26. Montano N, Ruscone TG, Porta A *et al.* Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation* 1994; 90: 1826–1831
27. Vita G, Bellinghieri G, Trusso A *et al.* Uremic autonomic neuropathy studied by spectral analysis of heart rate. *Kidney Int* 1999; 56: 232–237
28. Weinrauch LA, Kennedy FP, Gleason RE *et al.* Relationship between autonomic function and progression of renal disease in diabetic proteinuria-clinical correlations and implications for blood pressure control. *Am J Hypertens* 1998; 11: 302–308
29. Burger AJ, D'Elia JA, Weinrauch LA *et al.* Marked abnormalities in HRV are associated with progressive deterioration of renal function in Type 1 Diabetic patients with overt nephropathy. *Int J Cardiol* 2002; 86: 281–287
30. Brotman DJ, Bash LD, Qayyum R *et al.* Heart rate variability predicts ESRD and CKD-related hospitalization. *J Am Soc Nephrol* 2010; 21: 1560–1570

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Testosterone deficiency is a cause of anaemia and reduced responsiveness to erythropoiesis-stimulating agents in men with chronic kidney disease

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Abstract

Background. Hypogonadism or testosterone deficiency is a prevalent condition in men with chronic kidney disease (CKD). Testosterone stimulates erythropoiesis via production of haematopoietic growth factors and possible improvement of iron bioavailability. We hypothesized that testosterone deficiency predisposes to anaemia and reduced responsiveness to erythropoiesis-stimulating agents (ESAs) in CKD men.

Materials and methods. We studied associations between endogenous testosterone and haemoglobin in 239 ESA-naïve nondialysed CKD Stages 1–5 male patients. Additionally, we studied associations between endogenous testosterone levels and ESA dose (U/kg/week) in 126 ESA-treated men undergoing haemodialysis (HD).

Results. Among ESA-naïve males, patients with anaemia presented lower testosterone values. Endogenous testosterone was negatively associated with haemoglobin levels in uni- and multivariate models. Testosterone-deficient patients (total testosterone <10 nmol/L) were 5.3 (95% confidence interval 2.2–12.5) times more likely to be anaemic (Hb < 13.0 g/dL) than testosterone-sufficient patients. In ESA-treated men undergoing HD, higher ESA doses (above the median value of 121 IU/kg body weight/week) are associated with lower testosterone levels and higher percentage of hypochromic red blood cells (RBC). The inverse association between testosterone levels and ESA doses persisted after multivariate adjustment for age, sex hormone-binding globulin, comorbidities, C-reactive protein and s-albumin but was lost after further adjustment for iron medication and hypochromic RBC.

Conclusions. Hypogonadism may be an additional cause of anaemia and reduced ESA responsiveness in men with CKD. Our results raise the possibility that restoration of testosterone levels in hypogonadal CKD males may translate into lower prevalence of anaemia and better ESA responsiveness.

Keywords: androgens; chronic kidney disease; erythropoietin; sex hormones

Introduction

Anaemia and hyporesponsiveness to erythropoiesis-stimulating agents (ESA) are important phenomena

affecting patients with chronic kidney disease (CKD), which portend increased morbidity, mortality and a significant health care economic burden [1–3]. There are multiple causes of ESA hyporesponsiveness, the most important being, blood losses, iron-restricted erythropoiesis and inflammation [4–7]. Other less common and/or not consistently observed factors that may modify ESA responsiveness include other putative causes of anaemia, such as haematological diseases, malignancies, noncompliance, severe secondary hyperparathyroidism, high altitude, insulin resistance, statins and vitamin-D deficiency [8–11].

The association between androgens and erythropoiesis has been known for more than seven decades, and one common effect of testosterone therapy in non-renal populations is an increase in haemoglobin levels [12]. The exact mechanism(s) by which testosterone stimulates erythropoiesis is however, not yet evident. Although androgens have been reported to have myelostimulating effects by inducing production of haematopoietic growth factors in bone marrow stromal cells [13], it has been speculated that testosterone may also influence iron bioavailability [14]. Before the introduction of recombinant human erythropoietin into clinical practice, androgens were sometimes used to treat anaemia in dialysis patients [15, 16]. Androgens have also been suggested as adjuvants to ESA in the treatment of CKD patients with anaemia [17–19], but the risk of side effects has precluded its use in large numbers of dialysis patients [20].

Although testosterone deficiency is a prevalent condition in men with CKD [21–26], it has so far received scarce medical attention. The aims of this study were to evaluate if endogenous testosterone levels are associated with haemoglobin and ESA responsiveness in men with CKD and if the condition of testosterone deficiency contributes to the high prevalence of anaemia. This hypothesis is supported by a previous small sample size study, suggesting that endogenous testosterone may participate in the ESA resistance associated with renin–angiotensin system blockade in patients on haemodialysis (HD) [27]. We studied this in two carefully phenotyped cohorts of male CKD patients in whom testosterone levels had been analysed; one selected group of ESA-naïve CKD Stages 1–5 male patients and a prevalent group of ESA-treated men undergoing HD.

