

Erythrocytosis in a large cohort of trans men using testosterone: a long-term follow-up study on prevalence, determinants and exposure years

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Disclosure summary

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Abstract

Context: Erythrocytosis is a known side effect of testosterone therapy that can increase the risk of thromboembolic events.

Objectives: To study the prevalence and determinants in the development of erythrocytosis in trans men using testosterone.

Design: A twenty year follow-up study in adult trans men who started testosterone therapy, and had monitoring of hematocrit at our center (n=1073).

Results: Erythrocytosis occurred in 11% (hematocrit>0.50 l/l), 3.7% (hematocrit>0.52 l/l) and 0.5% (hematocrit>0.54 l/l) of trans men. Tobacco use (OR 2.2, 95%CI 1.6-3.3), long-acting undecanoate injections (OR 2.9, 95%CI 1.7-5.0), age at initiation of hormone therapy (OR 5.9, 95%CI 2.8-12.3), BMI (OR 3.7, 95%CI 2.2-6.2) and pulmonary conditions associated with erythrocytosis and polycythemia vera (OR 2.5, 95%CI 1.4-4.4) were associated with hematocrit >0.50 l/l. In the first year of testosterone therapy hematocrit increased most: 0.39 l/l at baseline to 0.45 l/l after 1 year. Although there was only a slight continuation of this increase in the following 20 years, the probability of developing erythrocytosis still increased (10% after 1 year, 38% after 10 years).

Conclusion: Erythrocytosis occurs in trans men using testosterone. The largest increase in hematocrit was seen in the first year, but also after the first years there is a substantial number of people that present with hematocrit >0.50 l/l. A reasonable first step in the care for trans men with erythrocytosis while on testosterone is to advise them to quit smoking and to switch to a transdermal administration route and if BMI is high, to lose weight.

Keywords: erythrocytosis, hematocrit, trans men, hormone treatment, gender dysphoria, time-relation

Introduction

People diagnosed with gender dysphoria (trans people) experience distress due to an incongruence between a subject's gender identity and the sex assigned at birth.¹ To reduce this distress, gender affirmative hormone therapy can be given to induce physical and mental changes towards the experienced sex.² In trans men, who are assigned as female at birth, testosterone therapy induces virilization.^{3,4} This includes deepening of the voice, an increase in facial and body hair, a more masculine body composition and psychological and sexual changes.⁵⁻⁷ Also, hematological and biochemical changes occur due to the treatment, and therefore regular laboratory monitoring is recommended.³

It is known from studies in hypogonadal cis men (birth-assigned male, male gender identity) that testosterone has a dose-dependent stimulating effect on erythropoiesis.⁸ Hence, one of the changes due to testosterone therapy is the rise in hematocrit levels. This results in secondary erythrocytosis, a potentially serious adverse effect of testosterone therapy as it is associated with an increased risk of thromboembolic events by an increase in blood viscosity.^{9,10} Hematocrit is one of the major determinants of blood viscosity with an increased risk of coronary heart disease (relative risk of 1.16)¹¹ and unprovoked venous thromboembolisms (2.4-fold increased risk in the 20th highest percentile compared to lowest 40th percentile in men).^{12,13}

Erythrocytosis is previously described in both cis men and trans men on testosterone therapy.¹⁴ Prevalence of erythrocytosis (hematocrit >0.50) in testosterone treated hypogonadal cis men is described between 5% and 66%. The largest increase was seen in the first year after initiation of testosterone therapy.¹⁴⁻¹⁷ Prevalence of erythrocytosis (hematocrit >0.50) in testosterone treated trans men is 11.5% in previous literature.¹⁸ Studies in testosterone treated hypogonadal cis men described highest risk of erythrocytosis with injectable testosterone therapy (both short acting esters and long acting undecanoate) when compared to transdermal administration. Studies in testosterone treated trans men also described the highest risk of erythrocytosis (hematocrit >0.50)

with injectable testosterone (both short acting esters and long acting undecanoate) compared to gel and the biggest increase in the first three months to one year (follow-up 1-5 years in these studies).¹⁹⁻²¹

