

## CLINICAL STUDY

# Trough serum testosterone predicts the development of polycythemia in hypogonadal men treated for up to 21 years with subcutaneous testosterone pellets

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## Abstract

**Objectives:** Testosterone formulations that have more steady-state pharmacokinetics, such as subcutaneously implanted testosterone pellets, may cause less erythrocytosis than i.m. injections of shorter acting androgen esters. We, therefore, sought to define the prevalence, predictors, and proximate basis (role of erythropoietin) for polycythemia (hematocrit >0.50) in hypogonadal men receiving testosterone implants long term.

**Design:** A cross-sectional study was conducted in an academic andrology center with a longitudinal subgroup analysis.

**Patients:** A total of 158 hypogonadal men aged 14–84 years (mean age 46.7 years) treated on average for 8 years (range 0–21 years).

**Measurements:** Trough blood testosterone and hematocrit. Serial serum erythropoietin concentrations were measured in 16 volunteers.

**Results:** Positive univariate associations between polycythemia (hematocrit >0.50) and log(testosterone) (odds ratio (OR) 24.7, 95% confidence interval (CI): 4.3, 141.2,  $P < 0.01$ ) and age (OR 1.1, 95% CI: 1.0, 1.1,  $P = 0.03$ ) and a borderline relationship with current smoking (OR 4.2, 95% CI: 0.9, 20.0,  $P = 0.08$ ) were unveiled. A sensitivity analysis using alternate definitions of polycythemia was performed to capture all potential covariants. Multivariate regression analysis incorporating all potential covariants disclosed the independent OR of developing polycythemia (after adjusting for smoking and age) for log(testosterone) to be 15.0 (95% CI: 2.5, 90.8). Duration of testosterone therapy did not alter the risk of polycythemia. A direct relationship between testosterone and erythropoietin was observed ( $P = 0.05$ ).

**Conclusions:** Higher trough serum testosterone concentrations but not duration of treatment predict the development of polycythemia in men receiving long-acting depot testosterone treatment.

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## Introduction

Secondary polycythemia can occur after testosterone therapy, although the risk varies depending on the actual formulation. Randomized studies in hypogonadal men show that more erythrocytosis is induced with i.m. injections of 200 mg testosterone enanthate every 2 weeks compared with 5 mg testosterone patches applied transdermally daily (1) or with 100 mg compared with 50 mg of transdermal testosterone (2). On the other hand, i.m. injections of 250 mg testosterone enanthate every 3 weeks, oral testosterone undecanoate 160 mg/day, or subcutaneous implantation of 1200 mg crystallized testosterone are equivalent (3). As a unifying hypothesis, these data suggest that both the dose and pharmacokinetics of

specific testosterone formulations (rather than the actual route of administration) are important because both influence the relative amount of time blood testosterone concentrations remain in the supraphysiological range. Indeed, others have postulated that the increased rates of polycythemia with short-acting testosterone esters are due to supraphysiological levels of testosterone (1, 4, 5). If true, longer acting testosterone preparations such as testosterone implants and i.m. injections of testosterone undecanoate, which do not cause prolonged supraphysiological testosterone levels when administered at appropriate doses (6), may therefore result in a lesser degree of erythrocytosis. In support of this hypothesis, direct relationships between testosterone and hematocrit have already been demonstrated (2, 3, 7).

A number of other biological factors modulate the relationship between testosterone and hematocrit. Of these, age (7), smoking (8, 9), and obesity (10) are the most important factors. Increasing age is associated with a well-defined decline in systemic testosterone exposure (11, 12) and a less well-defined decline in hematocrit in very old age (13, 14). Current cigarette smoking is associated with both increased testosterone concentrations (9, 11, 15) and polycythemia (8). Finally, body mass index (BMI) is inversely related to blood testosterone concentrations (11, 12) and confounds measurement of red cell mass (16).