Material and methods

This study includes *post hoc* analyses in two independent cohorts (one from Turkey and one from Sweden) described as follows.

Cohort 1: ESA-naïve men with CKD Stages 1–5 non-dialysis

The ethical committee of Gulhane School of Medicine (Etlik-Ankara, Turkey) approved the study, and informed consent was obtained from each subject. Subjects were 239 prevalent male patients recruited between March 2006 and June 2010, who were referred to the Renal Unit of the Gulhane School of Medicine Medical Center, Ankara, Turkey for the first time because of suspected or manifest renal failure. The protocol of this cohort has been described in more detail elsewhere [25]. All patients were diagnosed as having CKD according to their estimated glomerular filtration rate (eGFR) and the presence of kidney injury as defined by National Kidney Foundation K/DOQI Guidelines [28]. Since this cohort was originally designed to study factors influencing endothelial dysfunction, patients taking the following medications at time of inclusion were excluded from the analysis: angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), statins, ESA or supplemental vitamin pills. In addition, exclusion criteria included acute infections and unwillingness to participate in the study. General characteristics of the patients included are detailed in Table 1. Thirty-eight of the patients were on antihypertensive therapy (21 patients were treated with calcium channel antagonists, five with beta-blocker agents, three with alpha blockers and nine with loop diuretics). Fifty-three of the patients were on antidiabetic therapy (21 patients were treated with oral antidiabetics and 32 with insulin). As soon as diabetic nephropathy was diagnosed, all patients with oral antidiabetics were changed to insulin.

Blood specimens were obtained in the morning after overnight fasting. Routine biochemical measurements included serum albumin, haemoglobin, parathyroid hormone (PTH) and high-sensitivity C-reactive protein (CRP). Serum total testosterone levels were assessed *post hoc* from frozen samples. Quantification was done in a Modular Analytics E170 Module (Roche Diagnostics, Indianapolis, IN), using a radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX). The reference range according to the kit manufacturers was 10–28 nmol/L (or 288–800 ng/dL).

Cohort 2: ESA-treated prevalent male patients undergoing HD

The Ethics Committee of Karolinska Institutet Hospital and Uppsala University Hospital approved the study protocols. Informed consent was obtained from all patients before inclusion in the study. Subjects were 126 prevalent men undergoing HD recruited between October 2003 and March 2004. The protocol of this cohort has been described in more detail elsewhere [29]. Since this cohort was originally designed to study factors influencing variability of the inflammatory status over time, exclusion criteria applied were missing repeated CRP values, acute infections, HIV, hepatitis B and unwillingness to participate. General characteristics of the included patients are detailed in Table 3. Patients were treated with HD three times a week (4–5 h/session) using bicarbonate dialysate and high-flux (22%) or low-flux (78%) dialysis membranes. Most patients were on antihypertensive medications (β-blockers, $n = 69$, calcium-channel blockers, $n = 33$ and ACEIs/ARBs, $n = 46$) as well as other commonly used drugs in terminal CKD (such as phosphate and potassium binders) and vitamin B, C and D supplementation. Forty-three patients were on lipid-lowering medication (statins). All patients were receiving ESAs at time of evaluation. The ESA dose was recorded for each patient as international units administered per week (U/week). Weekly doses of darbepoetin in micrograms were converted to international units of erythropoietin by multiplying with a conversion factor of 200. The median ESA equivalent dose was 10 000 (6 000–14 750) U/week, which was normalized for body weight and presented as U/kg/week in the following analyses. Intravenous iron sucrose (Venofer®) was routinely given to the patients on clinical indication as judged by the nephrologist in charge of the patient. Sixty-nine percent of the patients received iron sucrose at the onset of the study.

Blood specimens were collected before the HD session after the longest interdialytic period. High-sensitivity CRP, albumin, haemoglobin concentrations and the percentage of hypochromic red blood cells (RBCs) (percent of RBCs with Hb concentration <28.0 g/dL) were analysed using routine methods in the Department of Laboratory Medicine at Karolinska University Hospital or Uppsala Academic Hospital. Serum total testosterone levels and sex hormone-binding globulin (SHBG) were assessed *post hoc* from frozen samples, using certified routine methods in the Department of Laboratory Medicine at Karolinska University Hospital. The sensitivity of the method was <0.4 nmol/L and the coefficient of variation 7.6%. For the purpose of this study, testosterone deficiency was defined as <10 nmol/L in both cohorts according to the definitions of each local Laboratory Department and the Endocrine Society [30]. A cutoff of 10 nmol/L was shown to be the level where symptoms of hypogonadism started to be significantly more prevalent in elderly patients [31].