Current guidelines on the management of secondary erythrocytosis in trans men on testosterone therapy refer to the guideline for testosterone treated hypogonadal cis men and consider hematocrit levels >0.50 l/l as potentially dangerous as it has a very high risk of adverse outcome.^{3,22} This guideline advises a cessation of testosterone therapy, a dose reduction, or therapeutic phlebotomy when hematocrit levels exceed 0.54 l/l to reduce the risk of adverse events. For levels between 0.50 - 0.54 l/l no clear advice is given in these guidelines. Question remains whether these guidelines are applicable to trans men as the duration of testosterone therapy is much longer in trans men and hormone treatment can often not be discontinued.

In light of these consideration the aim of this study is twofold:

1. To study the prevalence and determinants in the development of erythrocytosis in trans men on testosterone therapy.
2. To study the time relation between duration of testosterone therapy and hematocrit levels over the course of 20 years.

Based on the results of this study we discuss how these findings affects current practices regarding erythrocytosis in trans men

Methods

Study design and population

This study is part of the Amsterdam Cohort of Gender Dysphoria study (ACOG). This is a large cohort study containing medical data of 6793 people who visited the Center of Expertise on Gender Dysphoria of the Amsterdam UMC, Vrije Universiteit, Amsterdam, between 1972 and 2015.²³ Laboratory measurements of these people between 2004 and 2018 were available for analysis.

For this current study, trans men who started testosterone, and had at least one follow-up visit after starting testosterone therapy were included. People were excluded if testosterone therapy was discontinued during follow up, laboratory results or starting date of testosterone therapy were missing.

Information on medication use, medical history, duration of hormonal treatment, testosterone administration route, body mass index (BMI, calculated as the weight in kilograms divided by the square length in meters), tobacco use, alcohol use, and laboratory results of testosterone levels and hematocrit was obtained retrospectively from medical files. Chronic pulmonary diseases (e.g. asthma, COPD, chronic bronchitis), sleep apnea, and polycythemia vera were considered as a medical history predisposing to the development of erythrocytosis.

Hormonal treatment

In our Center of Expertise on Gender Dysphoria, testosterone was most commonly started as transdermal gel, intramuscular injections (both short acting esters and long acting undecanoate), or, in the past, oral administration. The starting dose of transdermal testosterone gel was 50 to 60 milligram (mg). Intramuscular administration as a 250mg short-acting testosterone ester mix was injected every two to four weeks. Long-acting intramuscular testosterone undecanoate injections were dosed 1000mg every 10 to 14 weeks. If used, oral administration was most frequently prescribed as testosterone undecanoate capsules in a dose ranging from 40 to 360mg daily. Dosages and administration intervals were adjusted on an individual basis by monitoring testosterone levels, aiming levels between 10 and 30 nmol/L for transdermal gel and levels of 10-15 nmol/L prior to injection for injectable administration routes.

Laboratory measures

Blood samples of the included trans men were available from January, 2004 until December, 2018. Total testosterone levels and hematocrit levels were obtained by venous blood samples drawn when people visited the endocrine outpatient clinic. The first year after initiation of testosterone therapy, trans men visited the center every three to six months. Thereafter the monitoring frequency was reduced to yearly, every two years, or more frequently if indicated. Therefore, the number of measurements per person varied.

Until January 2013, total testosterone levels were measured using a Coat-A-Count radioimmunoassay (RIA) (Siemens, USA) with a lower limit of quantitation (LOQ) of 1 nanomol per liter (nmol/L) and an inter-assay coefficient of variation (CV) of 7 to 20%. Thereafter, the Architect competitive immunoassay (Abbott, USA) was used to measure testosterone levels with a LOQ of 0.1 nmol/L and an inter-assay CV of 6 to 10%. Coat-A-Count RIA values measured before 2013 were converted to Architect values using two formulas. For testosterone levels <8 nmol/L, the formula Architect = $1.1 \times \text{RIA} + 0.2$ was used. For testosterone levels >8 nmol/L, the formula Architect = $1.34 \times \text{RIA} - 1.65$ was used.