Although these biological factors modulate the relationship between testosterone and erythrocytosis, the exact mechanism or mechanisms by which this occurs requires further evaluation. Erythropoietin is the key regulator of erythrocytosis and therefore a likely candidate. However, the currently available data examining its role are contradictory (7, 17–20). Whether these contradictory findings arise from differing study populations, study design, duration of therapy, or pharmacokinetics of testosterone administration are unknown. Indeed, the three available randomized studies ceased existing testosterone therapy for only 4–6 (1, 2) or 12 weeks prior (3), which may not have been sufficient, given that a single hematopoietic cycle requires 90 days to complete. Furthermore, only one large long-term study of androgen therapy using longer acting preparations is currently available (10), and there are no larger studies of longer duration examining subcutaneously implanted testosterone pellets.

For these reasons, our aim was to define the prevalence, predictors, and potential mediators of polycythemia in androgen-deficient men being treated long term with subcutaneous testosterone pellets. Such information would be useful in formulating surveillance strategies for long-term testosterone replacement with longer acting preparations as well as to inform understanding of the physiological actions of testosterone.

## Materials and methods

One hundred and fifty-eight men with established hypogonadism due to hypothalamo-pituitary or testicular disease (excluding andropause) were treated with subcutaneously implanted testosterone pellets at the Department of Andrology (Concord Hospital, Sydney, Australia) from March to November 2006. Men received testosterone pellet therapy primarily based on patient preference and had easy access to alternative forms of testosterone therapy if desired. A cross-sectional analysis was undertaken to define the characteristics of these men in order to investigate the effects of testosterone pellet therapy on hematocrit levels. Ethical approval was provided by the Sydney

South West Area Health Service Human Research Ethics Committee (Concord Hospital).

Blood was collected immediately prior to implantation of testosterone for later measurement of reproductive hormones (total testosterone and sex hormone-binding globulin, SHBG) and hematocrit. Anthropometric data (including height and weight) as well as demographic data (including age and smoking status) were collected during each visit.

Mean corpuscular volume (MCV) and red cell count (RCC) were measured by Cell-Dyn 4000 (Abbot Diagnostics) from which the hematocrit was calculated using the formula:  $MCV \times RCC / 100$ . The coefficient of variation (CV) of this assay was 1.2%. Hematocrit exceeding 0.50 was defined as polycythemia in accordance with our laboratory reference range, as there is no universally accepted definition of polycythemia in investigating androgen-induced erythrocytosis. However, other thresholds were also evaluated in a sensitivity analysis. Trough testosterone assays were performed using the ImmLite 2000 (DPC, Alameda, CA, USA). The inter-assay CV was 10.5% and the intra-assay CV was 8.7% for testosterone. Serum erythropoietin assays were performed on a Beckman Coulter UniCel 800 (Beckman Coulter, Fullerton, CA, USA). The inter-assay CV of this assay was 4.2% and the intra-assay CV was 3.3%.

In the longitudinal study, 16 men volunteered to allow blood to be collected serially (generally monthly) to explore the relationship between testosterone and erythropoietin. In these samples, erythropoietin and reproductive hormones were also measured.

## Statistical analysis

Age, trough testosterone concentrations, SHBG, BMI, height and weight, smoking status, diagnosis, and duration of treatment were considered to be possible predictors of polycythemia. The duration of treatment was calculated based on an average 6-month duration between implantation of testosterone pellets (6). Smoking and primary diagnosis were modeled as categorical variables. Univariate analyses were conducted on the covariates listed above.

Logistic regression was used to assess factors that alter the odds of polycythemia (thereby modeling hematocrit categorically). Multivariate models were built by forward stepwise logistic regression and confirmed by best subset analysis. A sensitivity analysis was also performed using different cutpoints for the definition of polycythemia to ensure that all potential covariates that might modify the relationship between testosterone and hematocrit were captured.

In order to confirm the results of logistic regression, univariate and multivariate linear regressions were then performed analogously to define continuous relationships of study variables with hematocrit.