Statistical analysis

The variables are expressed as means ± standard deviations, medians (interquartile ranges) or percentages, as appropriate. Statistical significance was set at $P < 0.05$. As many values were not normally distributed, Spearman's rank correlation (Rho) was used to determine correlations between testosterone and selected variables. The clinical phenotypes of the groups considered were compared using the Kruskal–Wallis test (for continuous variables) or χ^2-test (for nominal variables). A multivariate linear regression analysis was performed to test the independence of the association between haemoglobin and ESA with testosterone levels. Data is presented as betas (β) and standard errors (SEs). A logistic regression

analysis was performed to test the probability of testosterone-deficient patients to have anaemia. Data is presented as odds ratios and 95% confidence intervals. Statistical analyses were performed using STATA version 11.1 (StataCorporation, College Station, TX).

Results

In order to study the association of endogenous testosterone levels with haemoglobin in CKD patients, we used a cohort of ESA-naïve CKD Stages 1–5 male patients. Table 1 depicts general characteristics of the patients stratified according to the presence/absence of anaemia (defined according to the World Health Organization as Hb $\leq$13 g/dL for men). As expected, patients with anaemia had lower eGFR, had more often diabetes mellitus, presented with higher degree of inflammation (CRP levels) and tended to have higher PTH levels. Patients with anaemia also had 35% lower testosterone levels than those without anaemia.

In univariate analysis, testosterone negatively correlated with haemoglobin (rho $= -0.23$, $P < 0.001$, Figure 1). PTH (rho $= -0.38$; $P < 0.001$) and CRP (rho $= -0.33$, $P < 0.001$), while positively with eGFR (rho $= 0.37$; $P < 0.001$). The association between testosterone and haemoglobin persisted in multivariate analysis after correction for the abovementioned variables (PTH, CRP and eGFR) plus age, body mass index (BMI), diabetes, cardiovascular disease and albumin (Table 2, Panel A). As sensitivity analysis, results were confirmed after dividing the population into patients with and without reduced kidney function ($<$ and $>$ 60 mL/min/1.73 m^2, respectively, not shown). Using the same confounders in a logistic analysis, we could observe that patients with testosterone deficiency (defined as total testosterone $<$10 nmol/L) had 5.3 times greater probability to have anaemia than testosterone-sufficient patients (total testosterone $>$10 nmol/L, Table 2, Panel B). The results were similar if a cutoff value for haemoglobin of 12 g/dL was used instead (data not shown).

In order to study the association of endogenous testosterone levels with ESA responsiveness, we used a cohort of ESA-treated prevalent patients undergoing HD. Table 3 depicts general characteristics of these patients stratified according to median weekly ESA dose/kg of body weight (U/kg/week). Patients with ESA dose above the median value had lower total testosterone concentration and increased percentage of hypochromic RBCs.

In univariate analysis, testosterone concentration was negatively associated with age (rho $= -0.23$, $P < 0.05$), hypochromic RBCs (rho $= -0.41$, $P < 0.001$, Figure 2), ESA dose (rho $= -0.24$, $P < 0.01$) and CRP (rho $= -0.38$, $P < 0.001$) but positively associated with the testosterone transporter SHBG (rho $= 0.31$, $P < 0.01$). Testosterone did not differ in patients with or without iron medication. The association between testosterone and ESA dose persisted after multivariate adjustments for age, SHBG, comorbidities, CRP and albumin (Table 4, Models 1–4). Additional adjustment for ACEI or statin use did not alter the results (sensitivity analysis not shown). However, further adjustment for iron medication and hypochromic RBCs made this association become nonstatistically significant, with the regression coefficient of the association (β) being reduced by half (from 0.28 to 0.14) (Table 4, Model 6).

Discussion

In the present study, we demonstrate that endogenous testosterone associates with haemoglobin levels in ESA-naïve CKD Stages 1–5 patients also following correction for age, eGFR, inflammation, s-albumin, PTH and comorbidities. CKD patients with testosterone deficiency were five times more likely to be anaemic than patients with normal testosterone levels. Our data accord with previous reports showing an association between testosterone levels and haemoglobin in other patient groups. The study by Grossmann *et al.* [32] showed that testosterone deficiency contributed to an increased frequency of anaemia in men with type-2 diabetes mellitus. In accordance, Bhatia *et al.* [33] demonstrated that both low testosterone and chronic inflammation contributed to mild anaemia in type-2 diabetic men. Other studies support that not only healthy middle-aged and older men [34] but also women [35] with low testosterone levels are at higher risk of anaemia. As there is a clinically and statistically significant decrease in haemoglobin concentration in men after orchidectomy [36], the differences in haemoglobin levels between the sexes have in part been attributed to the higher testosterone levels (usually at least 5–10 times higher) in men.

