PILOT OPEN STUDY OF TESTOSTERONE REPLACEMENT IN NON-ALCOHOLIC STEATOHEPATITIS

Final Report

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Introduction

20-35% of adults have non-alcoholic fatty liver disease (NAFLD) (1-3). NAFLD often leads to liver inflammation and damage and sometimes to cirrhosis, liver failure and liver cancer and is now a common indication for a liver transplantation in the UK (4-6). No medical treatment has yet been shown to be effective in preventing this.

Some men with NAFLD have low levels of testosterone (male hormone). Often, levels are only slightly low and do not cause symptoms. However, these low levels may be aggravating the liver disease. NAFLD is thought to be caused by resistance of tissues to the actions of the hormone insulin (Insulin resistance or IR) (7-9). Low testosterone level is associated with and may cause IR (10-14) Treatments for prostatic cancer which lower testosterone levels result in both IR and in NAFLD (16,17). Mice who cannot produce testosterone also develop NAFLD and this is reversed by testosterone replacement. Testosterone administration to hypogonodal men(18-21)

We speculated that testosterone replacement in men with NAFLD and low blood testosterone levels will reduce liver fat content and also improve liver inflammation and fibrosis. Bayer awarded us funding for an open study of 10 patients.

We planned to study 10 men with NAFLD and some inflammation or scarring (proven on liver biopsy performed for clinical diagnosis) and who had mildly reduced testosterone levels. We planned to see if giving a 12-month course of Testosterone Replacement Therapy (TRT) to these men will lessen the severity of their liver damage.

Aims

To assess if, In hypogonadal men with non-alcoholic steatohepatitis (NASH), does Testosterone Replacement Therapy (TRT), given for 12 months

(a) improve severity of steatosis on liver biopsy (Primary Question)?

(b) improves severity of associated steatohepatitis on liver biopsy?

(c) reduce liver fat content as assessed by proton Magnetic Resonance Spectroscopy (^{1}H-MRS)?

The work is an open pilot study of 10 patients, the main aim of which is to assess the effect size of TRT in regard to these end points (regarding which there are no published data), thereby allowing power calculations for a more definitive phase II

trial. Other aims would be assessing recruitment and consent rates, which would also inform the design of the larger study.

Methods

Inclusion Criteria:

1. Abnormal serum ALT on >2 occasions over at least 3 months, despite standard lifestyle advice when appropriate, in regard to moderation of alcohol intake, weight reduction and exercise.
2. Negative serological tests for hepatitis Bs ag and C antibody.
3. Alcohol consumption >21 units per week for no more than 2 week in the last year and for no more than 3 months of the past 5 years, assessed using a lifetime alcohol consumption questionnaire [44].
4. Liver biopsy, performed as part of clinical management within 6 months of recruitment, which shows all of: (a) steatosis (Brunt grade 2 or 3)(22); (b) NASH (combined intralobular inflammation and hepatocyte ballooning score of $\geq$1)(22); (c) fibrosis Ishak stage $\leq$4(23); and (d) no evidence to suggest another major liver disease.
5. Hypotestosteronaemia, defined by total serum testosterone <11 ng/L (24) . We predict that this will include about 25% of men with NAFLD as defined above.

Exclusion Criteria:

1. Inability to give informed consent.
2. Age <18 or >75 years.
3. Symptomatic sexual dysfunction.
4. Cirrhosis either on baseline liver biopsy (Ishak score 5-6) or suggested by presence of varices, by ultrasound (small shrunken liver, ascites, splenomegaly) or by liver decompensation (encephalopathy, abnormal serum direct bilirubin, albumin or prothrombin time).
5. Space occupying lesion on ultrasound with any suspicion of malignancy.
6. Evidence of other chronic liver diseases pace occupying lesion on ultrasound with any suspicion of malignancy.
7. Prostatic nodule or mass on PR examination unless full urological examination rules our prostate cancer
8. Serum PSA or alpha feta protein above the age-specific normal range
9. Carcinoma of male breast
10. Taking medications (amiodarone, anti-retrovirals, sodium alproate, corticosteroids, tamoxifen) the previous 3 months (known to improve steatosis).
11. Diabetes or hyperlipidaemia, where therapy has been changed within the last 12 months or with suboptimal control anticipating the need for change in therapy during the study.
12. Severe or complicated obesity, likely requiring bariatric surgery in next 2 years.
13. LH/FSH levels reduced, raising the possibility of primary pituitary disease.
14. Subject trying to or hoping to conceive within next 18 months.
15. Haematocrit of >0.54
16. History of any of the following: Sleep apnoea, breast or prostate or liver cancer, congestive heart failure, chronic renal failure (serum creatinine >150), severe