**Table 1** Baseline characteristics of 158 men in cross-sectional analysis.

| Variable                             | Mean $\pm$ s.e.m. or n (%)                              |
|--------------------------------------|---|
| Age (years)                          | 47 $\pm$ 15<br>Median age 47 years<br>Range 14–84 years |
| Primary diagnosis                    |   |
| Primary hypogonadism                 | 60 (38%)  |
| Secondary hypogonadism               | 98 (62%)  |
| Treatment duration (years)           | 8 $\pm$ 5   |
| Smoking status                       |   |
| Current smokers                      | 23 (15%)  |
| Ex-smokers or never smokers          | 115 (73%)   |
| Unknown status                       | 20 (13%)  |
| Body mass index (kg/m <sup>2</sup> ) | 27.0 $\pm$ 5.3  |
| Weight (kg)                          | 85.7 $\pm$ 19.3   |
| Height (cm)                          | 177 $\pm$ 8   |
| Hematocrit                           | 0.44 $\pm$ 0.042  |
| Testosterone (nmol/l)                | 11.8 $\pm$ 7.1  |
| SHBG (nmol/l)                        | 27.5 $\pm$ 14.2   |

In the longitudinal substudy, data were analyzed using the mean or mean change of repeated measurements. Nonparametric Spearman correlation statistics, which are robust to outliers, were calculated to define the association between blood testosterone with hematocrit and erythropoietin.

Data are expressed as mean  $\pm$  s.e.m. For categorical variables, the number in each group and the percentage of the total are given. Two-tailed *P* values of <0.05 were considered statistically significant. Analyses were executed with proc logistic, proc reg, and proc corr using SAS version 9.1 (SAS Institute, Cary, NC, USA).

## Results

Baseline characteristics of the 158 men in the cross-sectional analysis are shown in Table 1. Eight of 158 men (5.1%) developed polycythemia (hematocrit exceeding 0.50 in accordance with our laboratory reference range). No cardiac or thromboembolic events occurred in those with secondary polycythemia.

**Table 2** Univariate odds for polycythemia.

| Variable                              | Hct > 0.49                   | Hct > 0.50                   | Hct > 0.51                   |
|---------------------------------------|------------------------------|------------------------------|------------------------------|
| Testosterone (nmol/l ln)              | 10.1 (2.5–40.6)*             | 24.7 (4.3–141.2)*            | 28.2 (4.4–182.1)*            |
| Age (years)                           | 1.1 (1.0–1.1)*, <sup>a</sup> | 1.1 (1.0–1.1)*, <sup>a</sup> | 1.1 (1.0–1.1)*, <sup>a</sup> |
| Current smoker versus nonsmoker       | 2.7 (0.6–11.8)               | 4.2 (0.9–20.0)               | 5.6 (1.1–29.7)*              |
| SHBG (nmol/l ln)                      | 1.0 (1.0–1.1)                | 1.0 (1.0–1.1)                | 1.0 (1.0–1.1)                |
| Body mass index (kg/m <sup>2</sup> )  | 1.1 (1.0–1.2)                | 1.1 (0.9–1.2)                | 1.1 (1.0–1.3)                |
| Height (m)                            | 1.0 (1.0–1.1)                | 1.0 (0.9–1.1)                | 1.0 (0.9–1.2)                |
| Weight (kg)                           | 1.0 (1.0–1.1)                | 1.0 (1.0–1.1)                | 1.0 (1.0–1.1)                |
| Treatment duration (years)            | 1.0 (1.0–1.1)                | 1.0 (1.0–1.1)                | 1.0 (0.9–1.1)                |
| Secondary versus primary hypogonadism | 0.7 (0.2–2.8)                | 0.5 (0.1–2.7)                | 0.3 (0.0–2.2)                |

The odds (point estimate and 95% confidence interval) of polycythemia is shown for biochemical (SHBG, testosterone) and clinical (height, weight, body mass index, smoking status, treatment duration, diagnosis, and age) factors using three different definitions of polycythemia: hematocrit > 0.49 (left), 0.50 (middle), and 0.51 (right). Statistically significant relationships are denoted with an asterisk.