Table 1. General characteristics of 239 ESA-naïve CKD Stages 1–5 male patients stratified according to the presence of anaemia in males (according to WHO)[a]

	All patients, $N = 239$	Haemoglobin $\leq$ 13.0 g/dL, $N = 56$	Haemoglobin $>$ 13.0 g/dL, $N = 183$	P-value
Age, years	53 (46–63)	53 (45–63)	55 (47–64)	0.4
eGFR, mL/min/1.73 m^2	48 (5–106)	44 (14–80)	69 (28–95)	$<$0.001
Diabetes mellitus, %	22	48	14	0.002
Previous CVD, %	33	34	32	0.6
BMI, kg/m^2	26.3 (24.1–28.3)	26.1 (23.8–28.1)	26.4 (25.2–28.2)	0.6
s-Albumin, g/dL	4.0 (3.7–4.3)	4.0 (3.7–4.3)	4.0 (3.7–4.4)	0.4
CRP, mg/L	13.1 (9.0–21.2)	16.0 (9.9–23.0)	11.0 (6.2–16.4)	$<$0.001
PTH, pg/mL	127 (55–168)	131 (59–178)	84 (51–151)	0.05
Total testosterone, nmol/L	12.4 (7.4–14.9)	8.4 (4.8–13.2)	12.8 (9.7–15.4)	$<$0.001

[a]Data are expressed as medians (interquartile ranges) or percentages. CVD, cardiovascular disease; BMI, body mass index.

Another important finding of our study is the association between ESA dose and endogenous testosterone levels in ESA-treated men undergoing HD, a finding that persisted also when correcting for the impact of confounders, such as age, the testosterone-binding protein SHBG, inflammation and comorbidities in multiple regression analysis. This concords with a previous report on 76 men and women on HD, where testosterone was associated with erythropoietin (EPO) dose independently of age, haemoglobin and ferritin [27]. When iron medication and hypochromic RBCs were introduced into the model, the significant association between testosterone levels and ESA dose was lost in our study. This suggests that testosterone deficiency-induced anaemia may, at least in part, be mediated via reduced iron availability for erythropoiesis. Indeed, already in 1967, Haurani and Green [37] showed that testosterone supplementation improved iron reutilization (but not the intestinal absorption). Results from the recent study by Bachman *et al.* [14] lend further support to the concept that testosterone administration leads to better iron mobilization. In their study of healthy young men, testosterone supplementation increased haematocrit in a dose- and age-dependent manner via suppression of the master iron regulatory peptide hepcidin. The levels of hepcidin are markedly elevated in CKD and have been thought to be a novel biomarker of iron status and ESA hyporesponsiveness [38]. Indirect support of this is the observation that high doses of testosterone normalized haematocrit values in 16 HD patients and replenished their bone marrow iron stores [39].

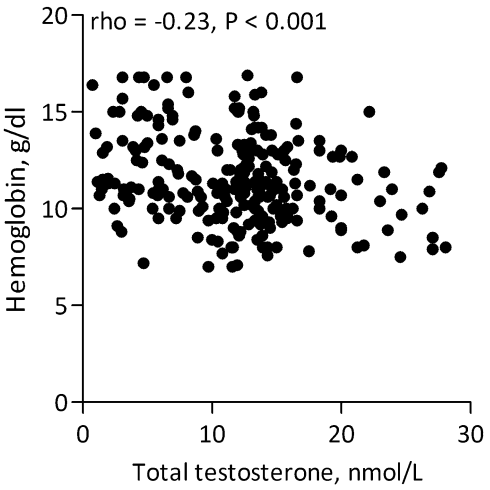

Fig. 1. Univariate Spearman correlation between testosterone levels and haemoglobin concentration in 239 ESA-naïve CKD Stages 1–5 male patients.

