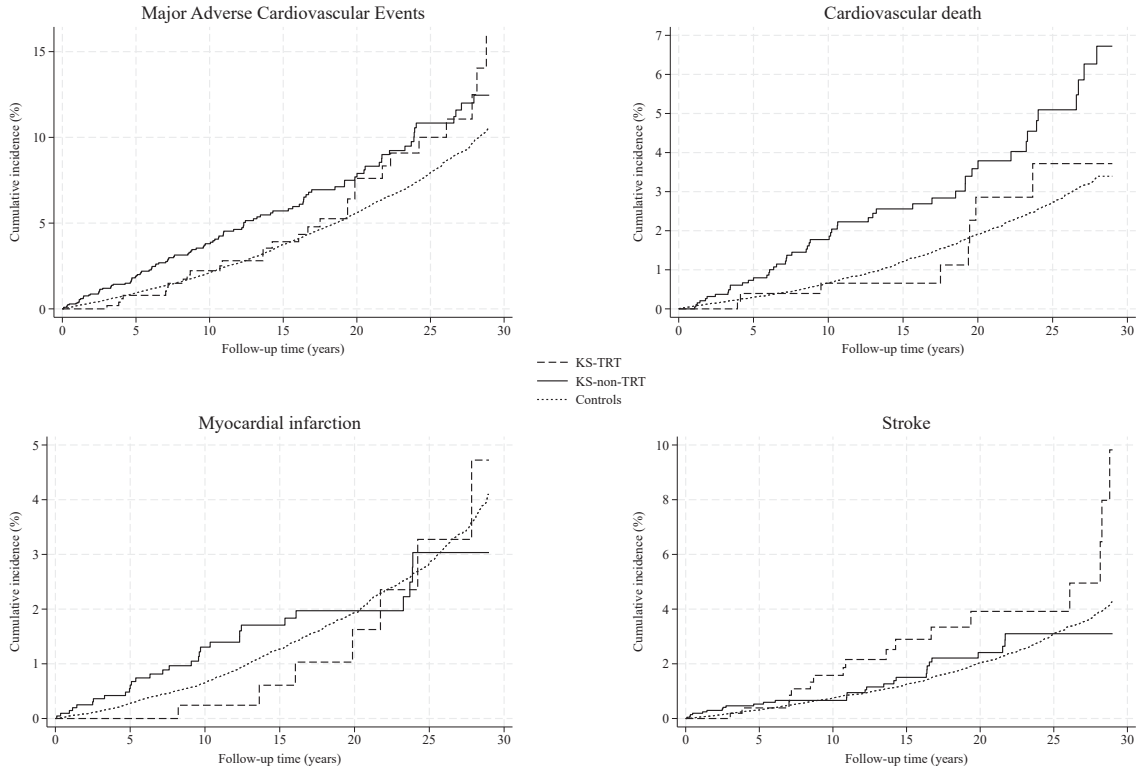

Fig. 4: Time-to-event analysis showing cumulative incidence estimates of cardiovascular outcomes in men with Klinefelter syndrome according to testosterone replacement therapy (TRT) status, and with death (all-cause mortality) as a competing risk.

Currently, to our knowledge, hard endpoints like mortality and MACE in relation to TRT in KS have not been studied outside the Danish setting, and such studies are warranted to support the current findings.

We applied an intention-to-treat analysis, which is insensitive to issues regarding adherence to treatment. From our many years of clinical experience treating hundreds of men with KS we generally see only very few men with KS opting to discontinue TRT. However, our study is not able to directly assess adherence to TRT among men with KS. We only have access to data regarding redeemed prescriptions and no information about actual administration of these prescriptions. However, we hypothesize that having paid for their own medication, patients would also be inclined to actual use. In addition, we demonstrate increased testosterone levels in men with KS immediately after TRT exposure, with sustained average testosterone levels in the middle of the normal reference range, and an inverse pattern observed for LH levels. We also demonstrate redemption of a relatively stable amount of testosterone DDD's over time. However, from our clinical practice, DDD's do not properly reflect the individual dosing regimens applied and likely underestimate the true treatment coverage. As an example, one injection with 4 ml Nebido corresponds to 55.5 DDD, while typical standard of care in our clinic would be one such injection every 12 weeks (84 days), however with a large variability (8–18 weeks).[25]

The data presented stem from a real-world setting reflecting actual applied standard of care in Denmark. The overall standard of health care in Denmark is high, free to all citizens, and prescription medicine is subsidized. As an example, the annual own payment for Nebido given every 12 weeks is approximately 2000 DKR (≈300 US $/270 €). In a global setting, we believe these to be relatively fortuitous circumstances for securing optimal treatment including TRT. As such, it is of course worrying that our data still find that onset of TRT is late, likely due to the concurrently late diagnostic age for men with KS,[21] and that many men with KS are not treated with testosterone despite being overtly hypogonadal.[3] The current study could therefore be underestimating the true effect of optimal TRT in KS.