chronic obstructive airways disease, uncontrolled hypertension epilepsy depression or migraine.

17. Severe co morbidity likely to reduce life expectancy to <10 years.
18. Hypersensitivity to active agent or to any of the excipients

Patients in whom H- MRS is not contraindicated (e.g. due to a pacemaker) were eligible for the trial but MR will not be performed

Individuals meeting inclusion and not exclusion criteria were invited to participate in the study. These criteria will confine patients to a limited severity range and so we do not anticipate any baseline parameters which will influence the main outcome. Therefore, randomisation will not be stratified. Patients unwilling to have a second biopsy but willing to have all other investigations, including ^{1}H-MRS (see below) would also be included in the study.

INTERVENTION

Testosterone Undecanoate (1 g in 4 ml oily base) was given as slow (2 minute) intramuscular injections (Nebido, manufactured by Bayer-Schering). These will be administered by the study investigator or designated nurse at time zero (baseline visit 2) and after 6, 18, 30 and 42 weeks.

In the event of a rise in Haematocrit to >0.54 the next Nebido injection was omitted and the subsequent scheduled injection given only if the Haematocrit falls below this value.

No changes were be made to concomitant medications at any stage of the study unless clinically indicated.

INVESTIGATIONS

Baseline

- Clinical assessment, including rectal examination
- Lifetime alcohol questionnaire[44]
- Aging male questionnaire
- SF-36 Quality of Life questionnaire
- Full blood count
- Serum liver enzymes
- Serum PSA (Prostate Specific Antigen marker for prostate cancer – see adverse effects)
- Serum alpha feta protein (marker for liver cancer – see adverse effects)
- HOMA index of insulin resistance (fasting blood glucose and insulin)
- Fasting serum lipids
- Serum Testosterone and bioavailable testosterone oestradiol
- Sex Hormone Binding Globulin (SHBG)
- Luteinising Hormone / Follicle-Stimulating Hormone (LH/FSH)

- Single-voxel localised ^{1}H-MRS of the liver to measure liver fat content performed , in University of Sheffield Academic Dept of Radiology, to estimate liver fat
- Anthropomorphic measurements to assess metabolic syndrome (weight, height, waist and hip circumference)

- Upper abdominal ultrasound (within 3 months of starting study)

- Liver biopsy will have been performed within the previous 6 months for clinical reasons. Although most patients will be newly presenting with NAFLD, we will also include patients with a diagnosis of NAFLD more than 6 months previously, who consent to repeat baseline liver biopsy with a view to participation in the trial.

- Liver total triglyceride content and polyunsaturation index, using H-Magnetic Resonance spectroscopy as recently described[49], performed in the Sheffield Teaching Hospitals Academic Department of Radiology.

Follow-up: Patients were seen after 6, 18, 30, 42 and 52 weeks when they completed the study. Subsequently, they will revert to standard clinical management (6 monthly clinic visits). If those subsequently found to be taking the active medication report benefits from it they will be referred to an Endocrine clinic for consideration of continuing TRT.

Clinical evaluation, anthropomorphic measurements compliance check and serum testosterone will be done at weeks 0, 6, 18, 30, 42 and 52. Anthropomorphic measurements and serum liver enzymes, lipid profile, testosterone, serum PSA and alpha feta protein will be done 6 monthly (Baseline visit 2, and on-treatment visits 2 and 5 at weeks 26 and 52 respectively.

After 52 weeks, H-1 MRS was repeated.