Hematocrit was measured using a CELL-DYN Sapphire (Abbott, USA), with an inter-assay CV of 1.1%. In our center, the upper limit of normal of hematocrit levels for cis men is 0.50 l/l.

Determinants

Clinical and laboratory parameters were studied as possible determinants of development of erythrocytosis. Studied determinants were: route of testosterone administration, age at initiation of testosterone therapy, duration of testosterone therapy, BMI, tobacco use, testosterone levels and a medical history predisposing to develop erythrocytosis including COPD, asthma, chronic bronchitis or polycythemia vera.

Statistical analysis

The results are presented as numbers, percentages, means with standard deviations or medians with interquartile ranges. The prevalence of erythrocytosis is presented as the percentage of trans men with erythrocytosis. Erythrocytosis was defined as a hematocrit level exceeding the upper limit of the physiological range for men (>0.50 l/l twice) conform the guidelines for the hormonal treatment of trans people.³ Also the prevalence of hematocrit levels of >0.52 l/l twice and >0.54 l/l twice were studied to give more insight into the prevalence of these high levels. For analysis of the determinants of erythrocytosis a hematocrit level of >0.50 l/l on a single measurement was used. Over the course of 20 years trans men could switch route of testosterone administration. Hence, hematocrit levels were linked to the route of testosterone administration prior to the time of laboratory measurement.

To study the effect of clinical and laboratory parameters on the risk of erythrocytosis development (hematocrit >0.50), logistic mixed model analyses were performed, with erythrocytosis

as outcome variable and tobacco use, age at initiation of testosterone therapy, BMI, testosterone levels, testosterone administration route, and predisposing medical history as determinants. Tobacco use was categorized to yes (for current en former smoking) or no. Age at initiation of testosterone therapy was categorized as <18, 18-30, 30-40, 40-50 and ≥50 years. BMI was categorized as underweight (<18.5 kg/m²), normal weight (18.5-25 kg/m²), overweight (25-30 kg/m²) and obese (≥30 kg/m²). Testosterone levels were categorized as under aimed range (0-10 nmol/l), within aimed range (10-20 nmol/l and 20-30 nmol/l), and above aimed range (>30 nmol/l). The results are presented as odds ratio's (OR) with 95% confidence intervals (95% CI). The analyses were adjusted for testosterone levels, testosterone administration route, predisposing medical history, tobacco use, age at initiation of testosterone therapy and BMI by analyzing all these determinants in one model. Linear mixed-model analyses with measurements clustered within participants were performed to analyze the change in hematocrit levels over time.

Failure analysis for the development of hematocrit levels >0.50 l/l were performed on all trans men of which baseline hematocrit measurement were available (testosterone therapy starting date after 2004)

Data analyses were performed using STATA Statistical Software, version 15.1 (Statacorp, College Station, Texas, USA).

Results

In this analysis 1073 trans men were included. The ACOG study database consists of 2398 trans men, 431 trans men were excluded due to missing starting date of hormone therapy and 894 trans men were excluded due to no available laboratory results. Baseline characteristics of the study population are shown in Table 1. Of all hematocrit measurements, 1087 were during the use of testosterone gel, 1826 under the use of short-acting injections, 345 under long-acting undecanoate injections, 150 under oral testosterone administrations and in 2120 measurements testosterone administration route was unknown.

Prevalence

A single measurement of hematocrit >0.50 l/l occurred in 24.0% of trans men, 11.1% had this measurement twice. Hematocrit levels of >0.52 l/l were seen in 7.6% of trans men in this cohort, 3.7% had this twice. Hematocrit levels of >0.54 l/l were seen in 2.2% once and 0.5% twice.