The association between biological sex, sex hormones and stroke is not clear.[26–28] In the unadjusted analysis, KS-TRT was associated with increased incidence of stroke. This potential risk should be noted, even if previous studies from non-KS cohorts do not find an increased risk of stroke with TRT[8,29] and although this association was lost after adjustment for preexisting comorbidities. In our data, TRT was not associated with an increased incidence of atrial arrhythmia, a major stroke risk factor. Thus, the mechanism potentially elevating risk of stroke with TRT is not clear. More data is needed to elucidate the risk of stroke in men with KS receiving TRT.

We find higher incidence of heart failure in KS-TRT. A previous study comparing untreated men with KS and men with KS who received TRT for a mean duration of 36 months did not find any effect of treatment on cardiac function evaluated by complete Doppler echocardiographic examination and a cardiopulmonary exercise test.[30] The association between TRT or testosterone and risk of heart failure in non-KS cohort is not clear.[8,31,32] Diagnosis of heart failure is complex with significant diagnostic delay and high non-diagnostic rates.[33] The finding of increased incidence of heart failure among KS-TRT, could be due to observation bias from seeing a doctor more regularly, increasing the chance of attention to symptoms of heart failure. Similarly, attention to routine monitoring of blood pressure in KS-TRT could explain the increased rates of prescriptions for antihypertensive drugs. Somewhat perplexing, optimized blood pressure controls in KS-TRT should theoretically alleviate the risk of heart failure, which does not seem to be the case, underlying the need for further studies directly investigating cardiac function in KS receiving TRT.

Many factors could influence the chance of being diagnosed with KS and eventually being treated. The KS phenotype is characterized by a large degree of inter-individual variation, but an increasing comorbidity burden could be a factor predicting diagnosis and TRT. However, comorbidity among undiagnosed men with KS in the UK Biobank resembled that of diagnosed cases to a large extend[20] and the comorbidity pattern at entry in our study was comparable between TRT exposed and unexposed men with KS. Poorer socioeconomic status in some men with KS could perhaps also preclude diagnosis and treatment. The available data for the current study does not allow evaluation of socioeconomic factors, and more pronounced difficulties with attending school, or coming from a low social status home could speculatively even raise the chance of being diagnosed due to the comprehensive social system in Denmark. Also, from our clinical experience, even men with KS and a low social status can prioritize affording TRT in the Danish setting, but even though the medication is subsidized, the cost of treatment could still be a barrier to some. Differences in utilization of health care provision among subgroups of men with KS could lead to observation bias with higher morbidity rates in the closest monitored groups affecting our estimates. We would, however, consider such bias as a positive indicator of TRT as a broader marker for optimized care including regular check-up etc. It is very likely that non-detection or delayed diagnosis of comorbidities in KS-non-TRT contributes to the higher mortality in the unexposed group. Accepting the estimated effects of TRT as also reflecting the compound effect of proficient medical attention in KS would further underline the need for centralization of KS care.

The strength of the present study includes the matched design, long follow-up and integration of national data from different sources to allow a comprehensive insight in the health effects of TRT among men with KS in Denmark. Naturally, the number of men eligible for inclusion could affect hazard estimates, although we are indeed able to demonstrate a significant effect on the ultimate endpoint of mortality. The nature of the Danish registries does not allow evaluation of life-style factors such as smoking, alcohol, physical activity and diet, which could be potential confounders across our inclusion groups. We speculate that life-style factors could contribute to the increased morbidity rates in KS vs controls, but there are currently no compelling data addressing these issues in KS in relation to treatment status. Also, KS-non-TRT and controls did not have regular biochemistry available, deeming comparison of testosterone and LH levels across cohorts impossible. Similarly, the nature of the data did not allow for sub-grouping of KS-TRT, which could have been of interest regarding for instance the effects of different TRT administration routes. Other study limitations include the lack of racial or ethnical information about the cohorts, and the lack of standardization of TRT. As follows, the data can only to a certain extent describe health effects of TRT in the idealized clinical setting, where standardized TRT is initiated in KS when signs of hypergonadotropic hypogonadism first emerges.[1,2] However, to our knowledge, such a standard of care is not provided anywhere in the world today. Also, the applied intention-to-treat analysis could underestimate both beneficial and unwarranted effects of TRT in the case of low adherence. However, as discussed above, in our experience adherence to TRT among men with KS is substantial. The greatest limitation to our study is the lack of randomization. Today, we simply do not understand why some men with KS are diagnosed and why most are not and secondly why not all men with diagnosed KS receive TRT. As such, it is possible that our cohort of men diagnosed with KS are not representative of men living with undiagnosed KS, and that KS-TRT represent a different subgroup than those who do not start treatment. Our design allowing men with subsequent TRT exposure as unexposed controls seek to compensate for this. Constructing a randomized controlled trial with a comparable number of participants and similar follow-up time to investigate TRT in men with KS would be unfeasible and unethical. We thus believe that the current design and similar ways of emulating randomized controlled trials using observational data might represent the closest approximation obtainable of TRT effects in KS.