Liver biopsy was repeated 12 months after the commencement of study treatment. Baseline and 12-month biopsies were interpreted by an a Specialist Liver Histopathologist, who was blind as to the clinical details. Steatosis (grade 0-3), inflammation (0-2), hepatocyte ballooning (0-2) and fibrosis (0-6) were be graded using the Brunt (22) and Ishak (23) scoring systems.

Endpoints (as per protocol)

Primary: Proportion of patients in whom steatosis grade on biopsy improved

Secondary

1. Proportion of patients in whom liver inflammation, ballooning and fibrosis improve on repeat biopsy (after 12 months)
2. Change in fat content of liver by MR spectroscopy 0n repeat scanning after 12 months) and its correlation with steatosis on liver biopsy
3. Change in HOMA index after 6 and 12 months
4. Changes in serum liver enzymes (timepoint not pre-specified)
5. Adverse events

Recruitment

The study opened in June 2013. Over the first year, two patients were recruited and monitored by a 0.2 WTE Hepatology Research Nurse, whose employment ceased in August 2014. A further patient was recruited in 2015 and completed the study in September 2016. No further patients were recruited. The study was closed in 2017.

RESULTS

All three patients met the study inclusion criteria and gave informed written consent. All completed the study, attended for all their clinic visits (albeit visits 1 and 5 delayed in Patient 3). wo had the full number of five Nebido injections as per protocol. In patient 2, injections were given at baseline and after 6 and 18 weeks but were omitted after 30 and 42 weeks because of haematocrit values marginally over 0.54, as per protocol.

Adverse events

Patient 1 remained well throughout and reported no side effects.

Patient 2 remained well throughout and had no symptomatic side effects but Haematocrit was 0.541 at on-treatment visit 3 (30 weeks), resulting in omission of the planned Nebido injection as per protocol. At visit 4 (42 weeks) Haematocrit was 0.549 and again, the Nebido was omitted. At visit 5 (52 weeks), Haematocrit had fallen to 0.536..

Patient 3 had one on-treatment visit 1 (at 6 weeks) delayed because of a chest infection, not requiring hospital admission and which had resolved by the time of his visit. This was considered to be unrelated to the treatment. He had no other side effects and subsequently remained well.

Clinical parameters

Table 1 shows the baseline demographic and baseline parameters of the three patients Two of the three patients gained weight (102 and 1.9 kg) on treatment in the third, weight was unchanged. Waist-hip ratio (measured in 2 patients) did not change.

Patient Number	Age	Weight (Kg)	BMI	Waist/hip ratio	Diabetic	Other metabolic syndrome features	Other Co-Morbidity
1	64	91.2	31.5	1.04	Yes	Hypertension On statin	
2	67	119.6	38.1	1.00	no	Hypertension On statin	Gout
3	45	100.9	34.1	nr	Yes		Psoriasis
Mean	58.7	103.9	34.6	1.02			

Table 1. Baseline demographic and clinical variables

Laboratory blood tests (Table 2)

	Baseline	**6 weeks**	**18 weeks**	**30 weeks**	**42 weeks**	**52 weeks**
ALT IU/L (0-41)	77 (56-98)	67 (28-122)	59 (52-67)	77 (68-88)	100 (35-103)	88 (51-133)
AST IU/L (0-40)	67 (52-83)	62 (42-82)	41 (32-45)	52 (45-53)	56 (27-58)	missing
Alk- Phos IU/L (30-130)	165 (79-184)	156 (72-197)	143 (76-160)	138 (75-154)	136 (75-146)	136 (75-150)
Haematocrit (0.38-0.48) L/L	0.405 (0.395-0.491)	0.442 (0.441-0.506)	0.429(0.426-0.554)	0.445(0.409-0.541)	0.430(0.415-0.549)	0.434 (0.428-0.536)
Testosterone nmol/L(8.6-29)	6.5 (4.8-8.2)	13.9(10.3-15.2)	10.3(5.9-21.8)	12.1 (6-25)	13.4 (8.8-17)	7.9(5.0-9.6)
SHBG nmol/L (18.3-54.1)	26.7 (15.1-29.2)	23.6 (18.1-30.7)	19.7 (18.5-26.6)	21 (18.7-22.1)	22(16.8-28.8)	22 (17.9-26.4)
Insulin pmol/L (7.8-173)	258.9 (244.3-258.9)			190.8(167.3-493.6)		262.4(240.7-274.5)
Glucose (11.1)	10.2 (6.8-12.3)			8.3 (5.4-8.5)		7.25 (6.5-8.0)
Cholesterol mmol/L	3.8 (3.0-3.8)			3.8(3.1-6.1)		5.1 (4.0-6.2)
HOMA index	19.5(12.5-73.8)			11.7(6.7-31.1)		14.4(13.2-15.5)
Triglycerides	2.2 (1.5-			1.7 (1.1-5.1)		5.1 (2.5-7.7)