Determinants

Table 2 shows the results of different determinants on the occurrence of a hematocrit of >0.50 l/l and >0.52 l/l. Compared to testosterone gel, the use of long-acting undecanoate injection showed highest odds for hematocrit levels >0.50 (OR 2.9, 95% CI 1.7-5.0). Short-acting ester injections (OR 1.1, 95% CI 0.7-1.6) and oral administration (OR 0.4, 95% CI 0.1-1.8) had similar odds for hematocrit levels >0.50 l/l compared to testosterone gel.

Tobacco use showed higher odds for hematocrit levels of >0.50 l/l and >0.52 l/l compared to no tobacco use. Higher age at initiation of testosterone therapy, a high BMI, and a predisposing medical history were also associated with high hematocrit levels. Table 2 also shows adjusted odds ratios for all determinants. After adjustment the odds of high hematocrit levels in the age range between 18-30 and 30-40 were much lower (OR 1.5, 95% CI 0.8-2.8 and OR 1.9, 95% CI 0.9-3.9). Lower than aimed testosterone levels had a low odds of hematocrit levels >0.50 l/l and >0.52 l/l compared to aimed levels. Higher than aimed levels of testosterone had similar and slightly higher odds of high hematocrit compared to aimed levels.

Time relation:

The time relation between the initiation of testosterone therapy and hematocrit is depicted in Figure 1. The largest increase in hematocrit level was seen in the first year after initiation of testosterone therapy. A slight increase was seen until twenty years after initiation of testosterone therapy. Figure

2 shows the probability of developing hematocrit levels >0.50 and >0.52 . In this subgroup analysis 776 trans men were included. After 1 year the cumulative risk of developing hematocrit levels >0.50 was 8%, after 10 years 38% and at the end of follow up, after 14 years, the cumulative incidence was 50%. For hematocrit levels of >0.52 the cumulative risk after 1 year was 4% and 16% after 10 years.

Discussion

Eleven percent of trans men using testosterone showed erythrocytosis (>2 measurements of hematocrit >0.50 l/l) during 20 years of follow-up. Determinants associated with hematocrit levels of >0.50 l/l besides testosterone therapy were smoking, higher age at initiation of testosterone therapy, higher BMI and a predisposing medical history. Higher testosterone levels per se were not associated with an increased odds of hematocrit >0.50 l/l.

The prevalence of erythrocytosis is similar to previous research in trans men.¹⁸ In testosterone treated hypogonadal cis men the prevalence ranges from 5-66% depending on the cut-off values (most studies used a cut of value of >0.50 l/l, one study >0.52 l/l) and study design.¹⁴ Jones et al reported a 5% increase in hematocrit levels for long-acting undecanoate intramuscular injections and a 6.4% increase in hematocrit for short acting ester intramuscular injections.¹⁷ In a study of Pastuzak et al. intramuscular injections had a very high prevalence of 66% of erythrocytosis (hematocrit levels >0.50).¹⁶ However, baseline hematocrit levels in this group were much higher. Besides the dosage of testosterone was higher and the mean age was much higher than in this study.

In this cohort long-acting undecanoate intramuscular injections showed the highest odds for hematocrit levels >0.50 l/l. Other studies reported a similar increase with long-acting intramuscular injections.^{18,21,24,25} However, some of these studies reported a higher or similar increase in hematocrit levels with short-acting intramuscular injections. All of these studies had smaller sample size and a shorter follow-up period. In line with previous studies, transdermal testosterone therapy showed least increase in hematocrit levels. Moreover, as described earlier, tobacco use, high BMI, a