<sup>a</sup>The lower 95% limit is at least 1.005.

## Logistic regression

Univariate regression consistently revealed an increased odds of polycythemia with higher trough blood testosterone concentrations, using the three cutpoints (hematocrit in excess of 0.49, 0.50, and 0.51) chosen for the sensitivity analysis (Table 2). Trough blood testosterone concentrations exhibited both the highest odds for polycythemia and the greatest significance amongst all covariables. Age was also a consistent univariate predictor of polycythemia (*P* < 0.03), whereas smoking history (current versus not) was a significant risk factor only for marked polycythemia (*P* = 0.04). Duration of treatment was not a risk factor for the development of polycythemia in any model when analyzed as a continuous variable, or dichotomized according to whether treatment duration was more than 1 year, or not (data not shown). Similarly, BMI did not increase the odds of polycythemia when analyzed as a continuous variable, or dichotomized according to whether obesity (BMI > 30 kg/m<sup>2</sup>) was present or not (data not shown).

Multivariate analyses were performed to assess the independent odds of polycythemia after adjusting for significant factors identified from the univariate analyses (Table 3). Trough testosterone concentrations remained a highly statistically significant predictor of polycythemia in all models (*P* < 0.001, for each) after adjustment for presumptive factors such as age or smoking. Age (*P* = 0.02) and smoking (*P* = 0.03) were significant predictors, but only with cutpoints of 0.49 and 0.51 respectively. Other presumptive factors (including obesity) were not independently significant, when added to any combination of testosterone, age, and smoking (not shown).

## Linear regression

Univariate linear regression confirmed that increasing trough blood testosterone concentration and age were the only factors significantly related to increasing hematocrit (Fig. 1). Multivariate linear regression disclosed an independent relationship only with trough blood testosterone (Table 4).

**Table 3** Multivariate odds for polycythemia.

| Variable                                 | Hct >0.49                   | Hct >0.50                   | Hct >0.51         |
|--|-----------------------------|-----------------------------|-------------------|
| Testosterone (nmol/l ln)                 | 6.3 (1.4–27.9)*             | 15.0 (2.5–90.8)*            | 16.7 (2.3–120.2)* |
| Age (years)                              | 1.1 (1.0–1.1)* <sup>a</sup> | 1.1 (1.0–1.1)* <sup>a</sup> | 1.1 (1.0–1.2)     |
| Current smoker versus never or ex-smoker | 4.6 (0.8–26.6)              | 6.4 (0.9–42.8)              | 11.4 (1.3–100.1)* |

The independent odds (point estimate and 95% confidence interval) of polycythemia for testosterone, age, and smoking status using three different definitions of polycythemia: hematocrit >0.49 (left), 0.50 (middle), and 0.51 (right). \*denotes statistically significant relationships.

<sup>a</sup>The lower 95% limit is at least 1.005.

### Longitudinal analysis of effects of erythropoietin

Sixteen men underwent serial blood sampling for measurement of erythropoietin, testosterone, and hematocrit. These men were on average 49.8 years of age (range: 26.8–83.9 years) with a BMI of 28.9 kg/m<sup>2</sup> (22.6–39.3 kg/m<sup>2</sup>) and treated on average for 7 years (0–20 years). Four of the 16 men were current smokers.