In conclusion, we present a nationwide matched-cohort study, for the first time demonstrating reduced mortality rate and non-increased incidence of MACE among KS-TRT. Our data support that TRT in men with KS is safe regarding overall cardiovascular risk. Knowing that most men with KS are left undiagnosed, our data stress the need for a conjunctive effort to raise diagnostic rates. Further, standardization of TRT in KS is needed and care should be taken to ensure that all men with KS and signs of hypogonadism have access to TRT.

Contributors

SC: conceptualisation, literature search, figures, study design, data collection, data analysis, data interpretation, project administration, writing–original draft, funding acquisition. LP: conceptualisation, study design, data curation, methodology, supervision, validation, visualisation, writing—review & editing. AS and AB: conceptualisation, project administration, data collection, data analysis, supervision, writing—review & editing.

CG: conceptualisation, study design, supervision, funding acquisition, resources, writing– review & editing.

Data sharing statement

The manuscript is based on publicly available data from Danish health registries. As such, the data basis for the current manuscript can be accessed upon reasonable request to the individual registries or The Danish Health Data Authority.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanepe.2025.101230.

References

1 Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebaek A. Klinefelter syndrome - integrating genetics, neuropsychology and endocrinology. *Endocr Rev*. 2018;39(4):389–423.
2 Zitzmann M, Aksglaede L, Corona G, et al. European academy of andrology guidelines on Klinefelter syndrome endorsing organization: European society of endocrinology. *Andrology*. 2021;9(1):145–167.
3 Chang S, Christiansen CF, Bojesen A, Juul S, Munster AB, Gravholt CH. Klinefelter syndrome and testosterone treatment: a national cohort study on thrombosis risk. *Endocr Connect*. 2020;9(1):34–43.
4 Chang S, Just J, Skakkebæk A, et al. Testosterone replacement therapy in Klinefelter syndrome - follow-up study associating hemostasis and RNA expression. *J Clin Endocrinol Metab*. 2024;109(4):978–991.
5 Chang S, Larsen OH, Hvas AM, Skakkebaek A, Gravholt CH, Münster AB. Platelet aggregation in Klinefelter syndrome is not aggravated by testosterone replacement therapy: a longitudinal follow-up study. *Andrology*. 2023;11(3):456–463.
6 Host C, Bojesen A, Erlandsen M, et al. A placebo-controlled randomized study with testosterone in Klinefelter syndrome -