medical history predisposing for erythrocytosis and higher age at initiation of testosterone therapy showed increased risk of high hematocrit levels.²⁶⁻³⁰ After adjustment for BMI and smoking the odds of high hematocrit levels for the age between 18-40 was much lower. Hence, higher weight and smoking partially caused increased odds in this age range. Previous research showed a dose dependent stimulating effect of testosterone on the erythropoiesis.³¹ In our study this dose dependent effect was seen when comparing lower than aimed levels of testosterone (<10 nmol/l) with aimed levels (10-20 and 20-30 nmol/l), our data also showed a slight increase in the odds of getting hematocrit levels >0.50 with increased testosterone levels. It is possible that dose-reduction has a lowering effect on hematocrit levels. Furthermore the aimed testosterone levels of 10-30 nmol/l for transdermal administration and 10-15 nmol/l prior to injection could give higher levels compared to levels in cis men (average 14-20 nmol/l in males in the age range 20-40).³² This could give an increased risk of high hematocrit levels but no additional benefit. However, it has to be noted that it is difficult to aim for the exact levels of the cis population due to the available testosterone administration types (especially with short acting esters as this gives a peak in testosterone).

Currently, the guidelines in transgender men³ for testosterone induced erythrocytosis are based on the guidelines on hypogonadal cis men from 2008.²² This guideline states that a hematocrit >0.50 l/l has a moderate-high risk of adverse outcome. If hematocrit levels exceed the level of >0.54 l/l testosterone therapy should be discontinued until hematocrit levels are normalized. However the new guideline for testosterone treated hypogonadal cis men from 2018 state that a hematocrit level of >0.48 l/l is associated with a moderate-high risk of adverse outcome.³³ The lowering of this upper limit is based on studies that show that older men have a higher risk of developing erythrocytosis.^{31,34} It is known from studies in general population that high hematocrit levels give a higher risk of cardiovascular events.¹¹ According to hematology guidelines (for people without polycythemia vera), the upper limit for hematocrit for cis males is defined as 0.52 l/l, for cis females this is 0.48 l/l.³⁵ Whether this upper limit for cis males is also applicable for trans men is not known.

It could be argued that the upper limit for cis females should be applied, as trans men are born with female genetics. This is a subject for further research, like the risk of thromboembolic events with different cut-off values. Furthermore, the difference in all these guidelines emphasizes the need for further research on this subject.

The results of this study show the largest increase in hematocrit in the first year after initiation of testosterone therapy, with a slight continuation of this increase up to 20 years. This is in line with previous literature and reflected in current guidelines.^{18,21,24,36} Our study confirmed that this is also the case in trans men. The probability of developing first-time erythrocytosis increases over the course of 14 years. It implicates that a person who is stable on testosterone therapy for years still can develop first-time erythrocytosis. Therefore, regular control of hematocrit seems warranted as long as people use testosterone.

Strengths and limitations

This is the first study in a large cohort of trans men on testosterone therapy with a follow up period up to twenty years. Because of this large sample size, statistical analysis with many determinants could be performed. Also, because of the long follow-up time, a clear time relation between initiation of testosterone therapy and hematocrit can be shown in this study.

A limitation of this study is that data are collected retrospectively. Because data were obtained from medical files and not via a study design this resulted in missing data. So, other possible confounders not present in our database could not be studied and often route of testosterone administration was unknown.

To reach acceptable group size for the analysis of the determinants of erythrocytosis upper limits of >0.50 l/l and >0.52 l/l for hematocrit were chosen. Current guidelines describe a hematocrit >0.50 l/l as a moderate-high risk of adverse outcome.³ If hematocrit levels exceed the level of >0.54

testosterone therapy should be discontinued until hematocrit levels are normalized. The group size of trans men with hematocrit levels >0.54 l/l was not sufficient to execute analysis on. Analysis on determinants were executed on hematocrit levels linked to testosterone administration route. Because there was a great variability in the amount of laboratory measurements per person it was not possible to analyze subsequent hematocrit levels >0.50 l/l. Therefore, these data give no insight in the duration of high hematocrit levels.