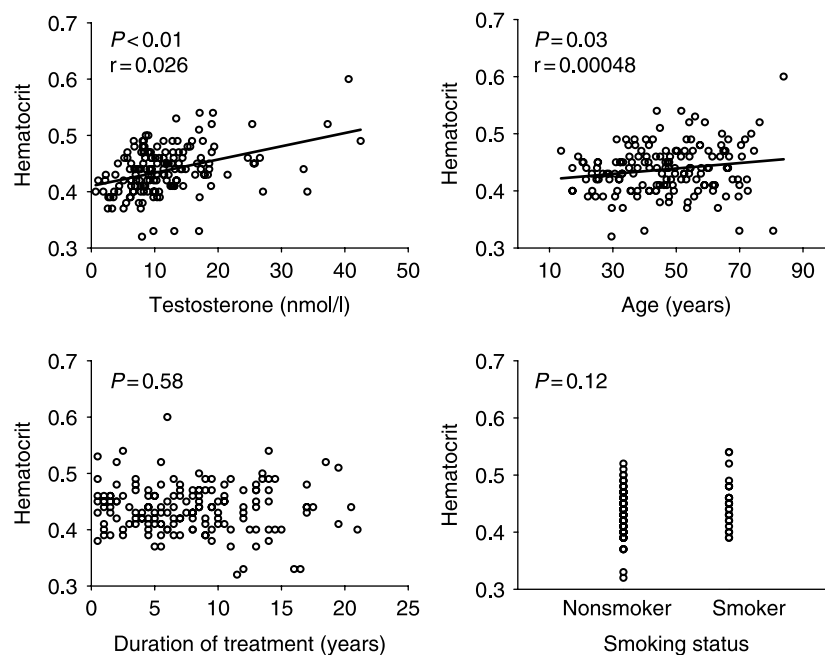
Mean testosterone and mean hematocrit ( $P < 0.01$ ), as well as the mean change in testosterone and mean change in hematocrit levels ( $P = 0.03$ ), were significantly related (Fig. 2). Mean serum erythropoietin levels were weakly associated with mean serum testosterone ( $P = 0.05$ ), but no association between the mean changes in serum testosterone and mean changes in erythropoietin levels were observed ( $P = 0.45$ ; Fig. 2).

### Discussion

The prevalence of secondary polycythemia was 5.1% in hypogonadal men treated with testosterone pellets for up to 21 years. Other studies have reported the

rates of secondary polycythemia that range from 2.5 to over 40% depending on the dose and testosterone formulation (1–3, 10, 21, 22). These individuals are still likely to be at increased risk for cardiovascular, cerebrovascular, and peripheral vascular mortality (23) or events that may occur due to augmented blood viscosity associated with polycythemia, despite testosterone-associated thromboembolic events not yet being reported in those receiving testosterone replacement therapy (4).

Higher trough blood testosterone concentration is the most important factor associated with higher hematocrit, consistent with other reports (2, 7, 10) and studies demonstrating a linear, dose–response relationship between testosterone and hematocrit (2, 3, 7). We show additionally through multivariate analysis that higher trough blood testosterone concentrations remain an important and significant risk for polycythemia, independently of a range of confounding clinical and biochemical factors. Together, these data suggest that the lowest efficacious dose required to achieve control of clinical features of androgen deficiency, e.g. hypogonadal symptoms, bone loss,



**Figure 1** Univariate linear regressions with hematocrit. The linear relationship between testosterone (left upper), age (right upper), treatment duration (left lower), and smoking status (right lower) and hematocrit is plotted for 158 men. Pearson's correlation coefficient and  $P$  values are shown. Regression lines are plotted for significant relationships.

**Table 4** Multivariate linear regressions with hematocrit.

| Variable                                 | Regression coefficient              |
|--|-------------------------------------|
| Testosterone (nmol/l ln)                 | 0.01937 (0.00851–0.03023)*          |
| Age (years)                              | 0.00039014 (–0.00003709–0.00081737) |
| Current smoker versus never or ex-smoker | 0.01565 (–0.00230–0.03360)          |

The independent regression coefficients of testosterone, age, and smoking status with hematocrit are shown. The point estimate is shown together with the lower and upper 95% confidence intervals. \*denotes statistically significant relationships ( $P < 0.05$ ).

fractures, should be prescribed in order to reduce the risk of polycythemia.