mmol/L (<1.7)	3.5)					
PSA μg/L (0.1-2.5)	1.2 (0.8-1.5)			1.4 (1.0-1.8)		1.5 (0.9-2.1)
α fetaprotein KU/L (3-8)	2 ((1-2)			1.5 (1-2)		2 (1-2)

Table 2. Laboratory tests (median (range))

In all patients, serum testosterone rose as expected, from (median(range) of 6.5 ng/L) at baseline to 13.1, 12.7, 14.3 and 13.4, after 6, 18, 30 and 42 weeks respectively, falling back however to 7.5 after 52 weeks. Patients 1 and 2 achieved values over 11 ng/L for 4 of the 5 on treatment occasions. In patient 3, levels rose but remained below 11 ng/dl. Serum SHBG remained unchanged throughout.

Serum Insulin and HOMA Insulin Resistance index were elevated in all three patients, suggesting insulin resistance, which is present in nearly all patients with NAFLD. These did appear to change during the study.

Although serum ALT and AST, seemed to fall modesty after 6 and 18 weeks, however, later values were unchanged from baseline.

Haematocrit rose slightly in all 3 patients from 0.41 at baseline to 0.43 and 0.44 after 18 and 30 weeks.

Cholesterol and triglycerides also rose slightly, though values after 52 weeks were missing in patient 3.

Serum bilirubin, and albumin, and the tumour markers: prostate specific antigen (PSA) and alpha feta-protein were unchanged and remained within the normal range throughout.

Liver histology (Table 3)

		Baseline biopsy	12 month biopsy
Steatosis	Graded 1-3	2(2-2)	1(1-1)
Hepatocyte	Ballooning	3(2-3) 233	1 (1-1) 111
	Necrosis	1(1-2)	2(1-2)
Neutrophils		2(0-3)	1(1-1)
Fibrosis	Central	5(4-5)	2(2-2)
	Portal	1(1-2)	1(1-1)
Brunt	Grade Inflammatory grade	3(2-3)	1(1-1)
	Stage of fibrosis	3(2-3)	2(2-2)
Ishak	Grade	1(0-1)	2(1-2)
	Stage	3(0-3)	2.5(1-4)

Table 3. Histology (median(range)

In all three patients, there were improvements in steatosis grade (1-: from 2,2,2 to 1,1,1), hepatocyte ballooning (1-3: from 2,3,3 to 1,1,1), in centi-lobular fibrosis (from 4,5,5 to 2,3,3) and in Brunt Inflammatory Grade (1-4: from 2,3,3 to 1,1,1)

There were questionable or no changes in neutrophil infiltration grade, in hepatocyte necrosis and in portal fibrosis.

Ultrasound of liver was in unchanged from baseline values, with no focal lesion after 6 months. Ultrasound after 12 months in patients 2 showed no focal lesion. In patient 3 ultrasound showed a x cm focal lesion after 12 months which was confirmed to be benign with a MRI scan. In Patient 1, ultrasound was incomplete after months (performed only with a view to liver biopsy). Subsequent ultrasound in 2018 in this patient showed no focal lesion.

Subsequent course.

All patients remained under follow up and clinically well until 2019

Discussion

This pilot study must be regarded as unsuccessful because of recruitment of only three of the planned 10 patients.