beneficial effects on body composition. *Endocr Connect*. 2019;8(9):1250–1261.
7 Gagliano-Jucá T, Basaria S. Testosterone replacement therapy and cardiovascular risk. *Nat Rev Cardiol*. 2019;16(9):555–574.
8 Lincoff AM, Bhasin S, Flevaris P, et al. Cardiovascular safety of testosterone-replacement therapy. *N Engl J Med*. 2023;389(2):107–117.
9 Chang S, Skakkebaek A, Davis SM, Gravholt CH. Morbidity in Klinefelter syndrome and the effect of testosterone treatment. *Am J Med Genet C Semin Med Genet*. 2020;184(2):344–355.
10 Pozza C, Sesti F, Tenuta M, et al. Testicular dysfunction in 47,XXY boys: when it all begins. A Semilongitudinal Study. *J Clin Endocrinol Metabol*. 2023;108(10):2486–2499.
11 Schmidt M, Schmidt SAJ, Adelborg K, et al. The Danish health care system and epidemiological research: from health care contacts to database records. *Clin Epidemiol*. 2019;11:563–591.
12 Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol*. 2015;7:449–490.
13 Schmidt M, Pedersen L, Sorensen HT. The Danish Civil registration system as a tool in epidemiology. *Eur J Epidemiol*. 2014;29(8):541–549.
14 Helweg-Larsen K. The Danish register of causes of death. *Scand J Publ Health*. 2011;39(7 Suppl):26–29.
15 Kildemoes HW, Sorensen HT, Hallas J. The Danish national prescription registry. *Scand J Publ Health*. 2011;39(7 Suppl):38–41.
16 Methodology WCCfDS. WHO ATC/DDD index. https://www.whocc.no/.
17 Heide-Jørgensen U, Adelborg K, Kahlert J, Sørensen HT, Pedersen L. Sampling strategies for selecting general population comparison cohorts. *Clin Epidemiol*. 2018;10:1325–1337.
18 Chuang-Stein C. Summarizing laboratory data with different reference ranges in multi-center clinical trials. *Drug Inf J*. 1992;26(1):77–84.
19 Bosco E, Hsueh L, McConeghy KW, Gravenstein S, Saade E. Major adverse cardiovascular event definitions used in observational analysis of administrative databases: a systematic review. *BMC Med Res Methodol*. 2021;21(1):241.
20 Zhao Y, Gardner EJ, Tuke MA, et al. Detection and characterization of male sex chromosome abnormalities in the UK Biobank study. *Genet Med*. 2022;24(9):1909–1919.
21 Berglund A, Viuff MH, Skakkebaek A, Chang S, Stochholm K, Gravholt CH. Changes in the cohort composition of turner syndrome and severe non-diagnosis of Klinefelter, 47,XXX and 47,XYY syndrome: a nationwide cohort study. *Orphanet J Rare Dis*. 2019;14(1):16.
22 Shores MM, Smith NL, Forsberg CW, Anawalt BD, Matsumoto AM. Testosterone treatment and mortality in men with low testosterone levels. *J Clin Endocrinol Metabol*. 2012;97(6):2050–2058.
23 Morgentaler A. Testosterone deficiency and cardiovascular mortality. *Asian J Androl*. 2015;17(1):26–31.
24 Hudson J, Cruickshank M, Quinton R, et al. Adverse cardiovascular events and mortality in men during testosterone treatment: an individual patient and aggregate data meta-analysis. *Lancet Healthy Longev*. 2022;3(6):e381–e393.
25 Kabilan A, Skakkebæk A, Chang S, Gravholt CH. Evaluation of the efficacy of transdermal and injection testosterone therapy in Klinefelter syndrome—a real-life study. *J Endocr Soc*. 2021;5(6):1–11.
26 Lopez DS, Mulla JS, El Haddad D, et al. Testosterone replacement therapy in relation with cardiovascular disease in cisgender women and transgender people. *J Clin Endocrinol Metabol*. 2023;108(12):e1515–e1523.
27 Rexrode KM, Madsen TE, Yu AYX, Carcel C, Lichtman JH, Miller EC. The impact of sex and gender on stroke. *Circ Res*. 2022;130(4):512–528.
28 Wu B, Lu J, Wei D, Wang C, Mao Z, Liu P. The association of cortisol, testosterone, and cortisol/testosterone ratio with stroke: a cross-sectional study. *J Stroke Cerebrovasc Dis*. 2024;33(9):107821.
29 Loo SY, Chen BY, Yu OHY, Azoulay L, Renoux C. Testosterone replacement therapy and the risk of stroke in men: a systematic review. *Maturitas*. 2017;106:31–37.
30 Pasquali D, Arcopinto M, Renzullo A, et al. Cardiovascular abnormalities in Klinefelter syndrome. *Int J Cardiol*. 2013;168(2):754–759.
31 Salzano A, D'Assante R, Lander M, et al. Hormonal replacement therapy in heart failure: focus on growth hormone and testosterone. *Heart Failure Clin*. 2019;15(3):377–391.
32 Luo S, Au Yeung SL, Zhao JV, Burgess S, Schooling CM. Association of genetically predicted testosterone with thromboembolism, heart failure, and myocardial infarction: mendelian randomisation study in UK Biobank. *BMJ*. 2019;364:l476.
33 Tisdale RL, Fan J, Calma J, et al. Predictors of incident heart failure diagnosis setting: insights from the veterans affairs healthcare system. *JACC Heart Fail*. 2023;11(3):347–358.