The duration of treatment was not associated with the development of secondary polycythemia. This novel finding has not been previously reported and may relate to the long average treatment duration of 8 years (range 0–21 years) experienced by our volunteers. In contrast, the next largest study treated men for  $< 2$  years on average and up to 9.5 years (10), and the available prospective randomized trials treated men for  $< 1$  year (1–3). These data provide some reassurance that prolonged treatment is not associated with adverse hematological outcomes, and is important given that hypogonadism requires lifelong therapy.

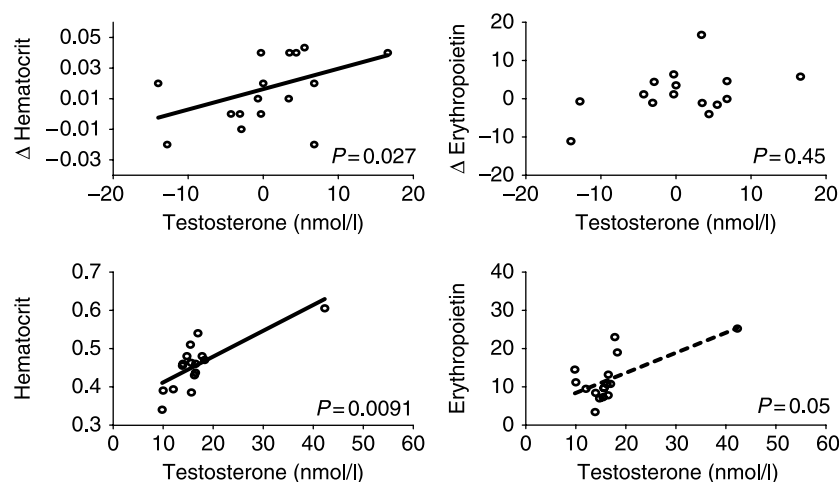
Increasing age was associated with increased odds for the development of polycythemia in androgen-deficient men treated with testosterone. Physiological experiments where normal men are rendered acutely androgen deficient, and then treated with graded and matched doses of testosterone confirm this age association (7).

Although testosterone therapy in androgen-deficient men clearly stimulates erythrocytosis and increases

the odds of polycythemia fourfold (24), the mechanism by which this occurs remains elusive. We show a direct relationship between testosterone and immunoreactive erythropoietin, which was of borderline significance ( $P = 0.05$ ), suggesting but not proving that erythropoietin concentrations may be a mechanism through which erythrocytosis is stimulated. Androgens also increase tissue sensitivity to erythropoietin in adults with renal failure (25), and this mechanism may also occur in androgen-deficient men treated with testosterone even in the absence of any change in bioactivity or immunoreactive erythropoietin. However, we did not assess erythropoietin sensitivity. On the other hand, physiological experiments in normal men do not show a relationship between testosterone dose and immunoreactive erythropoietin after 5 months of treatment (7). Whether these discrepant findings are due to study populations (acute versus chronic androgen deficiency), duration of therapy or other factors is unknown.

Strengths of this study include the large number of hypogonadal men studied and the long treatment duration, which are double the sample size and four times the treatment duration of the next largest study of a long-acting testosterone formulation (10). Caveats include the lack of determination of a number of factors which may influence erythropoiesis, including iron stores, folate, and renal function. However, these factors were not measured because they were considered likely to be normal in our population of otherwise well community-dwelling men.

We conclude that higher trough serum testosterone levels but not duration of treatment predict the development of polycythemia in men receiving long-acting depot testosterone treatment. These data inform clinical practice. Further investigation of the mechanisms by which polycythemia develops is required.



**Figure 2** Univariate relationships between testosterone, hematocrit, and erythropoietin. The univariate relationships between testosterone and hematocrit (bottom left), testosterone and erythropoietin (bottom right), and change in testosterone and change in hematocrit (upper left), and change in testosterone and change in erythropoietin (upper right) are shown for 16 men.  $P$  values from nonparametric Spearman correlation are shown. See text for further details.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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