Reasons for failure to recruit include:

1.The acquisition of a Fibrosan in 2013 (the year the study started) enabled assessment of fibrosis severity in NAFLD noninvasively, and resulted in a steep decline on the number of biopsies performed in patients with NAFLD.

2.Absence of a research nurse made monitoring of patients difficult, as it depended purely on a single Clinical Investigator (DCG), who saw the patients as part of his Hepatology outpatient clinics.

3. Some inclusion and exclusion criteria were more restrictive than had been anticipated. These included:

(a) Diabetes or hyperlipidaemia, with suboptimal control anticipating the need for change in therapy during the study or change within previous 6 months resting in delayed eligibility and the need for a second diagnostic biopsy (as happened with patient 3).
(b) Some drugs such as amiodarone, corticosteroids.
(c) Most commonly, unwillingness of patients to undergo additional liver biopsies: at least one and two, in the case of patient 3 (if recruitment was > 6 months since diagnostic liver biopsy).

However, all three patients completed the study without significant adverse events, consistent with the clinical safety of Nebido.

The 6 weekly followed by 3-monthly injection regime is commonly used to treat hypogonadism and the regime is consistent with recommendations on the British National Formulary (recommended injection interval 10—14 weeks). 12-13 week administration has previously been shown to be biologically effective, to achieve testosterone levels in the normal range, and to minimise the risk of testosterone n (26,27). Thus, as expected, serum testosterone levels increased following the initial injection, although achieving vales > 11ng/L in only two of the three patients.

Polycytaemia (defined as Haematocrit >0.54) occurs in about 40% of testosterone-treated patients (28) but is usually mild (29) and has not been associated with thrombo-embolic complications. In the present study, patients with pre-existing polycytaemia and with severe chronic obstructed lung disease were excluded. Haematocrit rose in all patients during the study. In Patient two, it exceeded 0.54 after 18 and 20 weeks, precluding administration of the Nebido (as per protocol), but had fallen back to 0.536 at the end of the study.

There were no clear changes in serum liver tests. Possible initial falls serum ALT and AST were not sustained and other tests were unchanged. Improvement in serum AST and ALT in NAFLD is not always accompanied by histological improvement.

The most striking changes were in liver histology. Statistical analysis was not feasible because only three patients were studied. However, in all three patients there were improvements between the baseline and 12-months biopsies in grade of steatosis. Secondly, there were (in all patients) improvements in grade hepatocyte ballooning, which is one component of steatohepatitis, although other components (neutrophil infiltration, hepatocyte necrosis) seemed unchanged. However there were also improvement in the Brunt inflammatory score, which assesses all these features.

Finally, there were (in all three patients), improvements in grade of centrilobular fibrosis. This feature of fibrosis around the central vein of the liver lobule is characteristic of NASH. In contrast, fibrosis severity in the periportal regions was unchanged.

There are several caveats however. This result, in only 3 patients could have arisen by chance. Also, as this was an open observational study and alternative explanations include

(a)an effect of time. Steatosis and steatohepatitis can improve spontaneously with time; however fibrosis tends to worsen with time.

(b) regression to the mean. Liver biopsy is subject to considerable sampling variation. This, if patients are pre selected on the basis of severity in regard to any parameter, this parameter would tend to “improve” on repeat biopsy purely as a result of samling variation. However, whilst this is a potential explanation for the observed improvement in hepatocyte ballooning, it seems unlikely to explain the changes in steatosis or fibrosis, as baseline values were in the middle of the severity ranges (indeed patients with severe fibrosis/cirrhosis were excluded).

Thus, it is possible that these observations represent a genuine improvement in histology as a result of Testosterone treatment.

Many drugs have been evaluated in regard to their effect on liver histology in NASH. However very few have been shown to improve all of steatosis, steatohepatitis and fibrosis.

Based on these three patients, there is therefore a case for repeating (or extending) this study to see if the effect on liver histology is real.