Recommendations for clinical practice

From this study a few recommendations for clinical practice can be formulated. Firstly, regular control of hematocrit seems warranted as long as people use testosterone. If hematocrit levels are between 0.50-0.54 l/l, reasonable first steps to prevent further increase would be:

- Consider switching injectable testosterone therapy to transdermal administration. We cannot exclude that dose-reduction has the same effect.
- If BMI levels are >25 the people should be advised to lose weight to reach a BMI in the healthy range (18.5-25)
- People should strongly be advised to stop smoking.
- Treatment optimization for chronic lung disease or sleep apnea should be pursued.

Declaration of interest

The authors declare to have no conflict of interest.

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Data availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Table 1 Baseline characteristics:

Characteristic	Total (n=1073)
Age at start of HT (median (IQR))	22.5 (18.4-31.8)
BMI at start of HT (mean (sd))	24.5 (+/-5.5)
Tobacco use (% yes)	38%
Conditions associated with erythrocytosis (% yes)	8.6%
Pulmonary condition associated with erythrocytosis*	7.6%
Obstructive sleep apnea	0.7%
Polycythemia vera	0.4%

IQR: Interquartile range

BMI: Body Mass Index

Sd: standard deviation

* Asthma, Chronic Obstructive Pulmonary Disease, chronic bronchitis

Table 2: Multivariable analysis: chance of high hematocrit for different determinants

	Hematocrit >0.50		Hematocrit >0.52	
	I/I (Crude OR(95%CI))	(Adjusted OR(95%CI))^	I/I (Crude OR(95%CI))	(Adjusted OR(95%CI))^
Tobacco use	2.2 (1.6-3.3)	2.0 (1.3-3.0)	3.2 (1.7-6.2)	2.7 (1.3-5.5)
Positive medical history*	2.5 (1.4-4.4)	2.0(1.1-3.8)	5.3 (2.3-12.3)	4.6 (1.9-11.2)
Age at initiation				
<18	ref	ref	ref	ref
18-30	2.5 (1.4-4.4)	1.5(0.8-2.9)	3.2 (1.0-9.9)	1.9 (0.5-7.0)
30-40	3.9 (2.0-7.5)	1.9 (0.9-4.0)	5.7 (1.7-19.4)	2.7 (0.6-11.3)
40-50	5.9 (2.8-12.3)	3.1 (1.4-7.2)	10.3 (2.8-38.8)	4.9 (1.1-22.5)
≥50	2.6 (0.8-8.6)	1.2 (0.3-4.5)	2.9 (0.3-26.4)	1.1 (0.1-13.5)
BMI				
≤18.5	0.4 (0.2-1.2)	0.6 (0.2-1.9)	-	-
18.5-25	ref	ref	ref	ref
25-30	3.4 (2.1-5.5)	3.0 (1.8-5.1)	5.3 (2.3-12.3)	4.3 (1.7-10.8)
≥30	3.7 (2.2-6.2)	3.1 (1.7-5.6)	6.1 (2.5-14.9)	4.6 (1.7-12.3)
Route of testosterone				
T gel	ref	ref	ref	ref
Short-acting im	1.1 (0.7-1.6)	1.1 (0.7-1.7)	1.5 (0.7-3.4)	1.5 (0.6-3.7)
Long-acting im	2.9 (1.7-5.0)	3.1 (1.7-5.6)	1.0 (0.3-3.4)	1.3 (0.4-5.0)
Oral T	0.6 (0.2-1.6)	1.3 (0.4-3.7)	0.2 (0.1-2.4)	0.5 (0.1-5.6)
Unknown	1.2 (0.8-1.7)	1.9 (1.3-2.8)	1.8 (0.9-3.8)	2.9 (1.3-6.6)
Testosterone levels				
0-10 nmol/l	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.2 (0.1-0.4)	0.1 (0.1-0.4)
10-20 nmol/l	ref	ref	ref	ref
20-30 nmol/l	1.3 (0.9-1.8)	1.3 (0.9-1.8)	1.3 (0.7-2.5)	1.4 (0.7-2.7)
>30 nmol/l	1.4 (1.0-1.9)	1.5 (1.1-1.8)	1.4 (0.7-2.5)	1.6 (0.8-3.0)

* COPD/Asthma, Sleep apnea, chronic bronchitis, polycythemia vera.