Altogether, our study identifies hypogonadal CKD men as a subgroup predisposed to impaired erythropoiesis and reduced ESA responsiveness, underlining the need for individualized and sex-specific therapeutic care and management [40]. As such, our results raise the question of whether restoration of testosterone levels to the normal range in these patients may translate into better ESA responsiveness. A number of studies in the past addressed androgen supplementation in dialysis patients (both men and women) as a mean to improve erythropoiesis [15, 16], with some [17–19], but not all [20] reports suggesting that the combined use of recombinant EPO and androgens elicit higher haematocrit than EPO alone. There are, however, few studies

Table 2. Multivariate regression model predicting haemoglobin (per g/dL) concentration (Model 1) and logistic regression analysis predicting the presence of anaemia (Hb $\leq$ 13.0 g/dL, Model 2) in 239 ESA-naïve CKD stage 1 to 5 male patients[a]

Model 1, prediction of haemoglobin (per g/dL) concentration, adjusted r^2: 0.35

	β (SE)	P-value
Total testosterone (nmol/L)	0.42 (0.02)	<0.001
Age (years)	0.07 (0.01)	0.2
BMI (kg/m^2)	−0.002 (0.04)	0.9
Diabetes mellitus (presence)	−0.19 (0.29)	<0.001
History of CVD	−0.005 (0.11)	0.5
eGFR (mL/min/1.73 m^2)	0.39 (0.01)	0.0004
s-Albumin (g/dL)	−0.08 (0.35)	0.1
CRP (mg/L)	−0.31 (0.02)	0.0003
PTH (pg/mL)	−0.16 (0.01)	0.09

Model 2, prediction of anaemia (Hb $\leq$ 13g/dL), pseudo r^2: 0.24

	OR (95% CI)	P-value
Testosterone deficiency (<10 nmol/L)	5.30 (2.23–12.56)	<0.001
Age (>median, 53 years)	1.22 (0.60–2.50)	0.6
BMI (>median, 26.0 kg/m^2)	0.77 (0.37–1.62)	0.5
Diabetes mellitus (presence)	5.03 (2.30–11.01)	<0.001
History of CVD	1.86 (0.87–3.05)	0.1
eGFR (per 10 mL/min/1.73 m^2 decrease)	1.43 (1.11–1.84)	0.005
s-Albumin (>median, 4.0 g/dL)	0.98 (0.46–2.06)	0.9
CRP (>median, 13.0 mg/L)	0.39 (0.13–1.17)	0.09
PTH (>median, 127 pg/mL)	3.66 (0.73–18.2)	0.1

[a]BMI, body mass index; CVD, cardiovascular disease.

Table 3. General characteristics of 126 ESA-treated prevalent male HD patients stratified according to median weekly ESA dose normalized per kg of body weight (IU/kg/week)[a]

	All patients, $N = 126$	ESA $\leq$ 121 IU/kg/week, $N = 63$	ESA $>$ 121 IU/kg/week, $N = 63$	P-value
Age, years	63 (49–73)	64 (47–76)	62 (49–70)	0.4
Vintage, months	25 (14–55)	28 (10–58)	24 (17–54)	0.8
Davies comorbidity score, %				
Low	19	20	17	0.1
Middle	54	60	49	
High	26	19	33	
BMI, kg/m^2	24.6 (21.3–27.5)	25.4 (22.5–28.2)	22.3 (20.0–25.7)	<0.0001
s-Albumin, g/dL	3.5 (3.2–3.8)	3.5 (3.3–3.8)	3.5 (3.1–3.8)	0.4
CRP, mg/L	6.8 (2.9–21.0)	6.4 (2.5–17.0)	8.7 (3.4–27.7)	0.2
Haemoglobin, g/dL	12.2 (11.0–13.4)	12.1 (11.1–13.3)	12.3 (11.2–13.3)	0.2
Hypochromic RBC, %	1.1 (0.5–4.1)	0.8 (0.4–1.5)	2.6 (0.6–5.9)	<0.0001
Intravenous iron medication, %	69	76	63	0.2
SHBG, nmol/L	27 (20–39)	27 (19–42)	26 (20–38)	0.9
Total testosterone, nmol/L	10.0 (7.2–12.0)	11.0 (8.4–13.0)	8.0 (6.6–12.0)	0.04

[a]Data are expressed as medians (interquartile ranges) or percentages. BMI, body mass index.

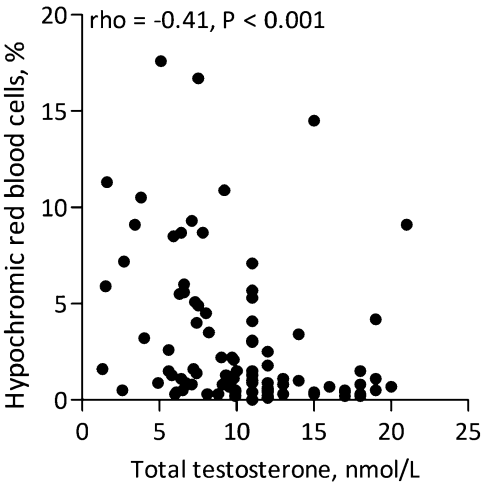

Fig. 2. Univariate Spearman correlation between testosterone levels and the percentage of hypochromic RBCs in 126 ESA-treated prevalent male HD patients.