References

1. Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000;**132**(2)**:**112-7.
2. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005;**42**(1)**:**44-52.
3. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;**40**(6)**:**1387-95.
4. de Ledinghen V, Ratziu V, Causse X, et al. Diagnostic and predictive factors of significant liver fibrosis and minimal lesions in patients with persistent unexplained elevated transaminases. A prospective multicenter study. *J Hepatol* 2006;**45**(4)**:**592-9.
5. Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J Hepatol* 2009;**51**(2)**:**371-9.
6. Soderberg C, Stal P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010;**51**(2)**:**595-602.
7.Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;**53**(2)**:**372-84.
8. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001;**120**(5)**:**1183-92.
9. Adiels M, Taskinen MR, Boren J. Fatty liver, insulin resistance, and dyslipidemia. *Curr Diab Rep* 2008;**8**(1)**:**60-4.
10.Muller M, Grobbee DE, den Tonkelaar I, Lamberts SW, van der Schouw YT. Endogenous sex hormones and metabolic syndrome in aging men. *J Clin Endocrinol Metab* 2005;**90**(5)**:**2618-23.
11 Traish AM, Feeley RJ, Guay A. Mechanisms of obesity and related pathologies: androgen deficiency and endothelial dysfunction may be the link between obesity and erectile dysfunction. *Febs J* 2009;**276**(20)**:**5755-67.
12. Isidori AM, Caprio M, Strollo F, et al. Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. *J Clin Endocrinol Metab* 1999;**84**(10)**:**3673-80.
13. Mohr BA, Bhasin S, Link CL, O'Donnell AB, McKinlay JB. The effect of changes in adiposity on testosterone levels in older men: longitudinal results from the Massachusetts Male Aging Study. *Eur J Endocrinol* 2006;**155**(3)**:**443-52.
14. Laaksonen DE, Niskanen L, Punnonen K, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care* 2004;**27**(5)**:**1036-41.
15. Kupelian V, Page ST, Araujo AB, Travison TG, Bremner WJ, McKinlay JB. Low sex hormone-binding globulin, total testosterone, and symptomatic androgen deficiency are associated with development of the metabolic syndrome in nonobese men. *J Clin Endocrinol Metab* 2006;**91**(3)**:**843-50.
16. Keating NL, O'Malley AJ, Smith MR. Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer. *J Clin Oncol* 2006;**24**(27)**:**4448-56.
17. Shahani S, Braga-Basaria M, Basaria S. Androgen deprivation therapy in prostate cancer and metabolic risk for atherosclerosis. *J Clin Endocrinol Metab* 2008;**93**(6)**:**2042-9.

18. Mayes JS, Watson GH. Direct effects of sex steroid hormones on adipose tissues and obesity. *Obes Rev* 2004;**5**(4)**:**197-216.
19. Jones TH, Arver S, Behre HM, et al. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). *Diabetes Care* 2011;**34**(4)**:**828-37.
20. Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol* 2006;**154**(6)**:**899-906.
21. Saad F, Gooren L, Haider A, Yassin A. Effects of testosterone gel followed by parenteral testosterone undecanoate on sexual dysfunction and on features of the metabolic syndrome. *Andrologia* 2008;**40**(1)**:**44-8.
22.Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;**41**(6)**:**1313-21.
23. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;**22**(6)**:**696-9.
24. Wu FC, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *N Engl J Med* 2010;**363**(2)**:**123-35.
25. Stanworth RD, Jones TH. Testosterone for the aging male; current evidence and recommended practice. *Clin Interv Aging* 2008;**3**(1)**:**25-44.
26. von Eckardstein S, Nieschlag E. Treatment of male hypogonadism with testosterone undecanoate injected at extended intervals of 12 weeks: a phase II study. *J Androl* 2002;**23**(3)**:**419-25.
27. Schubert M, Minnemann T, Hubler D, et al. Intramuscular testosterone undecanoate: pharmacokinetic aspects of a novel testosterone formulation during long-term treatment of men with hypogonadism. *J Clin Endocrinol Metab* 2004;**89**(11)**:**5429-34.
28. Rhoden EL, Morgentaler A. Risks of testosterone-replacement therapy and recommendations for monitoring. *N Engl J Med* 2004;**350**(5)**:**482-92.
29. Minnemann T, Schubert M, Freude S, et al. Comparison of a new long-acting testosterone undecanoate formulation vs testosterone enanthate for intramuscular androgen.