^Adjusted for all other determinants

T: testosterone

nmol: nanomol

im: intramuscular

-: no data available

Time relation of hematocrit and treatment years of testosterone

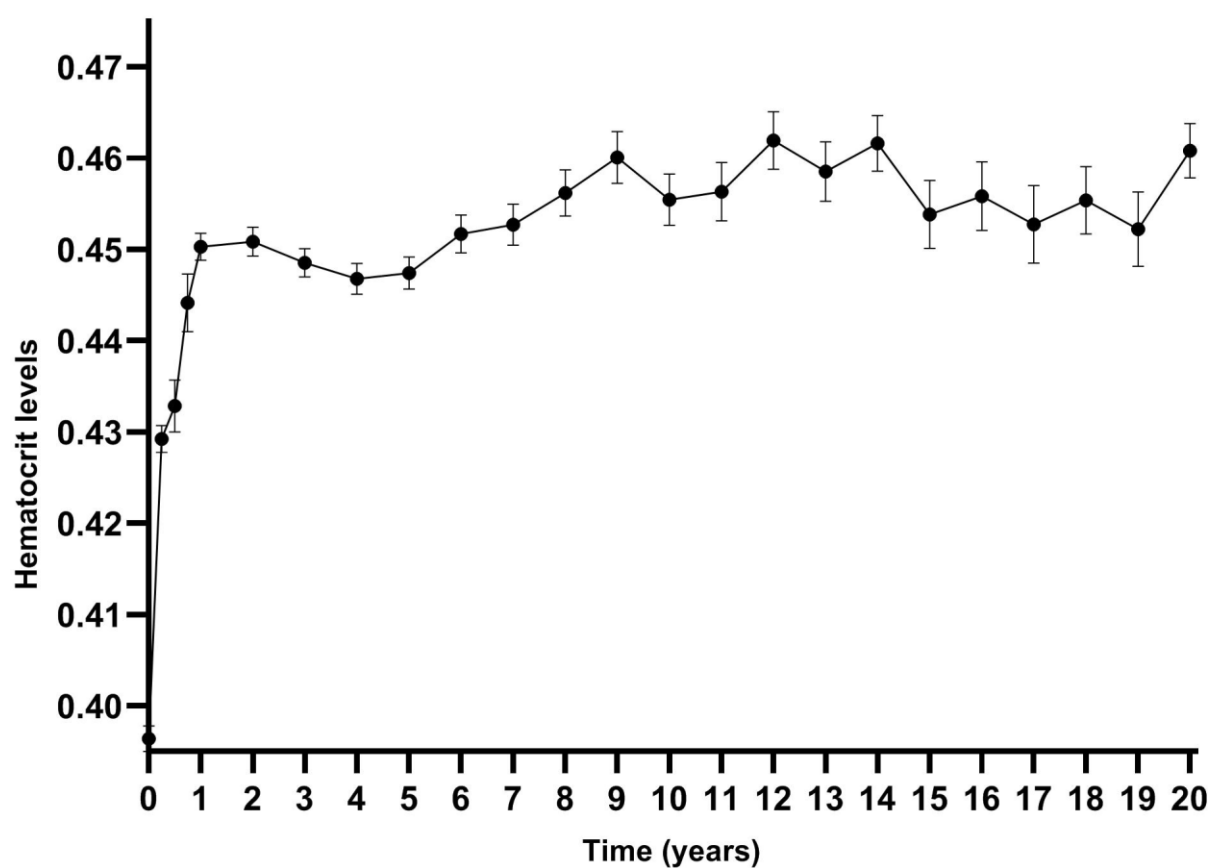


Figure 1: Time relation between hematocrit and duration of testosterone therapy (determined by mixed model analysis; shown as mean with standard error of the mean)