that have tested the implications of testosterone restoration in hypogonadal CKD men. Normalization of endogenous testosterone by topical gels in hypogonadal men with advanced CKD improved sexual function [41], but daily administration of 100 mg of 1% testosterone gel for 6 months in 40 hypogonadal CKD men neither increased serum testosterone levels nor had an impact on ESA requirements [42]. It is possible that greater testosterone dosages may be required to achieve a clinical benefit in this patient group. Additionally, alternative methods of administration, such as intramuscular, may ease compliance and bioavailability. Based on previous reports in non-renal patient groups, it can be anticipated that testosterone supplementation in hypogonadal men with CKD may have beneficial effects beyond anaemia, such as reducing inflammation [43], enhancing mood and cognition [44], improving bone strength [45] or endothelial function [46] and increasing both muscle mass and strength [47]. Although testosterone restoration in the hypogonadal range is in general considered safe, a recent small randomized controlled trial calls for caution: in older testosterone-deficient men with limitations in mobility and a high prevalence of chronic disease, the application of a testosterone gel was associated with an increased risk of cardiovascular adverse events [48].

This study has strengths and limitations that merit discussion. The inclusion of two independent carefully phenotyped cohorts, one of which included ESA-naïve CKD patients with a wide range of renal function strengthens the study, is one strength of the study. Additional exclusion of patients on ACEI or statins in the nondialysed patient material is an additional strength when assessing more unconfounded associations with haemoglobin [4]. The two main limitations of the study include a limited sample size (especially in the dialysis cohort) and the cross-sectional study design, which precludes from inferring the causality of the observed associations. Thus, this analysis should be regarded as hypothesis generating and hopefully, it will entice the renal community to initiate prospective studies on the effects of correction of hypogonadism in men with CKD. Moreover, a single low testosterone determination may be insufficient to define hypogonadism, and indeed, the association between an age-related decline in testosterone concentration and a clinically relevant entity of hypogonadism still remains controversial. Because our analysis relies solely on testosterone values, it may have led to misclassification. In particular, we do not have information on clinical signs of hypogonadism (in both cohorts) or free fraction (in the predialysis cohort). However, symptoms of hypogonadism have low specificity and coincide with those inherent to CKD, making its identification difficult. In any case, as this limitation may result in underestimation of the true prevalence of male hypogonadism, it rather reinforces the clinical implications of our results. We also acknowledge that measurements of testosterone were done with different commercial kits in each of the cohorts and that some potentially relevant parameters that may affect ESA responsiveness, such as transferrin, hepcidin, as well as PTH and serum EPO concentrations (the former in prevalent HD patients only), were not available in our patient cohorts.

Table 4. Multivariate regression analysis showing the association between endogenous testosterone and weekly ESA dose normalized per kg of body weight (U/kg/week) in prevalent HD patients

Model	Prediction of ESA dose	β (SE)	r^2	P-value
1	Total testosterone (in nmol/L)	−0.28 (2.3)	0.05	0.007
2	1 + Age (in years) and SHBG (in nmol/L)	−0.28 (2.5)	0.04	0.004
3	2 + Davies comorbidity score	−0.27 (2.4)	0.06	0.006
4	3 + CRP (in mg/L) and albumin (in g/L)	−0.26 (2.6)	0.06	0.02
5	4 + Iron medication (yes/no) and hypochromic RBCs (≤1.1 versus > 1.1%)	−0.14 (2.9)	0.18	0.2

To conclude, our results suggest that hypogonadism may be an additional and today neglected cause of anaemia and reduced ESA responsiveness in men with CKD, through mechanisms that may involve impaired iron availability. On the basis of this observation, it can be speculated that correction of hypogonadism could reduce the anaemia burden and improve ESA responsiveness in men. Interventional studies are needed to prove this hypothesis.

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Conflict of interest statement. Baxter Healthcare Corporation employs B.L. and P.S. is a member of the scientific advisory board of Gambro AB and have lectured at a meeting sponsored by Bayer. None of the other authors declare any conflicts of interest.

References

1. Kilpatrick RD, Critchlow CW, Fishbane S *et al.* Greater epoetin alfa responsiveness is associated with improved survival in hemodialysis patients. *Clin J Am Soc Nephrol* 2008; 4: 1077–1083
2. Solomon SD, Uno H, Lewis EF *et al.* Erythropoietic response and outcomes in kidney disease and type 2 diabetes. *N Engl J Med* 2010; 363: 1146–1155
3. Johnson DW, Pollock CA, Macdougall IC. Erythropoiesis-stimulating agent hyporesponsiveness. *Nephrology (Carlton)* 2007; 12: 321–330
4. Elliott J, Mishler D, Agarwal R. Hyporesponsiveness to erythropoietin: causes and management. *Adv Chronic Kidney Dis* 2010; 16: 94–100
5. Stenvinkel P, Barany P. Anaemia, rHuEPO resistance, and cardiovascular disease in end-stage renal failure; links to inflammation and oxidative stress. *Nephrol Dial Transplant* 2002; 17 (Suppl 5): 32–37
6. Carrero JJ, Stenvinkel P. Inflammation in end-stage renal disease—what have we learned in 10 years? *Semin Dial* 2010; 23: 498–509
7. Barany P, Divinho-Filho JC, Bergström J. High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. *Am J Kidney Dis* 1997; 29: 565–568
8. Tangdhanakanond K, Raja R. Effect of statins on erythropoietin responsiveness in type 2-diabetic versus non-diabetic hemodialysis patients. *Clin Nephrol* 2010; 73: 1–6
9. Abe M, Okada K, Soma M *et al.* Relationship between insulin resistance and erythropoietin responsiveness in hemodialysis patients. *Clin Nephrol* 2011; 75: 49–58
10. Brookhart MA, Schneeweiss S, Avorn J *et al.* The effect of altitude on dosing and response to erythropoietin in ESRD. *J Am Soc Nephrol* 2008; 19: 1389–1395
11. Kumar VA, Kujubu DA, Sim JJ *et al.* Vitamin D supplementation and recombinant human erythropoietin utilization in vitamin D-deficient hemodialysis patients. *J Nephrol* 2011; 24: 98–105
12. Fernández-Balsells MM, Murad MH, Lane M *et al.* Clinical review 1: adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2010; 95: 2560–2576
13. Kim SW, Hwang JH, Cheon JM *et al.* Direct and indirect effects of androgens on survival of hematopoietic progenitor cells in vitro. *J Korean Med Sci* 2005; 20: 409–416
14. Bachman E, Feng R, Travison T *et al.* Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. *J Clin Endocrinol Metab* 2010; 95: 4743–4747
15. Richardson JR Jr, Weinstein MB. Erythropoietic response of dialyzed patients to testosterone administration. *Ann Intern Med* 1970; 73: 403–407
16. Neff MS, Goldberg J, Slifkin RF *et al.* A comparison of androgens for anemia in patients on hemodialysis. *N Engl J Med* 1981; 304: 871–875
17. Gaughan WJ, Liss KA, Dunn SR *et al.* A 6-month study of low-dose recombinant human erythropoietin alone and in combination with androgens for the treatment of anemia in chronic hemodialysis patients. *Am J Kidney Dis* 1997; 30: 495–500
18. Lee MS, Ahn SH, Song JH. Effects of adjuvant androgen on anemia and nutritional parameters in chronic hemodialysis patients using low-dose recombinant human erythropoietin. *Korean J Intern Med* 2002; 17: 167–173
19. Ballal SH, Domoto DT, Polack DC *et al.* Androgens potentiate the effects of erythropoietin in the treatment of anemia of end-stage renal disease. *Am J Kidney Dis* 1991; 17: 29–33
20. Sheashaa H, Abdel-Razek W, El-Husseini A *et al.* Use of nandrolone decanoate as an adjuvant for erythropoietin dose reduction in treating anemia in patients on hemodialysis. *Nephron Clin Pract* 2005; 99: c102–c106
21. Albaaj F, Sivalingham M, Haynes P *et al.* Prevalence of hypogonadism in male patients with renal failure. *Postgrad Med J* 2006; 82: 693–696
22. Gungor O, Kircelli F, Carrero JJ *et al.* Endogenous testosterone and mortality in male hemodialysis patients: is it the result of aging? *Clin J Am Soc Nephrol* 2010; 5: 2018–2023
23. Carrero JJ, Qureshi AR, Nakashima A *et al.* Prevalence and clinical implications of testosterone deficiency in men with end-stage renal disease. *Nephrol Dial Transplant* 2011; 26: 184–190
24. Carrero JJ, Qureshi AR, Parini P *et al.* Low serum testosterone increases mortality risk among male dialysis patients. *J Am Soc Nephrol* 2009; 20: 613–620
25. Yilmaz MI, Sonmez A, Qureshi AR *et al.* Endogenous testosterone associates with endothelial dysfunction and predicts cardiovascular events in men with nondialysis chronic kidney disease. *Clin J Am Soc Nephrol* 2011; In press
26. Karakitsos D, Patrianakos AP, De Groot E *et al.* Androgen deficiency and endothelial dysfunction in men with end-stage kidney disease receiving maintenance hemodialysis. *Am J Nephrol* 2006; 26: 536–543
27. DeLong M, Logan JL, Yong KC *et al.* Renin-angiotensin blockade reduces serum free testosterone in middle-aged men on haemodialysis and correlates with erythropoietin resistance. *Nephrol Dial Transplant* 2005; 20: 585–590
28. National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2004; 42 (Suppl 3): S1–S202