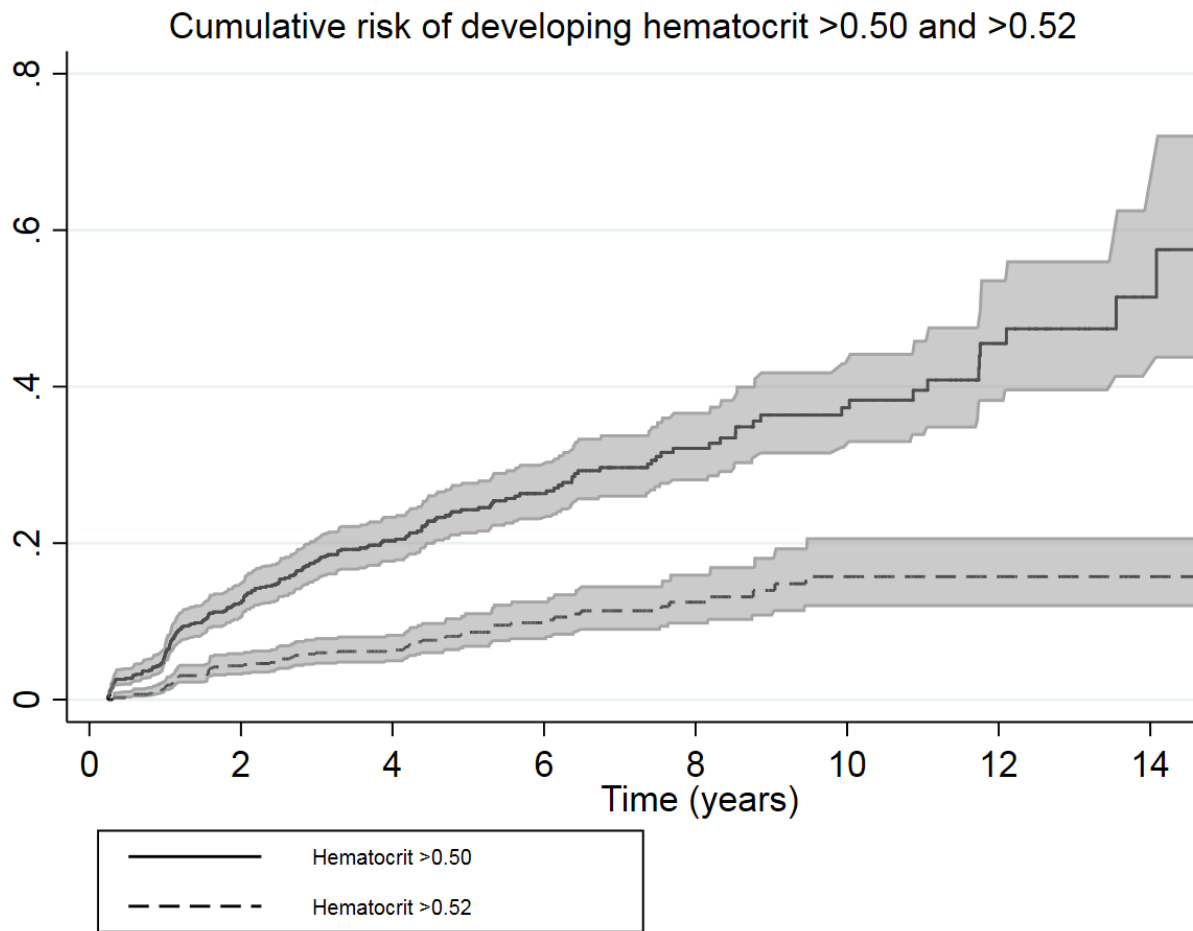


Figure 2: Nelson-Aalen curve for the cumulative risk of developing hematocrit levels >0.50 and >0.52.

*restricted to participants starting testosterone therapy after 2004 (baseline hematocrit levels were present)