29. Snaedal S, Heimburger O, Qureshi AR *et al.* Comorbidity and acute clinical events as determinants of C-reactive protein variation in hemodialysis patients: implications for patient survival. *Am J Kidney Dis* 2009; 53: 1024–1033
30. Bhasin S, Cunningham GR, Hayes FJ *et al.* Testosterone therapy in adult men with androgen deficiency syndromes: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2006; 91: 1995–2010
31. Zitzmann M, Faber S, Nieschlag E. Association of specific symptoms and metabolic risks with serum testosterone in older men. *J Clin Endocrinol Metab* 2006; 91: 4335–4343
32. Grossmann M, Panagiotopolou S, Sharpe K *et al.* Low testosterone and anaemia in men with type 2 diabetes. *Clin Endocrinol (Oxf)* 2009; 70: 547–553
33. Bhatia V, Chaudhuri A, Tomar R *et al.* Low testosterone and high C-reactive protein concentrations predict low hematocrit in type 2 diabetes. *Diabetes Care* 2006; 29: 2289–2294
34. Yeap BB, Beilin J, Shi Z *et al.* Serum testosterone levels correlate with haemoglobin in middle-aged and older men. *Intern Med J* 2009; 39: 532–538
35. Ferrucci L, Maggio M, Bandinelli S *et al.* Low testosterone levels and the risk of anemia in older men and women. *Arch Intern Med* 2006; 166: 1380–1388
36. Fonesca R, Rajkumar SV, White WL *et al.* Anemia after orchiectomy. *Am J Hematol* 1998; 59: 230–233
37. Haurani FI, Green D. Primary defective iron reutilization. Response to testosterone therapy. *Am J Med* 1967; 42: 151–158
38. Ashby DR, Gale DP, Busbridge M *et al.* Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. *Kidney Int* 2009; 75: 976–981
39. Schustack A, Meshiaj D, Waiss Z *et al.* Intramuscular iron replenishment and replacement combined with testosterone enanthate in maintenance hemodialysis anemia: a follow-up of up to 8 years on 16 patients. *Clin Nephrol* 1985; 23: 303–306
40. Carrero JJ. Gender differences in chronic kidney disease: underpinnings and therapeutic implications. *Kidney Blood Press Res* 2010; 33: 383–392
41. Canguven O, Aykose G, Albayrak S *et al.* Efficacy of testosterone gel in the treatment of erectile dysfunction in hypogonadal hemodialysis patients: a pilot study. *Int J Impot Res* 2010; 22: 140–145
42. Brockenbrough AT, Dittrich MO, Page ST *et al.* Transdermal androgen therapy to augment EPO in the treatment of anemia of chronic renal disease. *Am J Kidney Dis* 2006; 47: 251–262
43. Kalinchenko SY, Tishova YA, Mskhalaya GJ *et al.* Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clin Endocrinol (Oxf)* 2010; 73: 602–612
44. Almeida OP, Yeap BB, Hankey GJ *et al.* Low free testosterone concentration as a potentially treatable cause of depressive symptoms in older men. *Arch Gen Psychiatry* 2008; 65: 283–289
45. Cauley JA, Ewing SK, Taylor BC *et al.* Sex steroid hormones in older men: longitudinal associations with 4.5-year change in hip bone mineral density—the osteoporotic fractures in men study. *J Clin Endocrinol Metab* 2010; 95: 4314–4323
46. Malkin CJ, Jones RD, Jones TH *et al.* Effect of testosterone on ex vivo vascular reactivity in man. *Clin Sci (Lond)* 2006; 111: 265–274
47. Kenny AM, Kleppinger A, Annis K *et al.* Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty. *J Am Geriatr Soc* 2010; 58: 1134–1143
48. Basaria S, Coviello AD, Travison TG *et al.* Adverse events associated with testosterone administration. *N Engl J Med* 2010; 363: 109–122

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Predictors of outcome in paediatric IgA nephropathy with regard to clinical and histopathological variables (Oxford classification)

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Abstract

Background. There has been a lack of international consensus on the classification and the predictive value of the histopathology findings in IgA nephropathy (IgAN). Recently, the International IgA Nephropathy Network has developed the Oxford classification in which four histological variables with the most prognostic importance are identified (MEST score). Our objective was to validate these findings and to assess their predictive power in our cohort and to compare them to identified clinical predictors.

Methods. Ninety-nine children with a follow-up time >5 years were included and investigated with clearances of inulin or iohexol for glomerular filtration rate (GFR), proteinuria and blood pressure at biopsy and during follow-up. Biopsies (90/99) were re-evaluated and scored according to the Oxford classification.